

**Palacký University in Olomouc  
Czech Society for Plant Pathology**



**1st International Conference**

# **Wild Plant Pathosystems**

***Programme and Proceedings of Abstracts***

**Editors**

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**and**

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**July 2 – 5, 2013  
Palacký University in Olomouc  
Czech Republic**

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*1st International Conference*

### Wild Plant Pathosystems

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## The Topic

### **Wild Plant Pathosystems** (Olomouc, Czech Republic, July 2 - 5, 2013)

*Theory, communities and ecosystems, structure and variation, genetics and genomics, epidemiological and evolutionary dynamics, coevolution, agro-ecological interface, breeding applications*

This international conference address the theoretical and methodological aspects related to wild plant pathosystems, e.g.: ecological and genetic view on a coevolution, phylogenetics and speciation, general plant-pathogen perspective, ecological genomics of host-pathogen interactions, developing a common conceptual framework for antagonistic interactions, pathogens and community structure, epidemiological and evolutionary dynamics across the agro-ecological interface, trends in emerging and invasive pathogens, wild crop progenitors, sources of resistance, breeding applications

## Introduction and Welcome

Ladies and Gentlemen, colleagues and friends,

On behalf of the Organizing Committee of **Wild Plant Pathosystems**, the 1<sup>st</sup> International Conference on interactions between wild and weedy plants and their pathogens, I would like to welcome all of you to the Czech Republic and the historic city of Olomouc. This meeting is a first in addressing a broad swath of theoretical and methodological topics related to wild plant pathosystems - ecological and genetic views of coevolution, phylogenetics and speciation, general plant-pathogen interactions, ecological genomics of host-pathogen interactions, the development of a common conceptual framework for antagonistic interactions, interactions between pathogens and plant community structure, epidemiological and evolutionary dynamics across the agro-ecological interface, trends in emerging and invasive pathogens, wild crop progenitors, sources of resistance, and plant breeding applications. This broad area is a vibrant and fast developing area of plant biology and pathology which has been mostly neglected in various botanical, ecological and plant pathology meetings.

**Personally, I am delighted that I have the opportunity** to organise this conference in the city of Olomouc, within the historic Czech Lands, which became the Czech Republic in 1993. The history of this conference has two main basic roots. The first is connected with my own scientific activities which started in 1975 at the Plant Breeding Station in Smržice (just south of Olomouc). At that time I recognised that wild plant progenitors could be very interesting sources of disease resistance, later on I found these systems also exciting from general scientific viewpoint. The second aspect goes back to January/ February 2003 when I first met Jeremy Burdon during the ICPP 2003 in Christchurch in New Zealand. At that time we established very strong personal contacts which cemented links developed through correspondence in the 1980s. During a visit to CSIRO in Canberra in September 2005, and especially during our last meeting in July 2011 during 18<sup>th</sup> International Botanical Congress in Melbourne (Australia), Jeremy and I agreed such meetings often lacked strong participation by people focused on ecological, genetic and evolutionary aspects of interactions between wild and weedy plants and their pathogens. Jeremy concluded that I was a suitable person to organize such a type of meeting. It was great honour and challenge for me, but I was concerned as to the logistical difficulties in organizing a meeting and attracting an interesting and interested group of scientists. Nevertheless, during the autumn 2011 I made the final decision to take on this challenge and now after 18 months of planning we are at the front of gate of the first international meeting on wild plant pathosystems.

From this brief historical excursion it is evident that **compared with crop-pathosystems activities focused on research of wild plant pathosystems have been rather limited until recently** although there are two crucial books related to this topic. The first was Raoul Robinson's book: *Plant Pathosystems* (Springer-Verlag, Berlin, 1976); the second one which was more focused on topics of the current meeting was Jeremy Burdon's: *Diseases and Plant Population Biology* (Cambridge University Press, Cambridge, 1987). Both these books influenced me greatly; both were crucial to my future research development.

In the second half of 1970 I established strong contacts with Dr. Ian R. Crute (UK) in the area of research of interactions between wild *Lactuca* spp. and *Bremia lactucae*. These contacts yielded our first joint paper in 1981 (published in *Euphytica*). This coincides also with the period that I have been involved in this field of research, starting in 1975 not so far from the city of Olomouc, at the Plant Breeding Station at Smržice, which at that time was part of the Research and Breeding Institute of Vegetable Crops in Olomouc. Hence this region and Olomouc can be considered as the place where the first Czech experimental research on wild plant pathosystems was initiated.

In 1994 I transferred the research of wild plant pathosystems from the former Plant Breeding Station in Smržice to the Department of Botany, Faculty of Science, Palacký University in Olomouc. Soon, a research group focusing mostly on downy and powdery mildews was established, and research is now focused mostly on the ecology, plant pathology, physiology and genetics of host-pathogen interactions, population biology and epidemiology, and resistance breeding (mostly vegetable crops and their wild relatives).

**The historic city of Olomouc** is situated in the Central Moravia region, the main part of which is formed by the fertile and hospitable Haná lowlands. The name Haná was first used by Jan Blahoslav and Jan Amos Comenius on a map of Moravia in 1627. Historically the city of Olomouc is considered as the capital of Central Moravia, and a prominent centre of education and culture. It is well known that advances in culture and science in the Czech Lands have been, and are still, closely related to the developments of higher education and the University in Olomouc. In 1573 the city was granted university rights (i.e. this year we are celebrating 440 years of the University); the University in Olomouc is the second oldest university in the Czech Lands (after Charles University in Prague, established in 1348). The era of the old University is associated with the names of numerous outstanding scientists, including the founder of genetics Gregor Johann Mendel, who studied philosophy and theology here in 1840-1843. The recent name „Palacký University“ was given in recognition of the historian František Palacký, known as „the Father of the Nation“. At present, the University has seven faculties, with over 1500 teaching staff and nearly 25,000 students ([www.upol.cz](http://www.upol.cz)).

**The main venue of The 1<sup>st</sup> Conference on Wild Plant Pathosystems**, the monumental complex of baroque buildings named „Konvikt“, is very closely linked to the rich history of the city, the Jesuit Boarding School (Jesuit Seminary, Jesuit College) and the University in Olomouc. The early history of this building dates from medieval times. The present building was finished in 1708 and used for a long period by Olomouc University. Unfortunately, towards the end of 18th century the building of the former Jesuit College was handed over to the military for use as barracks. However, the building was returned to the University after the revolutionary changes in 1989. Extensive reconstruction and modernisation was completed in 2002, and it is once again used for its original academic purpose. Thus the building has extraordinary historic and artistic value. It is now used as the Art Centre of Palacký University (five art departments of the Philosophical and Pedagogical Faculties are located here), with the conference halls being used as a venue for international events such as the present symposium.

**Olomouc is well known both as a university city, and as a scientific centre.**

Research related to natural sciences, plant biology, breeding and growing of vegetable crops has a long tradition in this area. The Research Institute of Vegetable Crops (RIVC) was established here in January 1951, and in the end of 1950s the Institute of Experimental Botany of the Czechoslovak Academy of Sciences. However, the dramatic political and economic changes occurring after November 1989 resulted in closure of RIVC in 1994. The buildings and facilities were given to the Faculty of Science at Palacký University, which established a Biocentre here, focusing on biology and especially the plant sciences. Recently new facilities were built to house the Centre of the Region Haná for Biotechnological and Agricultural Research. This Centre combines activities of the University, the Institute of Experimental Botany of the Czech Academy of Science and the Gene Bank of the Research Institute of Crop Production in Prague.

