REPORT from

COST 850 – WORKING GROUP 4 MEETING

November 30 – December 1, 2001

At the
Royal Veterinary and Agricultural University
Copenhagen, Denmark

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INTRODUCTION

In connection to the COST 850 Management Committee meeting in Wageningen in August 2001 it was decided that Working Group 4 ("Interactions with field biota") should meet in Copenhagen in order to plan work within the group. Different ideas for future work was already suggested in Wageningen. This included several scientific projects, writing of review articles and a movie about entomopathogenic nematodes. These ideas were further discussed in Copenhagen and this report presents the outcome of the meeting.

MEETING PROGRAM

Friday, November 30, 14.00 - 20.00

Opening of meeting
Clarifying the frame of Working Group 4
Entomopathogenic nematode movie

Saturday, December 1, 8.30 – 17.00

Selection and discussion of activities within Working Group 4

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ABSTRACT: ***Wolbachia*, a reproductive parasite of *Rhagoletis cerasi* (Diptera, Tephritidae)**

Markus Riegler & Christian Stauffer

Wolbachia is an intracellular, maternally inherited α-proteobacteria occurring in arthropods and filarial nematodes. *Wolbachia* manipulates host reproduction in several ways favouring thereby its own dispersal in host populations (Bourtzis & O´Neill, 1998). A common phenotype of *Wolbachia* is cytoplasmic incompatibility (CI) (Hoffmann & Turelli, 1997). CI is embryonic lethality which occurs when infected males mate with uninfected females whereas reciprocal crosses between infected females and uninfected males do have offspring. In CI, sperm of infected males is free of *Wolbachia,* but is modified during spermatogenesis by the infection in the father. Infected eggs are able to rescue the imprinted sperm. Besides CI, other phenotypes caused by *Wolbachia* infections were described: thelytokous parthenogenesis in wasps, feminisation in isopods, male killing in insects and modifications of fecundity and fertility (Stouthamer *et al*., 1999).

Strong levels of unidirectional CI were detected between European populations of the cherry fruit fly *Rhagoletis cerasi* (Boller *et al*., 1976). All populations were found infected by two different strains of *Wolbachia*, *w*Cer1 and *w*Cer2. Individuals from one part of Europe were infected by *w*Cer1 and individuals from the other part were superinfected by *w*Cer1 and *w*Cer2. CI occurred between the infected and superinfected populations suggesting that *w*Cer2 is the inducer of CI. *w*Cer2 was also observed to currently disperse through *w*Cer1 infected populations (Riegler & Stauffer, 2001). *Wolbachia* infection types are linked to mitochondrial haplotypes of *R. cerasi*. CI also favours the dispersal of the haplotype linked with *w*Cer1&2 thereby replacing haplotypes linked with wCer1 infections. This results in a loss of mitochondrial polymorphism in European populations (M Riegler, unpubl. data). *w*Cer2 was transferred experimentally to *Drosophila simulans*, where it induced CI at a lower level than in its original host. Variation in the strength of CI was observed in different injected lines which could be due to differences in bacterial load or genetic variability in the transferred *w*Cer2 (M Riegler, S Charlat, C Stauffer, H Merçot, unpubl. data).

References

Boller EF, Russ K, Vallo V, Bush GL (1976) Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), their origin and potential use in biological control. *Entomologia Experimetalis & Applicata* **20**, 237-247

Bourtzis K, O´Neill SL (1998) *Wolbachia* infections and arthropod reproduction. *Bioscience* **48**, 287-293

Hoffmann AA, Turellli M (1997) Cytoplasmic incompatibility in insects. In: *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. (eds O'Neill SL, Hoffmann AA, Werren JH), pp. 42-80. Oxford University Press, Oxford

Riegler M, Stauffer C (2001) *Wolbachia* infections and superinfections in cytoplasmic incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). submitted

Stouthamer R, Breeuwer JAJ, Hurst GDD (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* **53**, 71-102

Review articles on *Wolbachia*

Bourtzis K, O´Neill SL (1998) *Wolbachia* infections and arthropod reproduction. *Bioscience* **48**, 287-293

Stouthamer R, Breeuwer JAJ, Hurst GDD (1999) *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* **53**, 71-102
Werren JH (1997) Biology of *Wolbachia*. *Annual Review of Entomology* **42**: 587-609

Book on *Wolbachia*
Influential Passengers: Inherited Microorganisms and Arthropod Reproduction. (eds O'Neill SL, Hoffmann AA, Werren JH), 214pp, Oxford University Press 1997, Oxford

THE FRAME OF WORKING GROUP 4 - BRAIN STORM (working paper)

