COST Action 0807

Integrated Management of Phytoplasma Epidemics in Different Crop systems

Working group 3
"Phytoplasma control in crop systems"

Working group meeting

Ancona, Italy, 23rd to 24th of September 2010

Convenors:
Wolfgang Jarausch & Ester Torres

Local organiser:
Gianfranco Romanazzi

Universita' Politecnica delle Marche,
Faculty of Agriculture,
Ancona, Italy

Program

Thursday, 23rd September 14:00 – 18:30

Task 1: Resistance

Presentations:
Breeding of apple proliferation resistant-rootstocks: where are we?
Wolfgang Jarausch

New insights into the nature of resistance to apple proliferation disease
Mirko Moser

- Wolfgang Jarausch:
  Progress report on subtask 1 "resistance to fruit phytoplasma diseases"
- Elisa Angelini:
  Progress report on subtask 2 "resistance to grapevine phytoplasma diseases"

General discussion

Task 2: Effects of biotic and abiotic environmental factors on disease and symptom development

Presentations:
Field treatment with resistance inducers to control grapevine Bois noir
Gianfranco Romanazzi

Nurse culture approach as a tool to study biotic and abiotic factors to control phytoplasmas
Tirtza Zahavi

- Rita Musetti:
  Progress report on subtask 2 "recovery"
- Wolfgang Schweigkofler:
  Progress report on subtask 1 "use of bioactive compounds for the control of phytoplasma diseases"

General discussion

Task 6: Cross protection strategies
General discussion

Friday, 24th September 8:30 – 13:00

Task 3: Improvement of vector control

Presentations:
Control of Cacopsylla pruni, the vector of European stone fruit yellows
Barbara Jarausch

Use of infochemicals for trapping phytoplasma vectoring psyllids
Jürgen Gross

- Michael Maixner:
  Progress report on subtask 2 “control of grapevine phytoplasmas”

- Tim Belien:
  Progress report on subtask 1 “control of fruit tree phytoplasmas”

General discussion

Task 5: Control strategies using the interactions of endophytes with the phytoplasma

Presentation:
Comparison of endophytic bacterial community associated with healthy, GY-diseased and recovered grapevine plants
Daniela Bulgari

- Piero Bianco:
  Progress report on task 5

General discussion

Task 4: Recommendations for best practices in disease control

Presentation:
‘Candidatus *Phytoplasma phoenicium’ in Lebanon: a threat for Mediterranean Countries*
Yusuf Abou-Jawdah

- Kadriye Caglayan:
  Progress report on task 4

General discussion

Minutes of the meeting

Thursday, 23rd September 14:00-18:30

Attendance:
- chair of the COST action: Assunta Bertaccini
- WG2 coordinator: Barbara Jarausch
- WG3 coordinators and subtask leaders (9)
- WG3 members (15)

Welcome by Wolfgang Jarausch

Objectives of WG3:

- exchange of scientific information
  - explication of observations
  - development of new control strategies
- network building
  - projects
  - experimental validation
  - STSMs
- collection of information
  - COST website
  - publications
- recommendations to plant health inspectors --> organization of a meeting in the framework of the COST action
- interaction with other WGs

Task 1. Resistance

Wolfgang Jarausch:
Presentation: *Breeding of apple proliferation resistant-rootstocks: where are we?*
- see abstract below

Mirko Moser:
Presentation: *New insights into the nature of resistance to apple proliferation disease.*
- see abstract below

Wolfgang Jarausch:
Progress report on subtask 1 “resistance to fruit phytoplasma diseases”:
a) resistance to phytoplasmas in fruit trees will be defined as absence of symptoms and low concentration of the pathogen
b) based on this definition data about “resistant” genotypes will be collected in order to establish a comprehensive list of material (“database”)

