IOBC-WPRS

Working groups
“Induced Resistance in Plants Against Insects and Diseases” and
“Multitrophic interactions in soil”

Proceedings of the meeting

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

at

Riva del Garda (Trentino, Italy)
18-20 October 2017

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IOBC-WPRS Bulletin
Bulletin OILB-SROP **Vol. 135, 2018**

The content of the contributions is in the responsibility of the authors.

The IOBC-WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC-WPRS).

Le Bulletin OILB-SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB-SROP).

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ISBN 978-92-9067-320-0

Web: http://www.iobc-wprs.org

Darmstadt, 2018

Preface

The VIII meeting of the IOBC Working Group of "Induced Resistance in Plants Against Insects and Diseases" was held in Riva del Garda in Italy at the nice location of Fiere Congressi Spa on October 18-20, 2017. The main focus of the meeting was on "Ecological perspectives of induced resistance in plants and multitrophic interactions in soil". It took place together with meetings of other working groups of the IOBC-WPRS under the name of "Future IPM 3.0 towards a sustainable agriculture". The sessions were organized combining two IOBC Working Groups: "Induced Resistance in Plants Against Insects and Diseases" and "Multitrophic Interactions in Soil".

Altogether, these joined activities of IOBC Working Groups attracted 524 participants from 21 different countries. Although most participants came from academia, there was a great attendance from industry and even growers, confirming the large interest in these meetings outside of academia. This also shows that the efforts of the IOBC concerning knowledge transfer are successful. The conference had the classical structure of keynote talks, oral presentations and poster sessions. Additionally, there was a plenary session offered by Dr. Pieterse to all participants in the different parallel IOBC groups.

More specifically, the group meeting on Induced resistance and Multitrophic Interactions in Soil had 69 participants with 37 talks and 31 posters. The contributions addressed four main topics: Novel and old players in plant-microbe interactions, Functional ecology of microbial interactions in soil, Ecology of microbial interactions in soil, Ecology and factors affecting induced resistance and multitrophic interactions and plant defense. All these sessions were covered with a total of 64 extended abstracts that collected the main novelties and findings exposed along the meeting.

The participants discussed hot topics such as the plant variability of the induced-resistance mechanisms according to the age of the plant and the relevance of the transcription factors in systemic immunity and induced resistance in monocots and dicots. The conference also included the latest findings on the new and old resistance inducers like non-protein amino acids, hormonal peptides, protein digests and also xyloglucans or new forms of chitosan preparations. A relevant number of contributions were dedicated to the deciphering of mechanisms of induced resistance in crops and woody plants in different plant-pathogen systems. Although there was a dedicated working group, several presentations in the IR group highlighted the induced resistance mechanisms and immunity in grapevine in which new receptors of chitin that trigger immunity in grapevine were presented. Notably, the contribution of omics techniques to induced resistance was acknowledged and also the use of novel microorganisms or the management of the soil microbiota to protect crops and induce the plant immune system. Note that the joint meeting with the Multitrophic Interactions in Soil offered a new perspective on the regulation of induced defense responses by soil microorganisms. This subject was not fully covered up to now in the IR working group.

All these aspects of induced resistance in plants among many other exciting findings, have contribut- ed to an enormously successful meeting reaching the high scientific standards that this working group is offering since it was created. For all this, we are deeply thankful to all attendees and especially to the local organizers that perfectly coordinated such an interesting event as the Future IPM 3.0.

The entire meeting was kindly sponsored by the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems.

Financial support and donations

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Novel and old players in plant – microbe interactions

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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 beta-amylases and impaired in beta-amylase mutants.
- Vesicular trafficking directed by syntaxyns is also more active upon I3CA treatments.
- This suggests that amylases and syntaxins 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 behaviour 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 beta-amylase overexpressing pants were generates by the constructs 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 *KO* 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 *beta-amylase* gene expression is induced. To test its relevance in the priming mechanisms, mutants impaired in beta-amylases, were treated with I3CA. These mutants were impaired in I3CA-IR and accordingly did not display callose priming.

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

Acknowledgements

This work was financially supported by the Plan de Promoción de la Investigación de la Universitat Jaume I UJI-B2016-43, MINECO AGL2015-64990-C2-2 and the Generalitat Valenciana AICO/2016/029.

