COST Action 0807
Integrated Management of Phytoplasma Epidemics in Different Crop systems

Working group 3
"Phytoplasma control in crop systems"

Working group meeting
"Prospectives of endophytes as biocontrol agents in the management of phytoplasma diseases"

San Michele all'Adige, Italy, 14th of November 2012

Convenors:
Wolfgang Jarausch & Ester Torres

Local organiser:
Mirko Moser

Research and Innovation Centre - Foundation Edmund Mach (FEM)
Via E. Mach, 1
38010 – San Michele all'Adige (TN) – Italy

FONDAZIONE EDMUND MACH

Program

Wednesday, 14th November

9:00

Welcome by the local organiser

Introduction by Wolfgang Jarausch

9:30 – 10:30

Marzachì C., D'Agostino G., Gamalero E., Bosco D., Berta G.
Beneficial rhizospheric microorganisms and control of phytoplasma diseases

Musetti R., Farhan K., Grisan S., Polizzotto R., De Marco F. Ermacora P.
Fungal endophytes as innovative tools for phytoplasma disease control

Bulgari D., Casati P., Quaglino F., Bianco P.A.
Characterization of beneficial bacteria isolated from grapevine leaves

10:30 – 11:00 coffee break

11:00 – 12:30

Naor V., Iasur-Kruh L., Zahavi T., Sharon R., Perlman S.J., Bordolei R., Zchori-Fein E.
The influence of bacteria from an insect vector on plants infected with phytoplasma

Romanazzi G., Murolo S., Feliziani E., Landi L.
Resistance inducer applications on the canopy of Bois noir infected plants cv Chardonnay: impact on the disease symptoms and on grapevine growth and production

Spinas N.L., Snyman M., Visser M., **Stephan D**., Burger J.T.
Can antimicrobial peptides be used to engineer resistance against the grapevine pathogen aster yellows phytoplasma?

General discussion

12:30 – 14:00 lunch

14:00 – 16:30

COST FA0807 WG3 subtask leader meeting

Beneficial rhizospheric microorganisms and control of phytoplasma diseases

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Bacteria living in the rhizosphere may influence plant growth and health by a number of mechanisms. Arbuscular mycorrhizal (AM) fungi form mutualistic associations with most terrestrial plants, including agricultural and horticultural crop species. This symbiosis not only influences plant nutrition, but also improves the plant's ability to overcome biotic and abiotic stresses. Hence, the presence of beneficial rhizospheric microorganisms, able to increase plant tolerance to biotic stresses or to behave as biocontrol agents, may represent a valid tool for controlling phytoplasma diseases in the frame of an IPM strategy. The effects of rhizobacteria, AM fungi, and mixed inoculum of rhizobacteria and AM fungi against the chrysanthemum yellows phytoplasma (CYP) infection of daisy (*Chrysanthemum carinatum*) was assessed. CYP is a strain of the '*Candidatus* Phytoplasma asteris' (16Sr-IB) which infects a variety of dicotyledonous plants, including daisy, causing very specific symptoms. CYP is transmitted with different efficiencies by several species of leafhoppers, which can be reared continuously under controlled conditions. Plant protection from the disease, pattern and severity of symptom expression, CYP multiplication and phytoplasma viability were evaluated in plant inoculated with the microorganisms in different combinations as well as in the non inoculated controls. A qualitative description of phytoplasma cell morphology by transmission electron microscopy was also performed. Single inoculations with *Glomus intraradices* BBE and *G. mosseae* BEG12 as well as inoculation with a mixed inoculum of *Pseudomonas putida* S1Pf1Rif and *G. mosseae* BEG12 resulted in some level of resistance to phytoplasma infection. CYP reduced plant growth and root development in non inoculated control plants. *P. putida* S1Pf1Rif, the AM fungi *G.intraradices* BBE and *G. mosseae* BEG12, and mixed inoculum with the pseudomonad and *G. mosseae* improved plant growth of CYP-infected plants. AM fungi, alone or in the mixed inoculum

also delayed symptom expression in non resistant plants. Senescent phytoplasma cells often were observed in fully developed leaves of CYP-infected plants inoculated with *P. putida* S1Pf1Rif and with the combination of the pseudomonad and *G. mosseae* BEG12. A more active and efficient root system in double-inoculated plants probably mediated the effects of the two rhizospheric microorganisms in the infected plants. The evaluation of the activity of rhizospheric microorganisms in mitigating phytoplasma damage under field conditions on grapevine is currently under investigation.