Elisa Angelini:
Progress report on subtask 2 “resistance to grapevine phytoplasma diseases”:

a) collection of data on the susceptibility/resistance of different cultivars and rootstocks: An excel sheet was sent out in order to collect data obtained from grey literature, local journals, leaflets, personal experience, unpublished data etc.. The behaviour of the same cultivar shall be compared in different countries. Actually, 7 publications about grapevine susceptibility to GY infection have been collected. It would be interesting to add information from personal experience.
b) establishment of a database of different cultivars and rootstocks and of genetic resources for resistance.
   There was a discussion about how to integrate the data and results in a table. In any case the table should include: phytoplasma strain, clone of cultivar, area, level of ‘inoculum’ of infection. It was suggested to establish a scale to classify the grade of susceptibility which should be used by everybody. E. Angelini offered herself to prepare such ‘susceptibility grade’ and to prepare a more detailed questionnaire.
   It is necessary to compile the information from the countries that have not attended to this meeting. The questionnaire shall also be translated by the WG3 members into the national language in order to be spread to advisors.
c) development of model systems to study the resistance mechanisms. E. Angelini discussed the difficulty of infecting grapevine plants and reported that her group is trying to infect micro-propagated grapevine plants with phytoplasmas by means of vectors. Sabrina Palmano and Assunta Bertaccini confirmed the difficulty in maintaining the FD in micro-propagated material due the high rate of recovery.
d) analysis of the genomic base of resistance. E. Angelini suggested the possibility of a joint meeting with WG4 to compare data, and W. Jarausch agreed to communicate this suggestion to Shaskia Hogenhout.

Task 2. Effects of biotic and abiotic environmental factors on disease and symptom development.

Gianfranco Romanazzi:
Presentation: *Field treatment with resistance inducers to control grapevine Bois noir*
- see abstract below

Tirtza Zahavi:
Presentation: *Nurse culture approach as a tool to study biotic and abiotic factors to control phytoplasmas.*

- see abstract below

A permanent GY-infected grapevine tissue culture could be established. Phytoplasmas could be maintained in infected shoots as well as in infected callus. FISH (Fluorescent In Situ Hybridization) *in situ* staining proved the presence of phytoplasmas in phloem of callus and shoots. Thus, tissue culture can be an efficient tool to work also with GY phytoplasma. It enables the testing of different compounds *in vitro*. For this, callus offers a higher surface area and, therefore, a better contact with the tested compound.

Wolfgang Schweigkofler:
Progress report on subtask 1 "use of bioactive compounds for the control of phytoplasma diseases"

a) analysis of the effectiveness of plant resistance inducers and similar bioactive compounds: W. Schweigkofler presented the results of the use of compounds in apple trees, including Regalis, Aliette, Dormex, Bion and Messenger.
W. Schweigkofler remarked that all compounds have effects on the symptoms, but not on the phytoplasma. When the application is stopped the phytoplasma will affect the plant again. W. Schweigkofler explained that sometimes there was no correlation between symptoms and concentration of phytoplasmas, and that in woody plants it should be taken in consideration that the titre of the phytoplasma varies with the season and the part of the plant.
Further strategies discussed:
- possible role of phytohormones (there is some effect by indole-3-acetic acid)
- effect of fertilisers on recovery rates of grapevine (not significant so far)
- direct antimicrobial activities
W. Schweigkofler stressed the observation that data obtained for recovery promotion for one crop are not directly transferable to any other plant-phytoplasma interaction.

b) establishment of a database for effective bioactive compounds: W. Schweigkofler asked for contributions by the WG3 members and received so far 4 publications.
W. Schweigkofler will try to set up an information sheet.

Rita Musetti:
Progress report on subtask 2 "recovery"

a) R. Musetti offered an overview about the recovery phenomenon. Recovery on grapevine is present in different regions of Italy, with different extent according to the area, cultivar and climatic/environmental conditions. In grapevine there are examples of recovery with high rates. With ESFY the recovery is rare. With AP symptom remission is possible.
b) R. Musetti suggested initiating an European project to study recovery and plant-pathogen relations. Recovery is a natural process that can be exploited to study how to control a phytoplasma disease.

There is a need to fix 'universal' rules to define recovery. The contributions to this discussion are summarized in the **general discussion** (see below).