**From the contents of the book of proceedings** it is evident that substantial progress has been made in various branches of wild plant pathosystems research and its application in plant breeding and disease control. The scientific and professional part of this meeting is based on 41 oral contributions and 22 posters, abstracts most of them are published in this book. Keynote lectures will be published separately as a Special Volume of the European Journal of Plant Pathology (Editors: A. Lebeda, J.J. Burdon, P.H. Thrall and M. Jeger). Some other papers will be published in a Special Volume of Plant Protection Science (published by Czech Academy of Agricultural Sciences). From the programme and the contents of the proceedings it is evident that there is a good balance between fundamental science, theoretical and methodological approaches, as well as practical applications of the results. Increasing international contacts and cooperation in wild plant pathosystems research provides an excellent foundation for future developments.

We are very happy that Palacký University in Olomouc, and the Czech Republic, will host this gathering of scientists and professionals from 15 countries and four continents. We will do our best to make **Wild Plant Pathosystems 2013** a most informative and enjoyable experience for all participants. We believe that the conference will take place in the spirit of cooperation and friendship, and that everybody will enjoy the science, the historic city of Olomouc, Moravia and the Czech Republic. We hope that you will find this to be a most memorable occasion.

Aleš Lebeda

Olomouc, June, 2013

## Programme

### Wild Plant Pathosystems, 1st International Conference (2-5 July 2013, Olomouc, Czech Republic)

#### *1 July, Monday*

*Hotel Flora*

*Accommodation*

15.00-20.00 *Registration*

#### *2 July, Tuesday*

*Palacký University, Konvikt*

8.00-10.00 *Registration, setting up the posters*

*Palacký University, Konvikt „Auditorium“*

10.00-10.30 *Opening ceremony, Introduction to the programme*

10.00-10.15 *Opening ceremony*

10.15-10.30 *Introduction*

**J.J. Burdon (Australia)**

Pathogens as driving forces in evolution

10.30-12.30 *Broad ecological / genetic view in a coevolutionary setting  
and host-pathogen interactions*

**Chairpersons: J.J. Burdon, J.K.M. Brown**

10.30-11.00 **J.K.M. Brown (UK)**

Arms race or trench warfare? Predictions about host-parasite coevolution in agriculture and nature

11.00-11.30 **U. Carlsson-Granér, B.E. Giles (Sweden), P.H. Thrall (Australia)**

Patterns of disease and host resistance in spatially structured systems

11.30-12.00 **E. Holub (UK)**

Following Biffen's footsteps with insights from *Arabidopsis-Albugo candida* interactions

12.00-12.15 **C.M. Higgins, J.D. Fletcher, B. Rodoni, F. Ochoa-Corona, M. Roossinck, U. Melcher, R. MacDiarmid** (New Zealand, Australia, USA)  
Knowledge of plant virus biodiversity and ecology is required for prediction of virus disease epidemics: an antipodean perspective.

12.15-12.30 **Y. Anikster, J. Manisterski, T. Eilam, P. Ben Yehuda, S. Ezrati and H. Sela** (Israel)  
Ecological adaptation of the leaf rust fungi attacking *Hordeum spontaneum* in Israel

**12.30-14.00 Lunch**

**14.00-15.45 *Phylogenetics & Speciation***  
**Chairpersons: E. Holub, M. Thines**

14.00-14.30 **M. Thines** (Germany)  
Phylogeny, evolution and diversity of oomycetes - a global perspective

14.30-15.00 **B. McDonald** (Switzerland)  
The role of effectors in the emergence of host-specialized fungal pathogens: a phylogeographical perspective

15.00-15.15 **A.-K. Buch, L. Nigrelli, M. Thines** (Germany)  
Diversity, phylogeny and host range of the *Pythium oligandrum* species complex

15.15-15.30 **Y.J. Choi, M. Thines** (Germany)  
Macroevolutionary strategy of downy mildew: A case study of *Bremia* and Asteraceae

15.30-15.45 **I. Solovyeva, A. Schmuker, M. Thines** (Germany)  
Evolution of *Hyaloperonospora* effectors: ATR1-effector genes in sister species of *H. arabidopsidis*

**15.45-16.15 Coffee break**

**16.15-18.30 *Ecological genomics of host-pathogen interactions***  
**Chairpersons: B. McDonald, L. Rose**

16.15-16.45 **P.H. Thrall, J.J. Burdon** (Australia)  
Integrated evidence for co-evolution in natural host-pathogen associations

16.45-17.15 **A.-L. Laine** (Finland)  
Life-history, evolutionary potential and environment as determinants of pathogen dynamics in a large natural metapopulation

17.15-17.45 **Y. Bai** (the Netherlands)  
Genetics and molecular aspects of resistance in wild *Solanum* spp. against powdery mildew

17.45-18.15 **L. Rose** (Germany)  
Evolutionary genetics of disease resistance in wild tomato species

18.15-18.30 **C. Tollenaere, A.-L. Laine** (Finland)  
Molecular tools for the study of the interaction between *Podosphaera plantaginis* and *Plantago lanceolata* in the Åland metapopulation

**19.00-21.30 Welcome Party at Historical Building of Archbishop Palace, Olomouc**

**3 July, Wednesday**

**Palacký University, Konvikt "Auditorium"**

**8.30-10.30 *Developing a common conceptual framework for antagonistic interactions Theoretical and modelling approaches and challenges***  
**Chairpersons: M.J. Jeger, E. Kosman**

8.30-9.00 **E. Kosman** (Israel)  
Diversity analysis of pathogens and their hosts: from individuals to populations

9.00-9.30 **M.W. Shaw** (UK)  
The population dynamics of disease on short and long time-scales

9.30-10.00 **R.K. Meentemeyer, S.E. Haas and T. Vaclavik** (USA, Czech Republic)  
Landscape epidemiology of emerging infectious diseases in ecological communities