Through group work and plenary discussions on the first day, a number of items that could be covered by Working Group 4 were listed and ordered under six different headings as presented below:

1. SURVIVAL & PERSISTENCE
Field activity under different conditions
Persistence in the field
Persistence/recycling
Relation with climatic conditions
Survival during winter
Influence of physical and chemical factors on interspecies coaction
Photorhabdus/xenorhabdus survival outside host?
Alternative hosts for EPNs during shortage of pests
Wider application range of EPN
Natural hosts?
Persistence time of EPNs after their application in fields
EPN dynamics in soil/water (relation with insects
Methods for assessing persistence of EPN

2. BIOLOGY AND INTERACTIONS WITH FIELD BIOTA
Multitrophical level of interaction
Natural interactions of EPN & entomopathogenic fungi, competition etc.
Host finding
Interspecies relation in the field
Competition between EPN species
Interactions of EPNs with other soil micro organisms (other nematodes)
Gnotobiology
Multitrophic endophytes, myccorrizae effect on susceptibility
Wolbachia plasmids: discovery and use as vectors
Mechanisms of EPN efficacy performance
EPN mobility in the field
Nematode behaviour attraction to insects
Effect of crop rotation on EPN populations
Multitrophic host plant species e.g. effect of strawberry cultivar on susceptibility of BVW
Relation EPN/insect/crops
Symbiont heterogenecity, effect on EPN pathogenecity

Pathogenecity of EPN when interacting with wolbachia or other parasites
Synergies/antagonists, effects – physical, biological

3. BIOCONTROL

EPNs in the frame of IPM systems
Combination with other methods
Interaction EPN-pesticides
Stimulation of EPN efficacy with other factors (chemical fertilisers/insecticides)
EPN/insects/cropping practices
Impact of EPN on plant parasitic nematodes
Synergistic and antagonistic interactions with other biocontrol agents
Heterosis breeding of EPN
Transgenic EPN (carrying desiccation tolerant gene)
Attempt to produce amphiploids for grub control
Isolation of large/long and small/short EPN mutants against large and small insects
Using the toolkit of genetics
Abiotic factors limiting EPN performance
Elaborate a joint integrated control of *Melolontha* in EU
Integration of biocontrol systems
Apple sawfly
Synergy

4. MONITORING

Modelling – forecasting tools, use strategies

5. SURVEYS & MONITORING

Quantitative methods for evaluation of EPN in soil
Identification, presence of real time PCR, EPN, bacteria, related to other analysis methods
Expedition to “white spots” of the world map (such as Mongolia) to discover new EPN
A systematic survey at altitude in the Carpatian mountains
Some experiments in different countries of Europe using the same methodology of sampling, evaluation
A systematic survey of the EPN distribution in EU countries
Natural populations
Effect of natural EPN on insect populations/pest species/ selected environment
Transgenic EPN(carrying desiccation tolerant gene)

5. INOCULATIVE RELEASE

Conditions enabling establishment
Alternate hosts for inoculative release
Inoculative application with long term evaluation of persistence
Re-colonisation
Inoculation, inundation, application methods
Methods of application EPN in forest, soil, trees
Studying requirements for formulation, slow release, storage
Buffer capacity of EPN keeps insect populations at a low level/no knock down effect
EPN recycling “conservative biological control”
EPN potential effects pest population development

6. SAFETY
Effects of EPNs on non-target organisms
Non-targets, including impact on native EPNs
Impact of Photorhabdus / Xenorhabdus on soil micro organisms
Use of exotic EPN, risk assessment and management
Registration issues, short-term/long-term toxicity etc.
Registration: make it uniform within EU

SELECTED ACTIVITIES IN WORKING GROUP 4 (Working paper)

During the second day of the meeting four groups were formed (Group A, B, C and D below) to follow up on the list of items above constructed during the first day. Each group discussed one of the following items: **A**, *Safety*; **B**, *Persistence and survival* (*in the soil*); **C**, *With EPN's – bringing science to practise*; **D**, *Multitrophic interactions*. The text below was presented by the respective groups and discussed in plenum.

A, Safety

- EPN have been released for biocontrol of insect pests in different crop systems for the last 15 years, and there use is increasing.
- They are excellent biocontrol agents and help to reduce the use of insecticides.
- Today EPN are the only alternative for control of some insect pests. In the future they have the potential to replace many insecticides in agricultural practices.
- According to our knowledge today EPN's are safe etc.(help)
- There is continuous work on looking for better nematodes (EPN) and we need more knowledge about the ecological (data) impact for these "new" EPN's. It may also be necessary to evaluate the use of EPN up to now to ensure that EPN's are environmentally sound biocontrol agents.