Task 6. Cross protection strategies

Emesse Kiss could not attend to this meeting.
An active discussion about this task took place. W. Jarausch proposed to abandon this task. He pointed out that recent research demonstrated the extremely high genetic variability of phytoplasmas, and that it will be not possible to assure that a mild strain will not become aggressive anymore. E. Angelini and B. Jarausch thought that cross protection with other phytoplasmas is not viable and that we should study other forms of cross protection, e.g. with bacterial endophytes or other organisms. This would be topic of task 5.

There was an agreement to abandon task 6.

The development of tools to distinguish mild from severe strains of a given phytoplasma are of further interest but should be treated rather in WG1 or WG4. After the meeting E. Kiss expressed her willingness to further contribute to this task by collecting the available data. Thus, her report will be discussed on the next meeting.

Friday, 24th September 9:00-13:00

Task 3. Improvement of vector control

Barbara Jarausch:
Presentation: *Control of Cacopsylla pruni, the vector of European stone fruit yellows*
- see abstract below

Jürgen Gross:
Presentation: *Use of infochemicals for trapping phytoplasma vectoring psyllids*.
- see abstract below

Michel Maixner:
Progress report on subtask 2 "control of grapevine phytoplasmas".

a) Collection of data about the currently applied control strategies: M. Maixner explained the questionnaire he established for the control strategies against BN and FD. The questionnaire was sent to all WG3 members before the meeting. The following data shall be collected:
    - data for as many viticultural areas as possible
    - focus on Bois noir and Flavescence dorée
    - information about presence and incidence of
        - vectors
        - diseases and phytoplasma strains
        - (alternative) host plants
    - information about direct and indirect measures to control vectors and disease spread
    - additional information about predominant cultivars, susceptibility, cultural practice
    - collection of published information, empirical data and ‚grey literature'

    The following topics related to the questionnaire were discussed:
    - the content of the questionnaire was approved
    - dissemination of the questionnaire: the questionnaire should also be send to extension services. For this, the questionnaire must be translated into the national language by the respective WG3 members
    - unpublished or "grey" literature: this kind of literature provides valuable information and should be specifically asked for
    - editing of the collected information: prior to publication the data have to be revised by national editors and have to be checked with regard to the official data for each region/country

- publication of the results: there was a discussion about the best way to publish the information and both versions, printed and internet (COST website, IPWG website) were adopted
- links to the Sanitary Services should be offered in order to get updated information

So far, only 5 questionnaires were sent back. They cover 6 regions in 3 countries.

Tim Belien:
Progress report on subtask 1 “control of fruit tree phytoplasmas”.

a) collection of data about the currently applied control strategies: T. Belien prepared a questionnaire on AP, PD and ESFY similar to the one established by M. Maixner. The questionnaire was sent to WG3 members before the meeting. So far, only 4 questionnaires were sent back. They cover 6 regions in 3 countries. Also this questionnaire must be translated into the national language by the respective WG3 members and send to extension services.
b) analysis of the effectiveness of available environmentally sustainable insecticides: T. Belien presented his results of trials with insecticides in IPM against *C. pyri* and informed about the establishment of a database for the effectiveness of insecticides and applied control strategies at pcfruit (Belgium).

Assunta Bertaccini proposed to put the questionnaires on the web.

Task 5. Control strategies using the interactions of endophytes with the phytoplasma

Daniela Bulgari:
Presentation: *Comparison of endophytic bacterial community associated with healthy, GY-diseased and recovered grapevine plants*
- see abstract below

Piero Bianco:
Progress report on task 5.

a) verification whether the interaction of endophytes with host plants and phytoplasmas can be used as control strategy: P. Bianco explained the term “endophytes” and the advantages and disadvantages to use endophytes as biocontrol organisms. He further presented the possible mechanisms in which endophytes can protect the plant against pathogens. The presentation of D. Bulgari showed that the bacterial diversity is higher in healthy plants than in infected ones. Two hypotheses were presented: a direct interaction between phytoplasma and microbial community, or that phytoplasmas induce a plant defense response leading to a reorganization of the microbial community.