- 10.00-10.15 **T. Koubek** (Czech Republic)  
Matrix models of plant infected by systemic pathogen show low impact of the disease and high influence of the environment
- 10.15-10.30 **P.S. Allen, S.E. Meyer, J. Beckstead** (USA)  
A predictive model for soil seedbank outcomes in the *Pyrenophora semeniperda*-*Bromus tectorum* pathosystem
- 10.30-11.00 Coffee break**
- 11.00-12.30 Pathogens and community structure**  
*Life-history attributes, evolutionary interactions and trajectories*  
**Chairpersons: A.-L. Laine, H.M. Alexander**
- 11.00-11.30 **M.J. Jeger, N. Salama, M.W. Shaw, F. van den Berg, F. van den Bosch** (UK)  
Effects of plant pathogens on population dynamics and community composition in grassland ecosystems
- 11.30-12.00 **J. A. Walsh, C. Obermeier, P.J. Hunter, R. Machado, K. Ohshima, M.J. Kearsey** (UK, Japan)  
Plant – virus co-evolution in wild brassicas
- 12.00-12.15 **N.A. Bosque-Pérez, L.L. Ingwell** (USA)  
*Barley/Cereal yellow dwarf* viruses in prairie and grasslands habitats in the US Pacific Northwest
- 12.15-12.30 **H. Susi, A.-L. Laine** (Finland)  
The effect of co-infection to pathogen epidemiology in *Plantago lanceolata* – *Podosphaera plantaginis* interaction
- 12.30-14.00 Lunch**
- 14.00-15.45 Epidemiological & evolutionary dynamics across the agro-ecological interface**  
**Chairpersons: M.W. Shaw, R.K. Meentemeyer**
- 14.00-14.30 **J.J. Burdon, P.H. Thrall** (Australia)  
Ecological and evolutionary processes across the agro-ecological interface
- 14.30-15.00 **H.M. Alexander, K.E. Mauck, A.E. Whitfield, K.A. Garrett, C.M. Malmstrom** (USA)  
Plant-virus interactions and the agro-ecological interface
- 15.00-15.15 **P. Bernardo, M. Golden, A. Mohd, E. Fernandez, M. Granier, A.G. Rebelo, M. Peterschmitt, D.P. Martin, P. Roumagnac** (France, South Africa, India)  
New insights into the evolutionary history of geminiviruses derived through the discovery of divergent viruses isolated from wild plants
- 15.15-15.30 **H. Finch, P.S. Allen, S.E. Meyer** (USA)  
Exposure to low water potentials and seed dormancy favor the fungus in the *Pyrenophora semeniperda*-*Bromus tectorum* pathosystem
- 15.30-15.45 **K. Hawkins, P. Allen, S. Meyer** (USA)  
Secondary dormancy of seeds in relation to the *Bromus tectorum*-*Pyrenophora seminiperda* pathosystem
- 15.45-16.15 Coffee break**
- 16.15-18.15 Trends in emerging and invasive pathogens**  
**Chairpersons: J.A. Walsh, L. Kiss**
- 16.15-16.45 **L. Kiss** (Hungary)  
Powdery mildew fungi as super-invasive plant pathogens
- 16.45-17.15 **K. Petržík, J. Vondrák, M. Barták, O. Peksa, O. Kubešová** (Czech Republic)  
Lichens - new source or host of higher plant viruses?
- 17.15-17.30 **O. Frenkel, M.T. Brewer, L. Cadle-Davidson, M.G. Milgroom** (USA)  
Population structure and biology of the grape powdery mildew fungus, *Erysiphe necator*, at its center of diversity
- 17.30-17.45 **R. Ben-David, R. Parks, C. Cowger, A. Dinoor, E. Kosman, T. Wicker, B. Keller** (Israel, Switzerland)  
Reciprocal virulence pattern among *Blumeria graminis tritici* isolates originating from domesticated wheat and its wild progenitor



17.45-18.00 **R. Sharma, B. Mishra, F. Runge, M. Thines** (Germany)  
Smut fungi are model systems for investigating the evolution of pathogenicity– insights from the genome of *Melanopsichium pennsylvanicum*

#### 18.15-19.00 *Poster Session*

### 4 July, Thursday

#### 8.00-21.00 *South Moravia - Excursion*

8.00 *Departure from Hotel Flora*

9.00-11.30 *“Austerlitz battlefield“ - The Cairn of the Peace Memorial and Museum*

12.00-14.30 *The Castle of Austerlitz (Slavkov), Lunch (supported by Rijk Zwaan)*

15.30-17.00 *Johann G. Mendel Museum of Genetics and Augustinian Abbey in Old Brno*

17.30-19.30 *Moravian wine (Vinselekt Michlovsky) tasting in Augustinian Abbey, Old Brno (Brno)*

19.30 *Departure from Brno to Olomouc*

21.00 *Arrival to Olomouc*

### 5 July, Friday

#### *Palacký University, Konvikt “Auditorium“*

9.00-12.15 *Applied aspects, wild progenitors, sources of resistance, resistance breeding*  
Chairpersons: Y. Bai, U. Carlsson-Granér

9.00-9.30 **L. Kiss** (Hungary)  
Mycoparasites: surprise elements in wild plant pathosystems

9.30-10.00 **A. Lebeda, E. Křístková, M. Kitner, B. Mieslerová, M. Jemelková, D.A.C. Pink** (Czech Republic, UK)  
Wild *Lactuca* species, their genetic diversity, resistance to diseases and pests, and exploitation in lettuce resistance breeding

10.00-10.30 **A. Dreiseitl** (Czech Republic)  
Wild pathosystem *Hordeum vulgare* subsp. *spontaneum* – *Blumeria graminis* f. sp. *hordei* and its position in resistance research and breeding applications

#### 10.30-11.00 *Coffee break*

11.00-12.15 *Applied aspects, wild progenitors, sources of resistance, resistance breeding* (continued)  
Chairpersons: Y. Bai, U. Carlsson-Granér

11.00-11.30 **A. Lebeda, B. Mieslerová, M. Petřivalský, L. Luhová, M. Špundová, M. Sedlářová, V. Nožková-Hlaváčková, D.A.C. Pink** (Czech Republic, UK)  
Resistance mechanisms of wild tomato germplasm to infection of *Oidium neolycopersici*

11.30-11.45 **A. Tahir, L. Nigrelli, F. Runge, A. Schmuker, I. Solovyeva, A. Çelik, M. Thines** (Germany)  
Diversity of *Arabidopsis thaliana* in Italy as inferred from next-generation genotyping in relation to biotic and abiotic factors

#### **Closing and Challenging lecture**

11.45-12.15 **M. Thines** (Germany)  
*Microthlaspi* – *Hyaloperonospora* as a new model system to investigate natural plant – pathogen interactions

#### 12.15-12.45 *Closing session and closing ceremony*

#### 13.00-14.30 *Lunch*

15.00-17.00 **Archdiocese Museum in Olomouc**  
(guided tour through the museum and historical buildings, for those who are interested)

#### *Farewell Evening, Palacký University, Konvikt “Atrium“ and surrounding area*

19.00-19.30 **Baroque music concert in Konvikt Chapel**

19.30-24.00 *Farewell dinner*

*\* some minor changes may appear in the programme structure/content*

**Abstracts**

**Keynote Lectures**

## Plant-virus interactions and the agro-ecological interface

H. M. Alexander<sup>1</sup>, K. E. Mauck<sup>2</sup>, A. E. Whitfield<sup>3</sup>, K. A. Garrett<sup>3</sup>  
and C. M. Malmstrom<sup>4</sup>

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<sup>3</sup>*Department of Plant Pathology, Kansas State University, Manhattan, KS U.S.A. 66506;* <sup>4</sup>*Department of Plant Biology, Michigan State University, East Lansing, MI 48824 USA*

As a result of human activities, an ever-increasing portion of Earth's natural landscapes now lie adjacent to agricultural lands. This border between wild and agricultural communities represents an agro-ecological interface, which may be populated with crop plants, weeds of crop systems, and non-crop plants that vary from exotic to native in origin. Plant viruses are important components of the agro-ecological interface because of their ubiquity, dispersal by arthropod vectors, and ability to colonize both crop and wild species. Here we provide an overview of research on plant virus dynamics within this interface in the context of ecological concepts of indirect interactions. We suggest three research priorities: (1) an increased effort to identify and describe plant virus diversity and prevalence in its entirety across agricultural and ecological boundaries; (2) multi-scale studies of virus transmission to develop predictive power in estimating virus propagation across landscapes; and (3) quantitative evaluation of the influence of plant viruses on host fitness in environmental contexts beyond crop fields. Multi-scale modeling will be invaluable for integration of research in these three areas.