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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 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 fertirrigated 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 4th to the 7th 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 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.

Acknowledgements

The authors thank the companies who kindly provided samples of the biocontrol products. Thanks are also due to the plant production team of the INRA Plant Pathology Unit for their excellent technical assistance. This study was supported in part by the H2020 European Project EUCLID (Grant #633999) and by the CASDAR French National project SWEET.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 7-8

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

Plants' 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 upon *Botrytis cinerea* infection. Two weeks after tomato plants germination, plants were mycorrhized with the arbuscular mycorrhiza fungus *Rhizoglomus irregularis*. Four weeks later, plants were infected with *B. cinerea*. We sampled for gene expression analyses and callose quantification after 72 h of infection.

Gene expression of sugar transporters, sucrose synthases, invertases, amylases and the callose synthase, and other genes important in the fungal penetration resistance were studied.

Two treatments were used to determine the importance of the callose synthase and the importance of vesicular trafficking in callose deposition. Plants were sampled 72 h after infection to study callose content and cell death.

Results and discussion

B. cinerea growth less in mycorrhizal plants (AM) compared with non-mycorrhizal plants (NM). Moreover, callose showed higher levels in AM plants following fungal attack. So, callose deposition is a likely mechanism of defence mediating MIR.

We hypothesize that starch can be source of sugars for a faster callose deposition. Mycorrhizal plants showed an enhanced β-amylase gene expression. After starch degradation, more free glucose is available which can be used in the callose deposition.

Accordingly, mycorrhizal plants showed an enhanced expression of sucrose transporters and the sucrose synthases irrespective to infection. These results suggest that changes triggered by the fungus in the plant sugar metabolism may have an impact in defence by activating a more efficient starch hydrolysis and a more efficient callose synthesis.

Some proteins implied in vesicular trafficking was suggested to play an important role in the callose deposition in *Arabidopsis*. In our study, we also studied the gene expression of some of these proteins in mycorrhizal tomato plants.

Regarding callose synthase gene expression, it was up-regulated in AM plants compared to NM plants with and without infection. Treatments to block the callose synthase activity and the vesicular trafficking were effective, showing that both processes are necessary for a correct callose deposition.

Acknowledgements

This work was financially supported by the Plan de Promoción de la Investigación de la Universitat Jaume I P1- 1B2015-33, the Spanish Ministry MINECO AGL2015-64990-C2-2-R and the Generalitat Valenciana project AICO/2016/029.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 9-11

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 crops, 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. 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 inter- acting 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.

Acknowledgements

The work by SWW and SP was supported by two Undergraduate Vacation Bursaries provided by the British Society for Plant Pathology (BSPP). The project was funded from a Sheffield Undergraduate Research Experience (SURE) grant and the BBSRC Future Leader Fellowship BB/P00556X/1 to EL.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 12-14

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 binding sites which suggests that the ERF 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*, 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.

Acknowledgements

The authors acknowledge financial support from the Deutsche Forschungsgemeinschaft through SFB924.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 15-16

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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 17-19

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 dextrose 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.

Acknowledgements

The study was funded by research grants to M. L., from the Chief Scientist at the Israeli Ministry of Agriculture (No. 823-0209-14) and from the Israeli Science Foundation (No. 1375/14).

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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 suggest- ing 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*.

Acknowledgements

This work was financially supported by ANR (PATRIC project, grant ANR-13-KBBE-0001), the Regional Council of Bourgogne Franche-Comté (PARI II Vigne and Vins, 50% of Justine Claverie's PhD) and INRA (50% of Justine Claverie's PhD).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 23-25

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

Tomato plants from *BetterBoy* variety and two transgenic lines, overexpressor *35S::PROSYS* and the antisense of PROSYS gene lines were used for these experiments. Both transgenic lines were provided by the Ryan's laboratory and referenced in McGurl *et al*., 1994 and 1992. Growth conditions were as follow: in a growth chamber under 16 h of light (300 µE/m^2/s) at 26 °C and 8 h of dark at 22 °C. Vermiculite was used as a substrate, and when cotyledons are fully developed, were transferred into a 300 ml pots, with vermiculite:soil (1:1) mixture. Three times a week plants were watered with Long Ashton solution (Hewitt, 1966). Six weeks after germination, leaves samples were harvested, and placed in -80 °C until analysis.