b) collection of data about microorganisms exhibiting inhibitory effects on phytoplasmas: P. Bianco referenced the currently available publications on the interaction of fungi and bacteria with phytoplasmas.

c) P. Bianco presented also strategies to use bacteria as biocontrol agent against phytoplasmas in insect vectors.
d) P. Bianco suggested the following future activities:
    - to assay suitable model systems for evaluation of the endophytes/symbionts as possible tester for microbe-phytoplasma interaction.
    - to achieve more information about endophytes/symbionts-phytoplasma-plant.
    - he proposed a Joined Meeting between COST 0807 (WG3, others?) and COST action FA0701: “Arthropod symbioses from fundamental studies to pest and disease management”.

Task 4. Recommendations for best practices in disease control

Y. Abou-Jawdah:
Presentation: *‘Candidatus Phytoplasma phoenicium’ in Lebanon*
- see abstract below

Kadriye Caglayan
Progress report on task 4
K. Caglayan reminded in her introduction that best practices in disease control are much more than insecticide treatments. They include clean propagation material, resistant plants, rouging, weed control, habitat management, mulching, physical control covering, barrier sprays, parasitoids/ predators and induced resistance.

a) collection of recommendations for different crop systems and countries: K. Caglayan has asked WG3 members for information about their national/regional recommendations. Two partners have sent recommendations for best practices. E. Angelini will sent recommendations to control phytoplasmas in grapevine. This task will also profit from the questionnaires sent out by M. Maixner and T. Belien.
b) definition of control measures to prevent the introduction of a new phytoplasma disease into a region (inside or outside of Europe). Almond witches’ broom disease in Lebanon can be regarded as case study. K. Caglayan suggested that if the infection of AlWB reaches other countries it should be considered as quarantine organism.

General discussion
With regard to the presentations in tasks 1, 2 and 5 there was an active discussion about the term **RECOVERY** among the participants. It became apparent that there is a need to fix ‘universal’ rules to define recovery. The following definitions were proposed:
- R. Musetti: asymptomatic for 2 or 3 consecutive years and negative by PCR
- P. Bianco: a spontaneous remission of symptoms in diseased plants; it may or may not involve the elimination of the pathogen from the host.

- M. Maixner proposed to think about different concepts: remission, recovery and no infection (e.g. remission = no symptoms).

The presentation of M. Moser demonstrated that natural resistance in apple might be correlated to recovery. R. Musetti commented that there is more than one kind of resistance and recovery and that these phenomena can be considered as different kinds of resistance. A. Bertaccini indicated that resistance is for definition linked to a genetic base. D. Bulgari asked that other aspects should be considered, such as the correlation between recovery and bacterial communities. She further proposed that recovery is natural and if it is induced by endophytes or other methods it should not be named recovery.

W. Jarausch summarized that all discussed aspects are linked to the success of the plant defense against phytoplasmas. Because of the importance of the question he proposed the creation of a subcommittee joining all the members that work on recovery-resistance.

Outlook

The planned meeting for plant health inspectors etc. will **not** be attached to the 2nd IPWG meeting held 12-16 September 2011 in Neustadt (Germany) but will be postponed to the end of COST action in order to provide as much as possible elaborated information to the public.

As consequence, an additional WG3 meeting is planned for 2011. Subject to agreement with the management committee this meeting will be attached to the 2nd IPWG meeting in Neustadt.

Breeding of apple proliferation resistant-rootstocks: where are we?