The development of new crops presents opportunities for research on agro-ecological questions. To illustrate, we provide examples using viral infection of switchgrass, *Panicum virgatum*, a native prairie grass in North America and a potential biofuel crop. We close by emphasizing that agro-ecological interfaces are dynamic. Climate change and unprecedented global movement of plants, vectors, and viruses (often human-mediated) are leading to shifts in species distributions. Consideration of virus interactions within these environmentally complex systems promises new insights into virus dynamics from molecular mechanisms to ecological consequences.

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## Genetics and molecular aspects of resistance in wild *Solanum* spp. against powdery mildew

Y. Bai

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Powdery mildews are obligate biotrophs, which establish long lasting interactions with living host tissue by forming haustoria in epidermal cells. Worldwide, approximately 500 PM species are able to colonize about 10,000 plant species. Pathosystems, involving tomato, barley and Arabidopsis, are well-studied and represent experimental interactions for understanding host and non-host resistances to powdery mildews.

Tomato powdery mildew caused by *O. neolycopersici* occurred for the first time in 1988 in The Netherlands and has spread within 10 year to all European countries and is nowadays a worldwide disease on tomato, except for Australia where another species, *O. lycopersici*, occurs. The origin of *O. neolycopersici* is still a mystery. It is supposed that a powdery mildew “jumped” from its host species to tomato. Upon the outbreak of *O. neolycopersici*, all tomato (*Solanum lycopersicum*) cultivars were susceptible and *O. neolycopersici* was the only fungus to be controlled by fungicides in greenhouse tomato production in Northwest Europe. By 1996, our group was invited by Dutch vegetable seed companies to search for resistance genes against *O. neolycopersici*. So far, we have introgressed six resistance genes (*Ol*-genes, *Ol-1* to *Ol-6*) and three quantitative trait loci (QTLs) from wild species into cultivated tomato to combat *O. neolycopersici*.

We generated a unique set of nearly isogenic lines (NILs) that harbor an introgression carrying the particular *Ol*-gene and QTL in the genetic background of *S. lycopersicum* cv. Moneymaker. Using the NILs, mechanisms underlying the powdery mildew resistance were studied. *Ol-1* enhances basal defense by inducing autophagic programmed cell death (PCD) in the late stages of pathogen infection. The recessive gene *ol-2*, which is a homologue of the barley *mlo* gene, mediates resistance by inducing callose deposition and cell wall fortification to stop powdery mildew at penetration stage. We have also shown that *Ol-4*, homologous to the *Mi-1* gene and encoding an NBS-LRR protein, triggers hypersensitive response (HR) and thereby prevents the fungal colonization after formation of primary haustoria. Also, we have investigated the role of different phytohormone pathways, salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) and ethylene (ET) in the response of tomato to *O. neolycopersici* (Seifi,

2011). Our results suggested that SA pathway is the main phytohormone pathway that is recruited in HR-associated resistance. For the resistance associated with autophagic PCD, the ET pathway is essential. The JA and ABA pathways are crucial for the resistance mechanism relying on callose deposition.

## Arms race or trench warfare? Predictions about host-parasite coevolution in agriculture and nature

**James K. M. Brown**

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In host-parasite coevolution, arms races lead to a profusion of diverse genes involved in disease, including parasite avirulence which are recognised by host resistance genes, thus eliciting defences in the host. In a trench warfare situation, by contrast, there is a quasi-stable equilibrium in gene frequencies in both species. Using the gene-for-gene model in plants as an example, the familiar boom-and-bust cycle is driven by indirect frequency-dependent selection, in which selection for resistance is strongest when pathogen virulence is rare and selection for virulence is greater when resistance is more common. In the simplest models, it is predicted that ultimately, one allele becomes fixed in each species, either resistance in the host and virulence in the parasite or susceptibility and avirulence.

In natural pathosystems, however, polymorphism in *R* genes is common and some *R* alleles are extremely long-lived. It is predicted that many ecological and epidemiological factors can lead to stable polymorphism in matching pairs of pathogenicity and plant resistance genes in a trench warfare scenario. This occurs by direct frequency-dependent selection, in which the contribution of a gene to an organism's fitness declines as its own frequency increases. Essentially any process which partially uncouples the life cycles of a host and a parasite can generate this type of frequency-dependent selection. The trench warfare scenario, with balanced, quasi-stable polymorphism, results from "messy ecology" in which one or more factors complicate the process of mutual natural selection by host and parasite species. While fitness costs do not themselves stabilise polymorphism, they affect the frequencies of host and parasite genes at equilibrium.

In agricultural pathosystems, by contrast, there has been rapid evolution of many genes involved in host-parasite recognition. The theoretical explanation of this is that many of the factors which generate direct frequency-dependent selection in nature and which stabilise host and parasite gene frequencies have been removed in order to increase the efficiency of food production. This generally leads to arms races in host-parasite coevolution rather than trench warfare. Better understanding of the processes which lead to trench warfare in nature may lead to ideas for achieving durable disease control and thus sustainable food production in agriculture.

## Pathogens as driving forces in evolution

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Over the last four decades studies of natural plant-pathogen associations, particularly with regard to the role they play in shaping plant community structure and eco-evolutionary dynamics have burgeoned. From an initial focus on basic issues to do with the effects of plant density on disease spread or pathogen impacts on host mortality, the field has expanded into a dynamic subdiscipline of coevolutionary biology as both pre-existing and novel concepts and lines of inquiry in theoretical population genetics, agriculture production, epidemiology, host-pathogen genetics, community and invasion ecology, and metapopulation theory have become interwoven. From this has emerged a broadly based focus on host-pathogen coevolution with considerable relevance to conservation biology, agriculture, and community and landscape biology.

## Ecological and evolutionary processes across the agro-ecological interface

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Anthropogenic impacts increasingly drive ecological and evolutionary processes at many spatio-temporal scales. This is particularly true across the interface between agricultural production systems and surrounding wastelands, roadsides and natural ecosystems. Shifting patterns of land-use in response to global drivers such as climate change and food security involve conflicting imperatives to expand or intensify production while simultaneously reducing environmental impacts. While the use of evolutionary principles is not new in agriculture (e.g. crop breeding, domestication of animals, management of selection for pest resistance), given these land-use trends and other transformative processes and management practices in production landscapes, ecological and evolutionary research in agro-ecosystems must consider such issues in a broader systems context. Here, we focus on biotic interactions involving weeds, pests and pathogens as exemplars of situations where integration of agronomic, ecological and evolutionary perspectives has practical value. Although their presence in agro-ecosystems may be new, many traits involved in these associations evolved in natural settings. We advocate the use of predictive frameworks based on evolutionary models as pre-emptive management tools and identify some specific research opportunities to facilitate this. We conclude with a brief discussion of multidisciplinary approaches in applied evolutionary problems.