Suggestion – Ecological impact study in sites where it is known that EPN has been used as a common practice for a long time. (This may facilitate the registration of exotic species- if we want to)

Continuing search for knowledge on ecological data, need a data base.
Safety - presentation

Exotic organisms have a large control potential

Arne is receiving information about natural infections of insects by EPN. This list can be used in evaluating the safety of EPN (effect on beneficial insects)

There is no risk for humans as far as we know – allergic reactions of person working in production observed.

Action plan:

1. Workshop on quantitative methods for assessing EPN in soil
   To use as a tool to assess the occurrence of EPN in soil
   A pre-requisite for identifying presence and spread of exotic EPN
   Also useful for other purposes

2. Ad hoc collection of data of persistence and spread of exotic EPN in current use. Exotic to country or region

3. Risk benefit analysis desk exercise on use of exotic EPN in Europe (workshop & proceedings)
Identify the risk to non-target arthropods and native EPN & entomopathogenic fungi
Identify what should be done, what information is needed

B, Persistence and survival (in the soil)

Information not needed for inundative release – "knock-down-effect" strategy

For inoculate release the successful establishment is important, less the persistence

The greatest potential for inoculate release is in the outdoor situation – recycling concept

Is re-colonisation possible ?

Predict EPN potential under variable field conditions
- crop
- host + alternative host
- distribution and density (monitoring based on incidence*)
- potential for EPN reproduction
- biotic and abiotic conditions
- agricultural practice
- application methods

*it is believed that incidence including the within-field distribution is the most appropriate method for quantifying EPN control potential in a given area.

The group working "Persistence and survival (in the soil)" made a joint action plan together with group C (see below).

C, With EPNs - bringing Science to Practice

SUSTAINABILITY

- REDUCING THE RISKS IN PLANT PROTECTION : IPM
  - EPNs integrated into Biocontrol systems
  - EPNs integrated with chemical pesticides
  - EPNs integrated with cropping practices

- NEW TARGETS
  EPNs solving intractable and emerging problems

  - difficult to control pests and diseases:
    . Apple sawfly
    . Fire blight
    . Nuts weevils
    . Phorids (EPNs with chemicals)
    . *Melolontha*

  - Pest control in critical environmental situation: the case of Urban Forests and Parks

- OPTIMIZING PERFORMANCES
  (EFFICACY: the major farmers requirement)

  - Factors limiting the Efficacy

  - Improving the Efficacy
    . Synergies vs. antagonisms
    . Using the genetic engine
      1) Classical genetics
        . mutations
        . Heterosis
        . Amphiploids
      2) Molecular genetics   Transgenic works

- DECISION SUPPORT TOOLS
  - Modelling   Pests – Crops – Environmental elements – EPNs
  - Forecasting
  - Simulation tools
  - Use strategies

Action plan (group B and C)

The concept is to work out parallel projects related to pests/cropping systems . Each of the project teams will at least consider a list of items which will be later shared in order to draw out general recommendations and/or scientific aspect relative to the use of EPNs in plant protection

	1	2	3	4	5	6	7	8	9…
	Nuts Weevils	Orchards Saw flies	Grubs	Pests in Urban Forests and Parks	Capnodis tenebrionis in Stone fruits	Otiorhynchus in Strawberries	Bacterial diseases	Phorids Glasshouses	
Alternative hosts									
Propagation Potential									
Pests density and distribution									
Biotic and abiotic factors - soil types - humidity…									
Agricultural practices Irrigation Weed control' fertili…									
Application methods Timing Formulation Dosage									
Monitoring Distribution and density									
Modelling forecasting									
Efficacy									

D, Multitrophic interactions

Basic research on multitrophic interactions (crops, pests, natural enemies, symbionts) with focus on long- term pest control in annual / permanent cultures is necessary to:

- Get a clearer view of how an agricultural, horticultural, forest ecosystem works
- Understand multitrophic interactions with the purpose of improving bio-control in the future
- Improve buffer capacity to avoid critical pest damage in ecosystems / keep ecosystems balanced

Research strategies:

Analysing Interactions in simple systems
Interactions after adding/removing factors
Interactions in complex systems

Laboratory studies / field studies

Modelling of systems (EPN, EPF, EPB models)

List of research areas:

Biocontrol agent (EPNs, Wolbachia & others) related	System related
Behavioural biology (host finding, mobility)	Cropping systems
Symbiont heterogenecity- influence on EPNs pathogenicity	pest species
Pathogenicity of EPN, EPF & EPB when interacting with each other	
Competition between EPN, EPF & EPB	
Interaction with indifferent (non economic) organisms	