Metabolomic analysis have 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, to finally detect the actual levels of the SYS, a chromatographic tandem mass spectrometry has been used. Form 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 in 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 has no detectable levels. Nevertheless, the levels were not as high as expected for a transgenic plant that overexpress a gene. This lead us to think that other posttranslational 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 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 more active the 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+. In 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. 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.

Acknowledgements

We thank the SCIC of the Universitat Jaume I for its technical support. This work was financially supported by the Plan de Promoción de la Investigación de la Universitat Jaume I P1-1B2015-33, the Spanish Ministry MINECO AGL2015-64990-C2-2-R and the Generalitat Valencia project AICO/2016/029.

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pp. 26-28

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 (Trdà *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.

Acknowledgements

This work was financially supported by the Conseil Régional de Bourgogne, INRA and the ANR Plant KBBE project PATRIC (Grant ANR-13-KBBE-0001-01; BP, DB).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 29-30

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.

Acknowledgements

This research was supported by grant TACR TG03010009.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 31-32

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 (21 °C) and 16 h night (18 °C) 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.

Acknowledgements

We thank Dr. Ana López Sánchez for technical and practical assistance. We also wish to thank the staff of the SCIC (Universitat Jaume I, Castellon) for technical assistance with mass spectrometry analysis. The research was supported by a consolidator grant from the European Research Council (ERC; no. 309944 "Prime-A-Plant"), a Research Leadership Award from the Leverhulme Trust (no. RL-2012-042) and a BBSRC-IPA grant BB/P006698/1 to J. T.

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pp. 33-34

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 chroma- tography 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.

Acknowledgements

The authors gratefully acknowledge the support of the Swiss National Science Foundation grant 31003A_140593 to B.M.M.

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Functional ecology of microbial interactions in soil

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 36-38

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.

Acknowledgements

This research was funded through the 2013-2014 BiodivERsA/FACCE-JPI joint call for research proposals, with the national funders BMBF (Germany), ANR (France), UEFISCDI (Romania), FWF (Austria, I2053), SNSF (Switherland).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 39-41

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 HiS-eq 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 log2-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.

Acknowledgements

S. C. and S. N. were funded by the Austrian Science Fund: grant Y0810-B16. We would like to thank the farmers who granted access to their land and Alfred Unmann (Landwirtschaftskammer Tirol) for support in identifying suitable sampling sites and Magdalena Pichler for lab support.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 42-44

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 pad- lock 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.

Acknowledgements

J. B. and S. N. were funded by the Austrian Science Fund: grant Y0810-B16.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 45-47

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). Twenty grams 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.

Acknowledgements

We thank Lallemand Plant Care and Triskalia as project partners.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 48-50

Weed microbiome characteristics and the development of bio-herbicides

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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 bioin-formatics 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.

Acknowledgements

We are grateful to Helmut Gangl (Gangl Wines) for the permission and help with sampling. The authors grateful to the Higher Education Commission (HEC) of Pakistan for financial support for A. Samad.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 51-53

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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pp. 54-55

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.

Acknowledgements

This project was funded by the European Union within the frame of FP7-KBBE-2013-7-single-stage (BIOCOMES; no. 612713).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 56-57

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 incorporatedat 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.

Ecology and factors affecting induced resistance

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 59-60

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 multi trophic 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

The project is funded by the U.S. National Science Foundation and the United States Department of Agriculture.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 61-63

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 (6 N 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.

Acknowledgements

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 265865 (project PURE, theme FP7-KBBE-2010-4) and no. 324416 (project INNOVA, theme FP7-PEOPLE-2012-IAPP), and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 722642 (project INTERFUTURE).

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

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Highlights

- *R. irregularis* colonization of tomato roots results in a defence priming increasing resistance of the shoots to *B. cinerea*.
- Perception of a nitrogen starvation has a negative impact in the plant resistance to pathogens.
- AM plants antagonize this negative impact of N starvation perceiving more efficiently N depletion than NM plants and activate the hormone regulation responses faster and stronger.