## Patterns of disease and host resistance in spatially structured systems

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We use data from populations of the anther-smut fungus *Microbotryum violaceum* and the host plants *Lychnis alpina* and *Silene dioica* to show that spatial structuring at different scales can greatly impact patterns of disease spread, disease dynamics and host resistance. *Microbotryum violaceum* is pollinator transmitted and causes permanent sterility in infected plants and has become a model system for studies of sterilizing vector-transmitted diseases. Patterns of disease and host resistance were surveyed in an archipelago subject to land-uplift where populations of *S. dioica* constitute an age-structured metapopulation, and in three contrasting areas within the range of *L. alpina* where populations range from continuous, through patchy but spatially connected to highly isolated.

In the archipelago system of *S. dioica*, disease levels depend on the age, size and density of local patches and populations. Disease is most predictably found in larger more dense patches and populations, and more frequently goes extinct in smaller populations. The rate of local disease spread is also affected by the levels of resistance; *S. dioica* populations showing an increase in disease over time are more susceptible than populations where the disease has remained at low levels. Among population variation in resistance is partly driven by founding events, and despite the potential for selection of resistance, populations remain differentiated due to limited gene flow between islands. As observed in the *L. alpina* system, when populations are more connected, a greater fraction of populations have disease present. Results from a simulation model argue that, while increased dispersal in well-connected systems can increase disease spread, it may also favour selection of host resistance which ultimately reduces disease levels within populations. This could explain the observed lower disease prevalence in *L. alpina* in regions where populations are more continuous. Consistent with this, a large scale inoculation experiment showed that isolated populations of *L. alpina* were more susceptible than more spatially connected populations. Clearly, the results obtained from the *S. dioica* and *L. alpina* systems confirm some of the predictions generated from spatial host pathogen models: the importance of population size and distance and the dynamic nature of disease.

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## The *Hordeum vulgare* subsp. *spontaneum* - *Blumeria graminis* f. sp. *hordei* pathosystem: its position in resistance research and breeding applications

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Barley (*Hordeum vulgare* L.) is an important cereal crop and powdery mildew, caused by *Blumeria graminis* f. sp. *hordei*, is one of the most serious diseases that occurs on barley throughout the world. In the Middle East, which is the centre of diversity for barley and its pathogens, the wild barley - powdery mildew pathosystem co-evolves resulting in many specific resistances in the host as well as corresponding virulences in the pathogen. Many specific resistances have been used in European breeding programmes and a centre of pathogen diversity has arisen also especially in the central Europe. This short review briefly summarizes the use of host resistances derived from wild barley and land races including the durable resistance gene *mlo*. The use of pathogen pathogenicity for studying new and unknown specific resistances and for identifying resistances in commercial varieties is described. However, highly heterogeneous wild barley is also characterized as a valuable source of minor genes for powdery mildew resistance. These might be exploited by barley breeders especially for winter barley improvement where the non-specific resistance gene *mlo* cannot be used.



## Following Biffen's footsteps, with insights from *Arabidopsis-Albugo candida* interactions

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Biffen's 1910 release of a yellow rust resistant wheat launched genetics as an applied discipline, for harnessing inheritance of natural variation to breed new animals and crops for human benefit. His legacy still informs modern translational research of rust diseases, including the use of *Arabidopsis thaliana* as a model for investigating how a plant restricts the host range of biotrophic fungi and oomycetes that cause rust and mildew diseases. For example, white blister rust caused by the oomycete *Albugo candida* provides is particularly useful for identifying genes that protect against pathogens from other wild relatives such as *Capsella bursa-pastoris*, and from crops such as vegetable & oilseed brassicas. White rust resistance (WRR) to *Al. candida* is polygenic in *A. thaliana* Columbia including the TIR-NB-LRR gene *WRR4*, which provides rapid pathogen recognition and resistance against a broad-spectrum of *Al. candida* from brassica species and *C. bursa-pastoris*. We have begun finding variants of *Al. candida* that can overcome WRR4 resistance, which will be useful for genetic characterization of the matching avirulence elicitors. This gene confers resistance in transgenic oilseed rape and oilseed mustard (*B. napus* and *B. juncea*). Two additional genes (*WRR5* and *WRR6*) have been identified which confer resistance against blister formation but associated with visible chlorosis surrounding infections. All three genes exhibit parallel loss-of-function including large deletions of coding sequence, similar to the bacterial resistance gene *RPM1* (see Rose et al. 2012. Frontiers Plant Sci doi: 10.3389/fpls.2012.00287). This suggests a recurring cost of resistance in the absence of disease, presumably caused by *A. candida*. Further *WRR* resistance in Columbia associated with pronounced wilting of infected tissue and partial blistering still needs to be characterized. The combined genetic evidence from host and pathogen will be useful for translational genetic development of crops for sustainable disease control.

## Effects of plant pathogens on population dynamics and community composition in grassland ecosystems

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Grassland ecosystems comprise a major portion of the earth's terrestrial surface, ranging from high-input cultivated monocultures or simple species mixtures to relatively unmanaged but dynamic systems. Plant pathogens are a component of these systems with a varying impact of disease depending on species diversity and community structure, the extent of managed cultivation and other disturbance, the invasiveness of introduced species, and the myriad interactions between plants, soil biota, disease vectors, and abiotic conditions including nutrient status. Plant pathogens are affected by these interactions and also act reciprocally by modifying their nature. Many studies have investigated these interactions: notable examples being the consequences of introduced species on viral infection of native grasses in the Pacific coast of N America, and diversity-productivity relationships found in grasslands affected by fungal plant diseases.

In this talk we introduce the 150-yr long-term Park Grass experiments (PGE) at Rothamsted Research and consider in detail two plant-pathogen systems, *Tragopogon pratensis*-*Puccinia hysterium* (TpPh) and *Holcus lanata*-*Puccinia coronata* (HIPc). These two systems have very different life history characteristics: the first, a biennial member of the Asteraceae infected by a systemic, host-specific, rust; the second, an annual grass infected by a non-host specific rust. We illustrate how observational, experimental and modelling studies can contribute to a better understanding of population dynamics, competitive interactions and evolutionary outcomes. With TpPh, characterised as an "outbreak" species in the PGE, we show that pathogen-induced mortality has minimal impact and unlikely to be involved in host population regulation. With HIPc, we show how nutrient conditions can affect adaptation in terms of host defence mechanisms, and that co-existence of competing species affected by a common generalist pathogen is unlikely.

## Powdery mildew fungi as super-invasive plant pathogens

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Powdery mildew fungi (*Erysiphales*) are ubiquitous obligate biotrophic plant pathogens as the symptoms they cause are obvious on leaves and other aerial parts of their host plants. Thus, their occurrence and spread can easily be monitored. Year by year, a high number of ‘new disease reports’ published in international journals, and reporting the emergence of new fungal and other plant diseases, refers to this group of fungal plant pathogens. In most cases, however, the origins of the powdery mildew fungi reported for the first time on a certain host plant species or in a given geographic area remain unclear. Sometimes it is likely that they originate from other geographic regions, e.g. other continents. Biological invasions caused by North American and Asian powdery mildew fungi in Europe have already been documented using molecular tools. The biological invasion caused by the grapevine powdery mildew pathogen, *Erysiphe necator*, in Europe in the 19. century is a well-known example, and the histories of two recent severe European powdery mildew epidemics, caused by *E. flexuosa* on horse chestnut (*Aesculus* spp.) and *E. elevata* on Indian beam (*Catalpa bignonioides*) trees, seem to be similar to that process. Some other species of the *Erysiphales*, thought to be native to North America or Asia, have also started to spread in Europe quite recently. Another source of ‘new’ powdery mildews could be a host range expansion of taxa already known from certain host plant species. Such an event has recently been documented for the first time in powdery mildews. However, an analysis of a few other ‘new’ powdery mildew fungi showed that these all should be regarded as distinct taxa with restricted host ranges. This suggests that case-by-case studies are needed to identify the sources of newly emerging powdery mildew fungi. The relatively easy monitoring of the invasive species of the *Erysiphales* should make them ideal targets for studies of biological invasions caused by plant pathogenic fungi.