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Apple proliferation (AP) is caused by the wall-less bacterium '*Candidatus* Phytoplasma mali' and is difficult to control because of its different means of spreading: transmission by psyllid vectors, by root anastomosis or diffusion by latently infected planting material. Previous work indicated that, due to the colonization behavior of the causal agent, the disease can be controlled by the use of resistant rootstocks. While extensive screening revealed no satisfactory resistance in established rootstocks, substantial levels of resistance were identified in experimental rootstocks derived from crosses of the apomictic species *Malus sieboldii* and genotypes of *M.* x *domestica* and *M.* x *purpurea*. However, trees on these hybrids are more vigorous and less productive than trees on standard stock M 9. For this reasons, a breeding program was initiated to reduce vigor and improve yield by crossing and backcrossing *M. sieboldii* and its apomictic hybrids with M 9 and other dwarfing stocks. From 2001 through 2006 a total of 36 crosses were made. However, only 23 progenies consisted of a substantial number of seedlings while the other crosses largely failed due to pollen incompatibility. The 3.500 seedlings obtained were DNA-typed using codominant SSR markers to distinguish apomicts and recombinants in the progenies. A total of 1.800 seedlings consisting of all recombinants and a representative number of apomicts were screened for AP resistance by graft inoculation. The evaluation of the progeny was done in two steps: all inoculated progeny was observed for a period of two years in a nursery scale, resistant genotypes were then transplanted into an experimental orchard scale and were grown under commercial growing conditions. In the latter step not only symptoms but also vigor and productivity of the tree were recorded. Several progenies showed a good inheritance of resistance. In two of them (4608 x M 9 and D2212 x M 9) more than 50% of the individuals never developed symptoms. The resistance phenotype could be characterized by the absence of AP symptoms and a low concentration of the phytoplasma as determined by quantitative PCR. After three to five years observation in the experimental orchard scale about 50 genotypes were selected so far which remained resistant and showed promising agronomic values. These genotypes are currently multiplied by micropropagation for further evaluation. Since 2007, selected progeny genotypes were also used as parentals in further crossings.

New insights into the nature of resistance to apple proliferation disease

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Apple proliferation (AP) disease is the most important graft-transmissible and vector-borne disease of apple in Europe. '*Candidatus* Phytoplasma mali'. ('*Ca.* P. mali') is the causal agent of AP. Resistance to AP was found in the wild genotype *Malus sieboldii* (MS) and in MS-derived hybrids. Differences in the gene expression between '*Ca*. P. mali'-resistant and -susceptible genotypes during infection were investigated through cDNA-Amplified Fragment Length Polymorphism (cDNA-AFLP) technique. Individuation and analysis of the differentially expressed genes was carried out exploiting an *in vitro* system. Healthy and '*Ca*. P. mali'-infected micropropagated plants were maintained under controlled conditions showing that the resistance phenotype could be reproduced in this system. In addition, *ex vitro* plants were generated as an independent control of the genes differentially expressed in the *in vitro* plants. The cDNA-AFLP analysis in *in vitro* plants yielded 63 bands characterised by over-expression in the infected state of both H0909 (MS-derived) and MS genotypes. The major part (37 %) of the associated sequences showed homology with products of unknown function. The other genes were associated with plant defence, energy transport/oxidative stress response, protein metabolism and cellular growth. Real-time qPCR analysis was employed to validate the differential expression of the individuated genes. Since no internal controls were available for the study of the gene expression in *Malus*, an analysis on housekeeping genes was performed. The most stably expressed genes were the elongation factor-1 α (EF1) and the eukaryotic translation initiation factor 4-A (eIF4A). Twelve out of 20 genes investigated through qPCR were significantly differentially expressed in at least one genotype either in *in vitro* plants or in *ex vitro* plants. Overall, about 20% of the genes confirmed their cDNA-AFLP expression pattern in *M. sieboldii* or H0909 while 30 % of the genes showed down-regulation or were not differentially expressed. For the remaining 50 % of the genes a contrasting behaviour was observed. The qPCR data suggest that the phytoplasma infection unbalance photosynthetic activity and photorespiration down-regulating genes involved in photosynthesis and in the electron transfer chain. As result, and in contrast to *M. x domestica* genotypes, an up-regulation of genes of the general response against pathogens was found in MS. These genes involved the pathway of H_2O_2 and the production of secondary metabolites. We hypothesise that a response based on the accumulation of H_2O_2 in MS would be at the base of its resistance. This resembles a phenomenon known as "recovery" where the spontaneous remission of the symptoms is observed in old susceptible plants but occurring in a stochastic way while the resistance in MS is an inducible but stable feature.