Fungal endophytes as innovative tools for phytoplasma disease control

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Plants frequently host endophytic fungi and/or bacteria which, for all or part of their lifecycle, colonize the tissues of living plants without causing disease. About fungal endophytes, they can result extremely diverse in the different plants, colonizing all the parts of the host. In spite of the knowledge about their biology still incomplete, it is recognized that endophytes are an important source of secondary metabolites and compounds of biotechnological value as antibiotics or antitumor drugs. Endophytes established mutualistic relationships with plants also inducing physiological modifications in their hosts, making them more resistant against biotic or environmental stresses.

As phytoplasma diseases are not curable and their control is exclusively based on vector control, the use of endophytic microorganisms could represent an alternative strategy against phytoplasmas. In fact, the actual concept of "management" and control of plant diseases implies the application of modern measures compatible with the environment, the cultural essential for the crops and economic thresholds.

Fungal endophyte strains have been isolated from grapevines and apple plants grown in areas where a spontaneous remission of disease phenomenon known as "recovery", was recurrent. Some of these endophytes, such as *Epicoccum nigrum* and *Aureobasidium pullulans*, are very interesting because already reported as biocontrol agents or resistance inducers. Recent research activities, performed using the model plant C*atharanthus roseus* infected with '*Candidatus* Phytoplasma mali', demonstrated that reduction in symptom severity and lower phytoplasma titre in host tissues occur when the plants were previously inoculated with an endophytic strain of *E. nigrum*. Aiming to assess the possibility of using *E. nigrum* in phytoplasma disease control, new investigation is in progress using the natural host of '*Ca*. P. mali', *Malus domestica*.

Characterization of beneficial bacteria isolated from grapevine leaves

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Bacterial endophytes are plant-associated bacteria that affect the plant life cycle in different manners such as the nitrogen fixation or the biocontrol of plant pathogens (Lugtemberg and Kamilova 2009). A basic point for the success of sustainable management of plant diseases based on biocontrol agents is the study of endophytic bacterial community associated with plants. Recently, endophytic bacteria known as putative biocontrol agents have been isolated from healthy, grapevine yellows (GY)-diseased and recovered grapevines (Bulgari et al., 2009; 2011). In the present work, such endophytic bacteria were characterized for beneficial traits related to mineral nutrition (phosphate solubilization, siderophores production), development (indolacetic acid-IAA synthesis), stress relief (1-amino-cyclopropane-1-carboxylate deaminase and catalase activity), disease control (chitinase activity, siderophores production). In detail, five bacterial isolates showed the ability to solubilize phosphate, react to stress and synthesize IAA. In addition, some of these strains are able to produce siderophores. Furthermore, the presence of the three best bacterial genera was analyzed in healthy, GY-diseased and recovered grapevine plants sampled from June to October. The analyses revealed a different bacterial distribution related to the season and to the plant sanitary status.

In conclusion, beneficial bacterial strains which have the potential to enhance plant growth and suppress diseases have been found in strict association with grapevine leaf tissues.

References

1. Bulgari D, Casati P, Brusetti L, Quaglino F, Brasca M, Daffonchio D, Bianco PA (2009) Endophytic bacterial diversity in grapevine (*Vitis vinifera* L.) leaves described by 16S rRNA gene sequence analysis and length heterogeneity-PCR. J Microbiol 47: 393–401.

2. Bulgari D, Casati P, Crepaldi P, Daffonchio D, Quaglino F, Brusetti L, Bianco PA (2011) Restructuring of endophytic bacterial communities in grapevine yellows-diseased and recovered *Vitis vinifera* L. plants. Appl Environ Microbiol 77: 5018–5022.

3. Lugtemberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63: 541-556.

The influence of bacteria from an insect vector on plants infected with phytoplasma

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The control of phloem restricted pathogens needs new strategies given that conventional application of chemical spray is inefficient. The role of endophytes in the induction of plant resistance against such pathogens including phytoplasma has been suggested. However, in order to proceed towards practical application, such a candidate should be cultivable and able to penetrate and survive within the plant for a reasonable time. We hypothesized that since phytoplasma is transferred by phloem feeding insects, the vectors, as well as the host plants, may serve as reservoirs of potential micro-organisms. Such a potential and beneficial bacterium was isolated from an insect vector (*Hyalesthes obsoletus* (Hemiptera: Cixiidae)), and grown on artificial medium. The isolate, belonging to the bacterial family Xanthomonadaceae, was introduced to healthy and phytoplasma-infected plants (periwinkle and grapevine) and its presence in plant tissues was confirmed by PCR analysis three weeks post inoculation. A change in the plant morphology was observed four and eight weeks post inoculation. The presence of the bacteria seems to affect the morphology of the infected plants but not the healthy ones. In some parameters, the effect of phytoplasma on plant morphology was markedly reduced. Further study is needed to examine the potential use of this isolate as a bio control agent against phytoplasma.

Resistance inducer applications on the canopy of Bois noir infected plants cv Chardonnay: impact on the disease symptoms and on grapevine growth and production

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Grapevine Bois noir (BN) is widespread in most viticultural regions of Europe and Mediterranean area, determining heavy detriments of yield production and affecting grapevine quality. At the moment, an effective strategy to reduce the incidence of BN-infected vines is not known. However, the interesting phenomenon called recovery, which naturally happens, seems to be related to systemic acquired resistance. Five commercial resistance inducers (Chito Plant, Olivis, Bion, Aliette, and Kendal) were weekly applied on the canopy of BN-infected vines cv. Chardonnay from beginning of May to the end of July in two different vineyards located in Abruzzi (2007-2008) and Marche (2009-2010), respectively. At the end of a 4 year-field trial, all elicitors reduced the number of symptomatic vines, limiting the incidence of dehydrated clusters. The best and constant performances were reached applying Olivis, Kendal and Bion, allowing to obtain a production in 1-year-recovered vines not significantly dissimilar as compared with healthy ones. The increase of natural recovery rate in BN-infected vines throughout the use of elicitors seems to be effective, even if further work is needed to establish protocols that can be applied by growers.

Can antimicrobial peptides be used to engineer resistance against the grapevine pathogen aster yellows phytoplasma?

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Phytoplasmas are of increasing significance around the world, and due to the recent discovery in South African vineyards, could be highly detrimental to the local wine industry (Engelbrecht et al. 2010). Antimicrobial peptides (AMPs) can offer protection against a wide variety of bacterial and fungal pathogens in plants (Rosenfield et.al., 2010). Due to fact that phytoplasmas lack an outer membrane they are an ideal target for AMPs. The current study intends to explore the efficacy of AMPs against Aster yellows phytoplasma (AYP) and whether AMPs could provide a mechanism to incorporate resistance in grapevine to control this devastating disease. Three AMPs were selected to be tested in a transient expression assay for their effect against AYP. Phytoplasma-infected grapevine plants (*Vitis vinifera* cv Chardonnay) were collected from a vineyard in the Vredendal region. These plants were cultured *in vitro* and kept in an incubator under controlled conditions. DNA was extracted from leaf and phloem scrapings at specific time intervals. This material was then screened for phytoplasma infection by a nested-PCR procedure using primers described by Lee et al. (1998). Semi-quantitative qPCR was performed using SYBR® Green II detection chemistry. Additionally, transmission experiments were carried out to infect periwinkle (*Catharanthus roseus*) and *Nicotiana benthamiana* with AYP through the insect vector *Mgenia fuscovaria* (Kruger et al. 2011). Over 90% of the *in vitro* infected plants developed fungal contamination, most probably as a result of endophytic fungal infection of the grapevine material. Leaf and phloem scrapings were taken from the remaining plantlets. In total, 134 *in vitro* plantlets were screened, but no AYP infection was found. Plantlets displayed a 'recovery phenotype', and until now no AY phytoplasma infected grapevine material could be established *in vitro*. As the establishment of AYP *in vitro* material proved to be challenging, alternative AYP infected plant material needed to be established. For this purpose, the natural AYP vector in South Africa, *M. fuscovaria*, was collected in a highly AYP infected vineyard and placed on *N. benthamiana*. These plants will be tested for AYP infection. Once phytoplasma infected plant material is available, the effect of the peptides D4E1, VvAMP1, SN1potato and SN1vitis on AY phytoplasma titres via a transient expression system will be evaluated using an established SYBR Green-based qRT PCR assay.