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## Mycoparasites: surprise elements in wild plant pathosystems

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Traditionally, the interactions between plant parasitic fungi and host plants are regarded as closed, two-species systems. However, both parasites and their hosts are, in fact, components of complex multitrophic interactions in which parasitic fungi are often attacked and suppressed by specialized fungal mycoparasites (i.e., hyperparasites) or other antagonists in the field. Parasites, by definition, have a negative effect on host fitness, so mycoparasitism / hyperparasitism may be favourable for plants infected with fungal parasites. However, there are only a few detailed studies on the role of mycoparasites in natural host-parasite relationships, although their use in biological control of plant diseases is largely based on the supposed importance of hyperparasitism in natural control phenomena. This talk reviews our current knowledge on specialized mycoparasites, such as *Ampelomyces* spp., well-known antagonists of powdery mildew fungi, *Eudarluca caricis* (anamorph: *Sphaerellopsis filum*), a mycoparasite of rust fungi, and *Coniothyrium minitans*, a mycoparasite of *Sclerotinia sclerotiorum* and other sclerotia-forming fungi, as parts of wild plant pathosystems.

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## **Diversity analysis of pathogens and their hosts: from individuals to populations**

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Making inferences about variation within and among various operational units in general and, in particular, in plant pathology may depend considerably on ability of an approach applied to diversity analysis to utilize correctly and in full extent information available in the raw data. Therefore, it is essential to select a suitable approach to measuring diversity in each specific study. Comprehensive analysis of the commonly used frequency-based genotypic and gene methods, as well as recently developed methods of 'true diversity' and functional diversity demonstrated both hidden and obvious limitations and possible inconsistency of these approaches in analyzing population diversity and structure. The proposed dissimilarity based methods can be separated into two groups according to the mode of dealing with a matrix of pairwise dissimilarities between individuals. The first group includes methods based on averaging pairwise dissimilarities. Many commonly used diversity parameters can be explained in terms of the average based measures. Methods from the second group are based on a solution of the corresponding assignment problem. Prior to analyzing diversity of various systems, the dissimilarity based approaches need a suitable assessment of dissimilarity between the corresponding individual operational units (individuals, communities, populations, clusters, functions, phylogenetic trees etc.). Choice or development of such coefficients of dissimilarity is the separate challenging problem that might be quite complicated. The dissimilarity based methods assess actually functional diversity because each individual is expressed by its functional profile in the space of the selected traits, and these approaches are also applicable to measuring complex diversity for combinations of several groups of descriptors of different types. New approach was proposed to decomposition of total  $\gamma$ -diversity into independent components of  $\alpha$ - and  $\beta$ -diversity. Mathematical validity of the average based methods depends on the selected dissimilarity measure, whereas the assignment based approaches are always valid. Finally, the assignment based methods are able to address shortcomings of the commonly used measures of population diversity, and they

are preferable in the case of possible association between traits. In light of these properties of dissimilarity based methods, it becomes clear that they may serve as universal tools for diversity analysis, which completely depend on a proper assessment of dissimilarity between elementary individual units.

### **Acknowledgements**

I am deeply indebted to Prof. Kurt Leonard (University of Minnesota) who focused my attention on problems of diversity analysis in the mid-1990s.

## Life-history, evolutionary potential and environment as determinants of pathogen dynamics in a large natural metapopulation

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Diseases pose a major threat to human, animal and plant health. Understanding what generates variation in epidemiological patterns, and variation in the harm pathogens impose on their hosts is one of the major challenges of disease biology.

I will discuss the key theories proposed for pathogen evolution –Red Queen hypothesis, life-history trade-offs and co-infection – and how they link with realized epidemiological dynamics in the interaction between *Plantago lanceolata* and its fungal pathogen, *Podosphaera plantaginis*.

Long-term epidemiological data collected from over 4000 host populations in the Åland archipelago coupled with ecological studies and genomic resources allow for a unique synthesis of the factors generating variation in both within and between season disease dynamics.

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## Wild *Lactuca* species, their genetic diversity, resistance to diseases and pests and exploitation in lettuce breeding - review

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Current knowledge of wild *Lactuca* L. species, their taxonomy, bio-geography, gene pools, germplasm collections quality, quantity and accession availability are reviewed in this paper. Genetic diversity of *Lactuca* spp. is characterized at the level of phenotypic and phenological variations, variation in karyology and DNA contents, biochemical traits, and protein and molecular polymorphism. The reported variation in reaction to pathogens and pests of wild *Lactuca* spp. is summarized, including viral pathogens (Lettuce mosaic virus - *LMV*, Mirafiori lettuce virus /Lettuce big vein virus – *LBV*, Beet western yellows virus - *BWYV*, Tomato spotted wilt virus – *TSWV*, Cucumber mosaic virus - *CMV*, Lettuce necrotic stunt virus – *LNSV*), bacterial pathogens (corky root - *Rhizomonas suberifaciens*, bacterial leaf spot - *Xanthomonas campestris* pv. *vitians*), fungal pathogens (downy mildew - *Bremia lactucae*, powdery mildew - *Golovinomyces cichoracearum*, anthracnose - *Microdochium panattoniana*, stemphylium leaf spot - *Stemphylium* spp., sclerotinia drop - *Sclerotinia* spp., verticillium wilt - *Verticillium dahliae*, fusarium wilt - *Fusarium* spp., pythium wilt - *Pythium tracheiphylum*, *P. uncinulatum*, nematodes (potato cyst nematode - *Globodera rostochiensis*, root-knot nematode - *Meloidogyne* spp., *incognita*, *hapla*, *javanica*, *enterolobii*), insects and mites (the green lettuce aphid - *Nasonovia ribisnigri*, the green peach aphid - *Myzus persicae*, the potato aphid - *Macrosiphum euphorbiae*, leafminer - *Liriomyza* spp., *L. langei*). The approaches used to exploit wild *Lactuca* spp. in lettuce breeding (interspecific hybridization, cell and tissue culture, transformation) are discussed, and known examples of lettuce cultivars with traits derived from wild *Lactuca* spp. are described.