Field treatment with resistance inducers to control grapevine Bois noir

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Bois noir (BN) is the main phytoplasma disease of grapevine in central Italy. It can induce severe losses of production, which mainly occur in sensitive cultivars like Chardonnay, where most of the clusters of the infected plants can dry out before the harvest. To date, there are no validated strategies to control phytoplasma diseases. However, it is possible to adopt an integrated approach that involves use of pathogen-free propagating materials and reduction in inoculum sources in the vineyard. A novel approach to control phytoplasma diseases consists of the exploitation of plant defences by the application of resistance inducers. These treatments have been tested in commercial vineyards on cv Chardonnay with an initial high number of plants with BN symptoms in the Abruzzi and Marche regions, central-eastern Italy. Under these conditions, the application of resistance inducers promoted significant increases in symptom remission, also known as recovery, in BN symptomatic plants.
The experimental vineyard was arranged in a randomised block design, and treatments were applied to single plants showing disease symptoms in the previous year. The tested resistance inducers included commercial products based on chitosan (Chito Plant, ChiPro GmbH, Germany), Phosetyl-Al (Aliette, Bayer Crop Science, Germany), two different mixtures of glutathione and oligosaccharines (Kendal, Valagro, Italy, and Olivis, Agrisystem, Italy, respectively), and benzothiadiazole (Bion, Syngenta Crop Protection, Switzerland). The compounds were sprayed on the canopy of the plants weekly, from the beginning of May to the end of July. This delivering the equivalent volume of the solution of 1,000 l/ha, using a motorized backpack sprayer. All of the treatments increased the number of plants that did not show disease symptoms in the year of the treatments, and which were thus termed “recovered”, as compared to the untreated control.
Over the four-year experiment, the best results were obtained with Kendal, Olivis and Bion, while Aliette and Chito Plant provided less constant results over the years. Molecular analyses of leaf-vein extracts from the recovered plants failed to detect the phytoplasma agent of BN. Although experimental data are not available, an induction of the host defences is likely to be responsible for the effectiveness of these resistance inducers in the control of grapevine BN. After four years of trials, we can conclude that an integrated strategy for the containment of BN in vineyards that are heavily infected can include the use of resistance inducers. However, further investigations are needed to determine the optimal number and timing of these resistance inducers applications.

Nurse culture approach as a tool to study biotic and abiotic factors to control phytoplasmas

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Phytoplasma are not cultivable, thus experimental systems are limited to whole plant and molecular studies. In our study, we are developing an experimental system to study phytoplsma. For this purpose we are growing phytoplasma in nurse culture of grapevine plantlets. We have 6 clones of infected Chardonnay more than 3 years in culture. In addition we have 16 clones of infected Chardonnay two years in culture, and 7 out 9 new clones of suspected Cabernet-Sauvignon were found positive. When introduced into tissue culture, healthy buds develop much quicker than infected buds and disease symptoms can be visualized. After several subcultures the characteristic symptoms diminish and infected plantlets look similarly to healthy ones. However, plant biomass is 40-45% lower in infected plantlets compared to healthy plantlets. To test the potential of various compounds as phytoplasma inhibitors we are developing an experimental system of callus as nurse culture for phytoplsma. To achieve this goal we developed a protocol for growing solid callus from grapevine with pro-meristem clusters. Furthermore, we developed a protocol to stimulate vascular differentiation in the callus in order to transfer phytoplasma into the callus tissue. Preliminary results show that: a. phytoplasma was introduced into the callus tissue during callus induction. b. phytoplasma reside and survived in the callus tissue more than two years and following ca.10 subcultures. Further study is currently performed to verify whether phytoplasma titer can be increased within the callus tissue.