Action plan:

- Exchange of information about research activities (e.g. COST 850)
- Involve expertise from other research fields (e.g. WG1 Symbiosis Biology)
- exchange of material / knowledge about EPN, EPF & EPB
- lobbying for long run experiments
- looking for possible project funding (national / international)
- combining results from different research fields (review paper)

Comments to above selected activities

Multithropic interactions

Key word: basic research

"Indifferent organisms" do not have a direct economic effect

Check publications already available

Use internet page (Ann Burnel)

With EPN´S, bringing science to practise (biocontrol)

Key words: Sustainable agriculture and forestry, synergy through combination of factors

Use of the Symbiont alone or its metabolites to control for instance fungal or bacterial caused plant diseases.

Combination of EPN with low amounts of certain chemicals (insecticides etc)

Discussion on terms : Biocontrol agents (BCA), biopesticides

BCA is recommended to be the term used

More meetings to exchange knowledge

Shown table is to be expanded.

Phorids in mushrooms instead of strawberry

Safety

Exotic organisms have a large control potential

Arne Peters is receiving information about natural infections of insects by EPN. This list can be used in evaluating the safety of EPN (effect on beneficial insects)

There is no risk for humans as far as we know – allergic reactions of person working in production observed.

Identify possible risks / what risks have to be considered

Side effects – influence from exotic EPN on native entomopatogenic fungi

Replacement of natives by exotic

There is a need for new data about risks

Publications about risk assessment are available – also within former COST actions

European video film on entomopathogenic nematodes (Report by Marek Tomalak)

During the Wageningen COST 850 meeting a group of WG4 and WG5 enthusiasts expressed its desire to produce an European video film on entomopathogenic nematodes. Discussion undertaken during the Copenhagen meeting has followed that idea and had the objective to view existing opinions.
In order to set a starting point for the successful discussion two video films were presented for the group. The first one prepared by American nematologists was dedicated to entomopathogenic nematodes. The second was produced by Europeans and concerned pest slugs. Both films have been rated by the group as very good and valuable examples to be consider during production of our own (COST 850) film. In relation to the first production it was suggested, however, that to improve perception of the message it would appropriate to avoid any “soap opera” acting parts which might seem somewhat “artificial” in such documentary presentation, unless they had been performed by really professional actors. The group discussion, well encouraged by a good supply of beer and wine brought a series of constructive suggestions.

1. First of all the main objective and addressee of the film should be clearly specified – should the film be addressed to plant growers, nematode distributors, scientists and students or to general public ?
2. Should it really be a film, internet web site with all the advantages of this communication medium or both of these forms ?
3. The film should not be excessively loaded with details of the nematode biology as it may be a little boring to non-specialist viewers.
4. In its construction the film should follow a logic sequence. One of such scenarios could start from the information about selected, important pests – at the beginning with information probably known to potential addressee (e.g. general image of the pest, known symptoms of its activity etc.). It should be followed by information usually not easily seen or known about the pest (e.g. hidden developmental stages of the pest, feeding activity, etc.). This trick usually helps to encourage attention of the viewer. The reasons to shift from chemical to biological control should be presented next. Some signals of the present role of entomopathogenic nematodes already played in the control of selected pests could be followed by basic information on hot spots in the nematode life-cycle, available commercial formulations, methods and hints on the field application, etc. At the end prospects for further developments, such as potential new pests and crops to be targeted in the nearest future could be presented in a kaleidoscopic way.
5. The internet web site should be constructed in a way allowing the viewer to easily find the pest and/or crop of interest. From this point it should lead to informative sections on available commercial formulations, methods of field application etc. All pages should be well illustrated with jpeg pictures and small volume mpg videos.
6. As there is some experience in video production and digital equipment available within the group first it would be appropriate to prepare "a working" version of the film through a gradual building and improving its form. If "perfect", such version could become a basis for a professional company to produce the European video film on entomopathogenic nematodes ordered by the COST Action 850.
7. To generate necessary funds for the final (highly professional) version of the film it would be appropriate to draw attention of nematode producing companies to this project.
8. The film should be prepared in a professional digital, beta or digital beta format to make it acceptable to TV presentations.
9. Finally, the group has agreed on the common involvement of all participants in the process of gradual building of the film scenario and subsequent verification of the outcome. The contact person responsible for co-ordination of this work would be Marek Tomalak from Institute of Plant Protection, Poznan, Poland, All suggestions on the film construction should be sent to the following e-mail address: M.Tomalak@ior.poznan.pl.
10. The progress in the nematode video film "construction process" will be discussed during subsequent COST 850 meetings and through e-mail communication.