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## Resistance mechanisms of wild tomato germplasm to infection of *Oidium neolycopersici*

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Tomato powdery mildew (*Oidium neolycopersici*) is one of the most devastating diseases of cultivated tomatoes worldwide. Although the first epidemics were recorded more than 25 years ago many aspects of this host-pathogen interaction are still not well understood. Detailed morphological and molecular studies of the anamorphs confirmed that *O. neolycopersici* is phylogenetically close to *Erysiphe aquilagiae* var. *ranunculi*. Host range is rather broad, apart from Solanaceae hosts were found in the families Apocynaceae, Campanulaceae, Crassulaceae, Cistaceae, Linaceae, Malvaceae, Papaveraceae, Pedaliaceae, Scrophulariaceae, Valerianaceae a Violaceae. Nonhost resistance within these families is not based on inhibition of formation of primary haustorium, however but on post-haustorial HR and another type of non-hypersensitive resistance. Screening of wild *Solanum* species (previous *Lycopersicon* spp.) germplasm revealed valuable sources of resistance (*S. habrochaites*, *S. pennellii*, *S. cheesmaniae*, *S. chilense*, *S. peruvianum*). The main resistance mechanism was found to be a hypersensitive response (HR), in some cases followed by limited development of the pathogen. However, there is a broad variation in resistance response on the histological and cytological level. Interaction between many wild *Solanum* spp. and *O. neolycopersici* is race-specific, at least three races were differentiated. In some interspecific crosses (*S. lycopersicum* × *S. habrochaites*) adult plant resistance was observed. biochemical studies focusing on production of reactive oxygen species (ROS) and peroxidase activity during infection of *O. neolycopersici* showed that production of ROS and activity of corresponding enzymes is related to activation of defense responses in genotypes of wild *Solanum* sect. *Lycopersicon*. The significance of NO in *O. neolycopersici* pathogenesis was supported by experiments with NO donors and scavengers. In

moderately resistant genotype *S. chmielewskii*, treatment by heat stress caused slight deceleration of pathogen development, increased production of JA and ABA and increased peroxidase activity in infected plants. The different degree of tomato resistance/susceptibility did not markedly change the rate and extent of photosynthetic response to *O. neolycopersici*; only minimal impairment of photosynthesis was found in both susceptible and moderately resistant genotypes during the first 9 days after inoculation. The accumulated evidence confirm a crucial role of localised increased production of ROS and RNS in response to pathogen penetration into plant tissue and its involvement in the plant resistance responses including the initiation and progression of plant cell death in host wild *Solanum* species. Crucial points of further research are discussed.

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## The role of effectors in the emergence of host-specialized fungal pathogens: a phylogeographical perspective

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Population genetic, phylogenetic and phylogeographical studies indicated that *Phaeosphaeria nodorum* (aka *Stagonospora nodorum*) is a part of a species complex that shares its center of origin with wheat. We investigated the evolutionary history of three necrotrophic effectors (NEs) produced by *P. nodorum*. We screened over 1000 global strains across seven major regions, including North America, Europe, Iran, Central Asia, China, South Africa and Australia for the presence/absence of each effector and assigned each individual to a multi-effector genotype. Diversity at each NE locus was determined by sequencing ~200 individuals. We found significant differences in effector frequency among populations and regions. We hypothesize that these differences reflect the presence/absence of the corresponding susceptibility gene in wheat cultivars. The population with the highest sequence diversity was different for each effector locus and never coincided with Fertile Crescent populations having the highest diversity at neutral loci. Coalescent analyses indicated that *P. nodorum* did not inherit NEs from a common ancestor, but instead acquired all three characterized NEs horizontally. Interspecific hybridization likely occurred between *P. nodorum* and its sister species, *P. avenaria tritici* 1, leading to a second horizontal transfer of all three NEs from *P. nodorum* to *Pat1*. We hypothesize that the horizontal acquisition of NEs was an important innovation that enabled the emergence of *P. nodorum* and *Pat1* as globally important wheat pathogens. There was no evidence for a fitness cost associated with the acquisition of these NEs.

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## Landscape epidemiology of emerging infectious diseases in ecological communities

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A principal challenge to studying emerging infectious diseases in wild pathosystems is a landscape dilemma: Our best empirical understanding of host-pathogen interactions occurs at fine scales, whereas pathogen invasions and management occur over broad spatial extents. The growing field of landscape epidemiology integrates concepts and approaches from disease ecology with the macroscale lens of landscape ecology, enabling analysis of disease across spatiotemporal scales in complex ecological settings. We review the status of the field and suggest analytical frontiers that demonstrate potential for advancement, concentrating on wild ecological communities. Concepts central to practicing landscape epidemiology are discussed, including spatial scale, static versus dynamic modeling, spatially implicit versus explicit approaches, selection of ecologically meaningful variables, and inference versus prediction. We highlight studies that have advanced the field by incorporating multiscale analyses, landscape connectivity, and dynamic modeling. Future research directions include understanding disease as a component of interacting ecological disturbances, scaling up the ecological impacts of disease, and examining disease dynamics as a coupled human-natural system.



## Lichens – a new source or yet unknown host of herbaceous plant viruses?

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Lichens are symbiotic associations of fungi with green algae or cyanobacteria. They have arisen independently several times within the *Ascomycota* and *Basidiomycota*. This symbiosis became with time one of the most successful life forms on Earth. Outside of the symbiotic algae and fungi, there are endophytic fungi, other algae, and lichen-associated bacteria present within lichen thalli. Till now, no lichen-specific pathogens have been reported among bacteria and viruses. Around 15 dsRNA viruses are known from *Eurotiomycetes* and another dsRNA and reverse transcribed ssRNA viruses from *Dothideomycetes* containing some lichenized fungal lineages. Algal viruses have been identified from less than 1% of known eukaryotic algal species but no virus has been found in *Trebouxia* or in *Trentepohlia* (Chlorophyta, Pleurastrophyceae, Pleurastrales), the most common green lichen photobionts. On the other hand, dsDNA viruses infecting related *Chlorella* algae are well known from freshwater phytoplankton.

However, high-molecular weight dsRNA isolated from different lichen thalli indicated us presence of ss or dsRNA viruses. A PCR-based search for viruses with genus-specific and species-specific primers resulted in amplification of genome segments highly identical with those of plant cytorhabdoviruses and with Apple mosaic virus (ApMV). The nucleotide sequence of the putative lichen cytorhabdovirus showed high identity (98%) with Ivy latent cytorhabdovirus. The nucleotide sequences of six Apple mosaic virus isolates from lichens showed high similarity with ApMV isolates from apple and pear hosts. The lichen ApMV isolates were mechanically transmitted to an herbaceous host and detected positive in ELISA 14 days thereafter, which support its infectivity on plants. We prepared axenic cultures of photobionts identified as *Trebouxia* sp. from this ApMV-positive lichen samples. All these cultures were positive for ApMV in RT-PCR test.

We suggest that lichens as a whole (or their photobionts, more specifically) could serve as reservoirs for viruses, despite that the way of transmission between different organisms is not clear. We showed that lichens could harbour several viruses simultaneously, as the plant cytorhabdovirus and ApMV were detected in the same host, also.

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## Evolutionary genetics of disease resistance in wild tomato species

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Resistance genes typically show evidence of rapid protein evolution in natural host populations. Variability at these loci may be the direct result of a co-evolutionary arms race between host and pathogen. However, the prevalence of pseudogenes also segregating in natural populations at well-known resistance genes indicates that the strength of natural selection likely fluctuates and these genes may be subject to bouts of relaxed constraint as well.