Introduction

Arbuscular mycorrhizal (AM) symbiosis is one of the most widespread mutualistic associations worldwide established. 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 *B. cinerea* in tomato, the mechanisms behind it and whether nitrogen sensing in the root environment have an impact on its functionality.

Material and methods

Tomato seeds (*Solanum lycopersicum* L. cv. Better Boy) were inoculated with the mycorrhizal fungus *R. irregularis* and maintained in a 25% in phosphorous Long Ashtom liquid solution with continuous aeration. 48 hours before *Botrytis cinerea* infection, part of the AM and NM tomato plants were watered with a free-N solution; 3rd and 4rd leaves were collected 72 h post infection to perform the analysis.

Cell death was detected by lactophenol Trypan Blue staining; *B. cinerea* fungal biomass was extracted as described by Sanchez-Vallet *et al*. (2010) and quantified by comparing the expression of the housekeeping genes of the fungus (BcTub) and the plant (LeEf-1) as described by Gamir *et al*. (2014). 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 leaves samples as described by Pastor *et al.* (2014). Targeted hormonal analyses were performed as previously described (Gamir *et al*., 2012). Non-targeted metabolic extraction and analysis was carried out as described by Pastor *et al*. (2014).

Results and discussion

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

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 OPDA were higher in infected mycorrhizal 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 mycorrhization 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 botrytis, 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 BBAM-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* colonization of tomato roots results in an increased resistance of the shoots to *B. cinerea*, likely through defense priming involving the induction of a set of secondary metabolites that appear to participate as an integral part of a complex tuning mechanism of the immune system in mycorrhizal plants. However, plants that sense potential N starvation reorganize their metabolism to prepare for a nutritional battle antagonizing defense responses against biotic stress but this defense repression observed in non-mycorrhizal plants is partially antagonized 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

This work was financially supported by the Plan de Promoción de la Investigación-Universitat Jaume I P1-1B2013-68 and the Spanish Ministry MINECO AGL2012-39923-C02 and AGL2015-64990-C2.

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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 resuspended (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). 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

The workshop on biocontrol experiments was organised thanks to the chair Agrosys. This work was supported by two PhD grants from the Vietnamese and Chinese governments. This work was supported by the GARP project from the ANR-SYSTERRA and MetaboHUBANR-11-INBS-0010 programs. Part of this work was supported by Natural Science Foundation of Yunnan Province of China (2016FD025).

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IOBC-WPRS Bulletin Vol. 135, 2018
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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 unknown.
- In adult plants I3CA-IR seems to be mediated by a faster callose accumulation.
- 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 20 °C day/18 °C 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 *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.

Acknowledgements

This work was financially supported by the Plan de Promoción de la Investigación de la Universitat Jaume I UJI-B2016-43.

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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 info-chemicals 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 108 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 re- sponse 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.

Acknowledgements

We thank Marlies Riedlmeier, Claudia Knappe, Kerstin Koch, Elisabeth Georgii, Sanjukta Dey, and Jane E. Parker for their contributions to this work. ACV thanks the Deutsche Forschungsgemeinschaft for funding through SFB924; ACV and JPS are thankful for financial contributions from the German Federal Ministry of Education and Research through the German Plant Phenotyping Network.

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Multitrophic interactions and plant defence

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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

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 722642 (project INTERFUTURE).

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pp. 78-80

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.

Acknowledgements

We are grateful to the Toulouse Genomics Platform (http://get.genotoul.fr) for access to the BioMarkTM HD System, to N. Ladouce (LRSV) for technical assistance, to L. Druelle for technical assistance in vineyard experiments, to L. De Bastard from Syngenta for supplying acibenzolar-S-Methyl. This work was supported by funding from four French ministries (FUI Neoprotec), the De Sangosse Company (Agen, France), the CIVB (Interprofessionnal Committee of Bordeaux Vines) and INRA.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 81-83

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-warmed (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*., 2015 b), 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*., 2015 a).

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.* (2015 a).

Acknowledgements

This study was funded by the LIFE financial instrument of the European Union (contract no. LIFE13 ENV/HR/000580). The authors are thankful for this financial support.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 84-86

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 below-ground.
- 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 hybridized 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 MeSA in the volatile root gall metabolome.