Control of *Cacopsylla pruni*, the vector of European stone fruit yellows

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European stone fruit yellows (ESFY) is one of the most important diseases in apricot and peach cultivation and causes severe damage in nearly all stone fruit growing regions in Europe. So far, only the psyllid species *Cacopsylla pruni* (Hemiptera, Psyllidae) has been identified as vector of this disease. *C. pruni* is an univoltine species which has one generation per year and which is strictly oligophaguous on *Prunus* species in all European stone fruit growing areas. Since several years regular captures of *C. pruni* have been conducted in selected orchards in southwest Germany and the life cycle of this psyllid species could be described by this means. Thus, the complete development occurs on *Prunus* species including 5 larval instars until emergence of the new imagines in summer which migrate after a short period of about 2-4 weeks on their *Prunus* hosts to conifers as overwintering sites. The highest density of *C. pruni* was found on wild *Prunus* species e.g. *P. spinosa* or *P. cerasifera* compared to lower populations on cultivated *Prunus* such as *P. armeniaca* or *P. persica*. Interestingly, important numbers of individuals were also collected from rootstock suckers of different rootstock genotypes.
The transmission parameters for the agent of the disease, '*Candidatus* Phytoplasma prunorum', by overwintered adults and by newly hatched imagines of *C. pruni* were determined in the greenhouse under standardized conditions as well as in semi-field trials. It could be shown that both generations were capable to transmit the phytoplasma successfully to healthy test plants. Directed control strategies against adults and the various larval instars were tested in semi-field trials. By this means the most promising results could be obtained with the substance abamectin by affecting the larval development and thereby reducing the emergence of new imagines of *C. pruni*. The most efficient application period for abamectin was between mid April and mid May when the first nymphs (L1+L2) started to hatch. The residue analysis revealed no risk if a waiting period of at least 28 days was respected. Consequently, abamectin can be used with exemption certificate for the control of the larval development of *C. pruni* in Germany.

Use of infochemicals for trapping phytoplasma vectoring psyllids

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We work on the chemical ecology of jumping plant lice (Hemiptera: Psyllidae) and their interactions with vectored phytoplasmas and their different host plants. We investigate the olfactory reactions of these insects to host plants used for reproduction or overwintering, and elucidate chemically mediated interactions between all players in such multitrophic systems. By identification of chemical compounds responsible for the migration of these insects between their different host plants, we make a valuable source accessible for the development of innovative strategies using attractive and repellent infochemicals for control of these insect vectors.
Here I present an example for our attempt to develop a biotechnical control method for psyllid species vectoring fruit tree phytoplasmas by sticky traps lured with newly detected infochemicals. The apple psyllid *Cacopsylla picta* is the main vector of '*Candidatus* Phytoplasma mali', the causal agent of the apple proliferation disease. Complex interactions between *Malus domestica,* the psyllid *C. picta*, and the phytoplasma were investigated in the laboratory and in the field. Results from Y-tube shaped olfactometer trials showed that immature adults of *C. picta* are able to distinguish the odours of healthy and infected apple trees and preferred the odours of infected trees. Thus, the phytoplasma directly manipulates both the plant physiology by producing an attractive compound and the psyllid behaviour, resulting in a better spread within its host plant population. The compound responsible for the attraction of the vector was collected from headspace of infected apple plants and identified by gaschromatography coupled with mass spectrometry. This sesquiterpen attracts both genders of *C. picta* and is now used for the development of traps for monitoring or mass trapping of this vector.

Comparison of endophytic bacterial community associated with healthy, GY-diseased and recovered grapevine plants