We aim to analyse whether volatile MeSA induces systemic resistance mechanisms in the root system. We hypothesize that released MeSA activates the SA signaling pathway demonstrated by the regulations of 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 x 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*., 2015, 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 log2FC; *VviSAMT2* 2.80 log2FC) providing evidence that nodosity formation triggered MeSA biosynthesis by the host plant via transcriptional stimulation. Microarray data (Griesser *et al*., 2015) confirmed the upregulations of *VviSAMT1* 3.24 log2FC and *VviSAMT2* 3.02 log2FC.

MeSA is reported to activate the salicylic acid (SA) signaling 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 log2FC; *VviPR5* 4.71 log2FC and *VviSTS* 2.28 log2FC) 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 mechanisms 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 or 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 log2FC and *VviSTS* 2.87 log2FC) in not infested root tips of infested plants reveal- ing that phylloxeration induced a systemic defense 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 signaling pathway in the global root system.

Acknowledgements

This project was funded by a DOC fellowship of the Austrian Academy of Sciences (ÖAW). The experiments were performed in the 2016/17 at the Division of Viticulture and Pomology at the BOKU in Tulln.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 87-88

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, *Trichoderma* (two treatments that can act as resistance inducers) and their combination were used for treatments in order to evaluate the potential effects on diseases and pests. The present paper reports the results obtained with the application of silicon.

Material and methods

To achieve the aims of this work, a single silicon foliar application was carried out 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. Traps 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 checked in the laboratory in order to evaluate the arthropods captured.

The most abundant taxa (Diptera: Nematocera and Phoridae), including the important groups which sustain ecosystem service in vineyard (Hymenoptera: Mimaridae, Chalcidoidea and Ichneumonoidea) were chosen for statistical analysis and a multifactorial analysis of variance (ANOVA) was carried out.

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 leafhopper 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 effective as resistance inducer. These results in fact 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). Attractivity of silicon (and other elicitors) would be confirmed in other studies, including the potential increase of ecosystem services. 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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 89-91

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

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Highlights

- Liquorice leaf extract and fraction 4 elicit early responses in treated, non-infected cucumber plants.
- Fraction 4 is likely 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* (Scherf *et al*., 2012). 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* (tests on the oomycete *Phytophthora infestans*). Furthermore, treatment of cucumber leaf discs resulted in an accumulation of H_2O_2, (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 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: dichloromethane was substituted by tert. butyl-methyl-ether), 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%), BABA (0.1%, POD trial), EtOH (2%, efficacy trial) or 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 peroxidase (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 fractions 4 and 6 were found. We now observed the same reaction after treatment of leaf discs of the cucumber cv. ‘Agnes’ with liquorice extract and these fractions.

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. Therefore, 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. Being toxic for the plant tissue, the plant protects itself from being destroyed, e. g. by depletion of ROS with the help of POD (Lin and Ishii, 2009).

The relative expression of POD was approx. 8 times higher in BABA and fraction 4 treated plants (6 hours 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 3 times higher compared to that in untreated plants. A similar peak occurred in untreated, infected plants 6 hours post inoculation. In untreated, infected plants, there was a second peak 3 times the height of the first one, 6 days post inoculation. This suggests that in the liquorice treated plants a second peak may occur after inoculation as well. This will be tested.

The effect of crude liquorice 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. Whereas fraction 4 moderately controlled the infection with *P. cubensis* (efficacy = 17%) in ‘Chinese Slange’, it seemed to promote the infection in ‘Agnes’ (disease severity: EtOH = 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 and hint induced self-defence as part of the mode of action of the liquorice leaf extract and especially of 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* (Scherf *et al*., 2016).

To verify the results of this preliminary study, further trials on the expression of other pathogenesis related protein genes, beyond POD, are in progress. 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

This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 28949.

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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.

Acknowledgements

This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 324416 (project INNOVA, theme FP7-PEOPLE-2012-IAPP).

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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 from 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.

Acknowledgements

Authors are very grateful to the National Plant Protection Institute (INPV) El Harrach, Algeria, for providing necessary facilities and chemical analysis of extracts and also, to the Department of Sustainable Ecosystems and Bioresources, Fondazione Edmund Mach, San Michele all' Adige, Italy, for molecular analysis.

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Multitrophic interactions and plant defence
Poster session

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pp. 98-100

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 (Shanker *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

Solanum lycopersicum Mill cv. Ailsa Craig plants were grown under growth chamber with a photoperiod of 16/8 h (light/darkness), temperature of 26/18 °C and relative humidity 60%. One-month-old plants were maintain as untreated (control) or under drought stress (lacking of water during one week), or temperature stress (5 °C temperature increase), or *Botrytis cinerea* infection (applying to the plants droplets containing 10^6 spores and sampling 24 hours post

inoculation; hpi), or *Pseudomonas syringae* pv *tomato* DC3000 infection (inoculation by dipping plant leaves into the 5×10^5 cfu/ml 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 plants, cDNA was synthesised, and *SlyWRKY75* gene expression was measured by qRT-PCR, using Power SYBR Green PCR MasterMix (Applied Biosystems) and gene specific forward and reverse primers. For each sample, 3 biological replicates were analysed. *RPS18* (ribosomal protein S18) gene was used for relative-fold calculations to normalize gene expression. qRT-PCR was also used to quantify miRNA expression.

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-hexa- polequadrupole 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.

Acknowledgements

This work was supported by "MINECO" and "FEDER" (AGL2013-49023-C3). M. J. López-Galiano was awarded with a University of Valencia Ph. D. fellowship. A. I. González-Hernández is currently supported by a grant from "Programa de apoyo a la formación e incorporación de personal investigador" of Jaume I University (UJI-PREDOC/2016/27). O. Crespo-Salvador was the recipient of a research contract from grant C1. We thank the Genomics, Proteomics and Greenhouse Facilities from the SCSIE of the University of Valencia and the SCIC from the University Jaume I.

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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.

Acknowledgements

We thank Stefan Kellenberger, Agroscope Changins, Nyon, for technical support advice to handle the leaf rust pathogens. F. B. gratefully acknowledges the financial support by the Swiss Federal Commission for Scholarships for Foreign Students.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 104-106

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 log10 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.

Acknowledgements

This work was supported by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 324416 (project INNOVA).

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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 d 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.

Acknowledgements

This project has been funded by Ministero delle Politiche Agricole Alimentari e Forestali, Italy, MIPAAF-ID052-NANO-TOX. We warmly thank Prof. Wilhelm Schäfer (Biocenter Klein Flottbek, Hamburg, Germany) for providing the GFP-engineered *Fusarium graminearum* strain, and Deam Laterza and Loris Previtali for helpful technical assistance.

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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 microorganisms' 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 as 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 are 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).

Acknowledgements

The research frameworks USAGE and SWEET benefit from the respective financial support of the “Office National des Eaux et des Milieux Aquatiques” and the “Ministère de l’Agriculture” on the Ecophyto plan.

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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 sensi- tivity 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.

Acknowledgements

The authors thank Melaku Demese of Holetta Agricultural Research Center for arranging the land for the experiments and VR-Uforsk grant “Effects of a low toxicity pesticide in an intercrop system” for funding.

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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 Blight. 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.

Acknowledgements

This research was funded by the Swiss National Research Programme 69 "Healthy Nutrition and Sustainable Food Production" (grant number: SNF 145210/1).

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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 (R2) 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

This work has been financially supported by the Free University of Bozen-Bolzano (IMPRESS- TN2071).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 119-121

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 fo- cused 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

This project has received funding from « Pour et Sur le Plan Ecophyto » PSPE 2_ONEMA.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 122-123

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. longibrachiatum*, *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.

Acknowledgements

This project was funding by ENSA (École Nationale Supérieure Agronomique).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 124-126

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.

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.

Acknowledgements

This research was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 127-129

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 crop 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 (2 m × 2 m 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 farmers' 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.

Acknowledgements

This research work is of collaboration between Wageningen University and Research Centre (WUR), The Netherlands and Bangladesh Agricultural University (BAU) and funded by NUFFIC-NICHE- BGD-156 program.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 130-132

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 are 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.

Acknowledgements

This project has received funding from BBSRC and is an industrial case studentship, Grant ref. BB/M016765/1. This project is undertaken at Newcastle University and twinned with Stockbridge technology Centre and Rothamsted research.