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Flavescence doree (FD) and Bois noir (BN), grapevine yellows (GY) diseases associated with phytoplasmas, induce severe crop losses. Therefore, their containment is a priority in the European wine producing areas. Until now, none grapevine varieties have been found resistant to phytoplasmas infection. FD control is based on chemical treatments against the insect vector, *Scaphoideus titanus* Ball; on the other hand, this strategy is not efficient for BN containment since biological complexity of this disease. In recent years, there has been an increasing interest about the recovery from GY diseases and in the role of endophytic bacteria as biocontrol agents. Endophytic bacteria can be termed all the bacteria colonizing the interior of plants without inducing diseases, including those that become pathogenic under certain conditions. Though their relationship with the host is not well understood, they may reduce the disease severity by activating systemic resistance, production of allelochemicals (biocidal volatiles, antibiotics, and lytic enzymes) and competition of nutrients and niches.
To investigate the possible role of endophytic bacteria in recovery from GYs, microbial community diversity and structure associated with healthy, GY-diseased and recovered grapevine plants were analyzed through Length Heterogeneity-PCR (LH-PCR) combined with statistical analyses. Endophytic bacterial community were examined in healthy (20 plants), GY-diseased (20 plants) and recovered grapevine plants (20 plants). LH-PCR of total DNA from grapevine leaves was used to generate bacterial amplicon profiles that were analyzed with statistical methods. These analyses highlighted that microbial community is different in healthy, GY-diseased and recovered grapevines. Further, LH-PCR electrophoretic peaks, assigned to isolated cultivable grapevine-associated single bacterial strains, were used to identify these peaks and to monitor bacterial species distribution in total DNAs from analyzed plants. Moreover, bacterial community associated with healthy plants was characterized by a greater diversity (major number of LH-PCR peaks) than that present in GY-diseased and recovered plants. Observed decrease in bacterial richness and different microbial composition in GY-infected and recovered plants suggests that (i) phytoplasma infection could restructured bacterial community and/or (ii) variation in the microbial composition could be a starting point for recovery.

‘*Candidatus* Phytoplasma phoenicium’ in Lebanon

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‘Candidatus Phytoplasma phoenicium’, a member of the 16S rRNA group IX, has been reported as the presumptive aetiological agent of the “Almond witches’ broom” disease in Lebanon, causing the death of more than 100.000 almond trees. After 10 years, the pathogen continues to spread to new cultivated areas and severe infections were also observed on peaches and nectarines, two important crops for the Lebanese agriculture. On the frame of the cooperation projects on rural development in Lebanon, financed by the Italian Cooperation (Ministry of Foreign Affairs), the NGO AVSI managed the research project on “Integrated management of stone fruits phytoplasma in Lebanon”, in collaboration with Italian and Lebanese Universities and research institutions. The ‘Almond witches’ broom issue’ was addressed by an innovative, integrated method, involving field monitoring, laboratory research and awareness training and information for farmers, thus shifting the focus of the project from solving an agricultural problem to dealing with the individual farmers and communities living “with” the problem.
In order to localize the new infected regions, a national survey, covering all the 26 Lebanese Cazas, has been conducted during the year 2010, allowing to draw the disease spread map. The project, in 12 months, involved 557 farmers, 910 orchards, regularly monitored, in 490 villages belonging to all the 26 Lebanese "Caza". A total of at least 40.000 new infected trees have been detected and localized by GPS in 16 Cazas.
Seasonal symptom evolution was described by one year-long observation of infected almond, peach and nectarine trees. On the basis of the symptoms, samples have been collected from symptomatic, doubtful and asymptomatic plants. Nested PCR, performed by using the universal primers P1/P7 followed by primers F2n/R2, or direct PCR, performed by using semi specific primers AlWF2/AlWR2, have been carried out for detecting phytoplasma in the analyzed samples. In detail, ‘*Ca*. P. phoenicium’ was detected in almond, peach and nectarine samples.
Since the insect vector is still unknown, a wide insect collection has been carried out in two infected orchards, in order to collect, identify and analyze the putative phytoplasma

vector/s. Fifty-nine species of leafhoppers and seven species of psyllids have been identified and analyzed by nested PCR using universal primers P1/P7 followed by F2n/R2. At the moment, no insects were positive in PCR reactions. Identification of cixiids and phytoplasma detection analyses are in progress.
Future researches will be focused on in-depth investigating ecologies and epidemics of '*Ca*. P. phoenicium' in Lebanon and in neighbouring countries.

Photo of the meeting