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Functional ecology of microbial interactions in soil
Poster session

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pp. 134-135

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 Enchytraeae 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 observations 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 was 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 which had the highest 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.

Acknowledgements

This research was funded through the 2013-2014 BiodivERsA/FACCE-JPI joint call for research proposals, with the national funders BMBF (Germany), ANR (France), UEFISCDI (Romania), FWF (Austria, I2053), SNSF (Switzerland).

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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 h at 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 found 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

This research was funded through the 2013-2014 BiodivERsA/FACCE-JPI joint call for research proposals, with the national funders BMBF (Germany), ANR (France), UEFISCDI (Romania), FWF (Austria, I2053), SNSF (Switzerland).

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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 was 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 root- stocks 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 in- creasing 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.

Acknowledgements

This work has been financially supported by Free University of Bozen-Bolzano (GRASP TN2053, RHIZOPRO TN2081).

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pp. 140-141

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/kg of 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

This work was financially supported by the European Union - Programme Life+: 2007-2013 (LIFE12 ENV/IT/000671 - LIFE-OPTIMAL2012).

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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 micro- biological 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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 144-145

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 favoured 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.

Acknowledgements

The authors wish to thank Lucie Caron from MAPAQ for their expertise. We also thank technicians from CRAM and INRS-IAF for their technical support. This project has received funding in part through the Agri Innovation Program (AIP) on behalf of Agriculture and Agri-food Canada.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 146-148

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.

Acknowledgements

Funding for this project has been provided by a private foundation, Agriculture and Agri-Food Canada and the BC Ministry of Agriculture through the BC Agriculture and Food Climate Action Initiative under Growing Forward 2, a federal-provincial-territorial initiative. The program is delivered by the Investment Agriculture Foundation of BC. In 2016, Paige Munro was the recipient of Natural Sciences and Engineering Research Council of Canada – Canada Graduate Scholarships – Master's (NSERC-CGS-M).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 149-151

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.

Acknowledgements

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 324416 (project INNOVA, theme FP7-PEOPLE-2012-IAPP).

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 152-154

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 m^2 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 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 endogaeic 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) cit. 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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pp. 155-156

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 nutri- ent 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 colour 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 as 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.

Acknowledgements

This work has been financially supported by the Free University of Bolzano (TN2023).

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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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 160-161

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

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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 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 *Fusarium* 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 caryophyllata*, *Lavandula stoekas*, *Allium sativum*, *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*.

Acknowledgements

The authors are grateful to the National Institute of Plant Protection (INPV) and the Laboratory of research of Medicinal and Aromatic Plants for the means and the facilities provided.

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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 162-164

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: T1: control (zero urea), T2: 3% neem oil coated urea (NOCU); three levels of T2: T2L1 (half dose of N: 0.055 g/kg of soil), T L (recommended dose of N: 0.111 g/kg of soil) and T2L3 (full dose of N: 0.222 g/kg of

soil); T : 3% moringa oil coated urea (MOCU) same levels used as in NOCU; T4: ordinary urea (without coating) same levels used as in NOCU and MOCU without coating. The rate of nitrogen application was different for all levels [L1: 0.055 g/kg (Half dose of N); L2: 0.111g/kg (recommended dose of N); L3:0.222g/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 -N and NH +-N, were analysed according to Soil and Plant Analysis Laboratory Manual (Ryan *et al*., 2001).

Results and discussion

Plant height significantly increased in T2L1 with the application of 3% neem oil coated urea with L1 (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 T3L3 and T4L3 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 the case of neem oil coated urea with level-1 (L1: 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 T2L1 where 3% neem oil coated urea was used. There was no 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 (0.41 g) was observed in neem oil coated urea T2L1 with half dose of the N (0.055 g) than the other two levels T2L2 and T2L3.

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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 165-167

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 24.7 kg per hectare. N, P and K were 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.

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.

Acknowledgements

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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IOBC-WPRS Bulletin Vol. 135, 2018
pp. 168-170

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 designated as 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 commercialized 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).

Acknowledgements

The three month internship of Pablo Garcia-Raya at Agroscope was financed by the Universidad de Almeria.

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