In my lab, we investigate the strength and target of natural selection within resistance genes present in natural host plant populations of *Arabidopsis* and wild tomatoes. Using a combination of population genetic studies of well-characterized pathogen resistance genes and functional studies involving protein expression, enzymatic assays, plant transformation and pathogen infection studies, we have determined how naturally occurring protein variation in host populations affects defense to pathogens at the individual level. I will describe our recent results from wild tomato species and how we are expanding these studies to include population genomic analyses to understand how resistance genes evolve as a result of host-parasite interactions.

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## The population dynamics of disease on short and long time-scales

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Observational evidence concerning the distribution of plant pathogen population sizes or densities as a function of time-scale or spatial scale is surprisingly thin, even for crop pathogens. For wild pathosystems we can only get indirect evidence from evolutionary patterns and the consequences of biological invasions. We have little or no evidence bearing on extermination of hosts by pathogens, or successful escape of a host from a pathogen. However, evidence over the last couple of centuries in crops suggest that the abundance of particular pathogens in the spectrum affecting a given host can vary hugely on decadal time-scales. This may be an artefact of domestication and intensive cultivation; understanding in a wider range of pathosystems would be very desirable.

Depending on dispersal method (for example, soil-borne mycelium or resting spores against aerially dispersed short-lived spores) we can consider pathogens as foraging in either time or space for new hosts. This situation can be formulated mathematically fairly easily – for example as SIR-type differential equation or difference equation models, and this has been the (successful) focus of recent work in crops. „Long-term“ is then discussed in terms of derived equilibria. However, both host and pathogen dynamics are driven by environmental factors as well as their mutual interactions, and both host and pathogen co-evolve, and evolve in response to external factors. We have virtually no information about the importance and natural role of higher trophic levels (hyperpathogens) and competitors, but they could induce long-scale fluctuations in the abundance of pathogens on particular hosts.

In wild pathosystems the host distribution cannot be modelled as either a uniform density or even a uniform distribution of fields (which could then be treated as individuals). Patterns of short-term density-dependence and the detail of host distribution are therefore critical to long-term dynamics. Host density distributions are not usually scale-free, but are rarely uniform or clearly structured on a single scale. In a (multiply structured) metapopulation with co-evolution and external disturbances it could well be the case that the time required to attain an equilibrium (if it exists) based on conditions stable over that timescale is longer than that time-scale. Alternatively, local equilibria may be reached fairly rapidly but the meta-population equilibrium be very slow; there is plausible support for this in some genomic parasites. In either case, meta-stability on various time-scales is a more relevant than equilibrium concepts in explaining observed patterns.

## Phylogeny, evolution and diversity of oomycetes - a global perspective

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Oomycetes are fungal-like organisms of the kingdom Straminipila, are related to diatoms and brown seaweeds, and have evolved several convergent traits with fungi of the kingdom Mycota. Oomycetes can be found in almost all marine, limnic and terrestrial ecosystems, where they play important roles as decomposers and pathogens. Currently about 2000 species of oomycetes have been described, most of which are plant parasitic.

The basal clades of oomycetes are containing mostly obligate biotrophic marine holocarpic parasites of algae, molluscs, crustaceans and nematodes. As there are saprotrophs and facultative pathogens among the most species-rich groups, the Saprolegniomycetes and the Peronosporomycetes, it can be assumed that oomycete diversity evolved from saprophytic or necrotrophic holocarpic organisms in a marine environment. Preliminary dating suggests the evolution of an ancestral oomycete in the Cambrian, and significant diversification in the Devonian and Carboniferous. Obligate plant parasitism has evolved more than once during oomycete evolution. While the white blister rust pathogens of the Albuginales apparently represent an ancient lineage, the downy mildews seem to have diversified comparatively recently from within the paraphyletic genus *Phytophthora*. The root of downy mildew evolution is probably within lineages parasitic to monocots, possibly grasses.

The vast majority of obligate plant parasitic oomycetes colonises short-lived angiosperms, in particular herbaceous plants. First analyses provide evidence that the evolution of diversity among the most species-rich group of oomycetes, the downy mildews, is not by long-term co-evolution and co-speciation, but rather by host jumping, subsequent radiation within the newly acquired host group and subsequent specialisation and speciation during shorter-term coevolution. In *Hyaloperonospora*, first insights into the molecular processes of the adaptation of pathogens to new hosts have been gained from comparative analyses within the *Hyaloperonospora arabidopsidis* species complex, which indicate that plant resistance genes do not necessarily protect the same defence reaction hubs in different species.

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## ***Microthlaspi* and *Hyaloperonospora* – a new model system for investigating natural plant-pathogen interactions**

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Members of the Brassicaceae are generally recognised as useful models for investigating functional and developmental genetics. In particular, research on *Arabidopsis thaliana* has led to fundamental insights into plant cellular processes. As a consequence, also pathogens of *A. thaliana* have received considerable attention, and several have had their genomes sequenced throughout the past five years. However, *A. thaliana* has several characteristics, which are not optimal for investigating plant-pathogen interaction genetics and ecology on a wider scale. Especially the often ephemeral nature of populations, the high dispersal potential by wind and human activity and the wide ecological niche with respect to soil types in disturbed habitats render it difficult to unravel range-wide patterns.

*Microthlaspi perfoliatum* is an annual Brassicaceae that disperses its comparatively large seeds by rain-splash, is usually found on slightly alkaline limestone-derived soils and forms relatively stable populations on rocky slopes with enough moisture throughout the year. Populations are usually comparatively stable and while the plant is commonly found also in vineyards and orchards, it is found much less frequently within urban areas and is less easily distributed because of its larger seeds.

*Hyaloperonospora thlaspeos-perfoliati* s.l. is an obligate biotrophic oomycete pathogen of *M. perfoliatum* and is regularly encountered in the host populations, depending on the geographic region and host population characteristics. It is closely related to *H. arabidopsidis*, which genome sequence has been released in 2010.

Throughout the past five years, we have built up collections of more than 5000 plant individuals from more than 400 populations throughout Europe and West Asia and have collected more than 100 pathogen strains. First range-wide investigations have shown that *Microthlaspi perfoliatum* s.l. populations are geographically structured, with several local lineages and one lineage that is present throughout the distribution range. Also *H. thlaspeos-perfoliati* s.l. populations are structured, and first infection trials on single plant lineages have revealed differential responses towards pathogen strains. Genome sequencing

and annotation of the about 320 Mb genome of the diploid type of *M. perfoliatum* s.l. and the 100 Mb genome of *H. thlaspeos-perfoliati* s.str. is currently under way and will offer an ideal tool for investigating evolutionary patterns in plant-pathogen molecular interactions and a basis for investigating the relationship between neutral and functional diversity.

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## Integrated evidence for co-evolution in natural host-pathogen associations

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Antagonistic coevolution is a critical force driving the evolution of host and pathogen diversity, yet the selective processes underpinning reciprocal adaptive changes are still not well understood. Thus, we have little knowledge of the relative importance of fluctuating selection vs. arms-race dynamics in maintaining polymorphism in host-pathogen interactions or how these are impacted by host resistance structure, pathogen life-history, and spatio-temporal environmental variation. We discuss these issues with a particular focus on the wild association between *L. marginale* and *M. lini* which we have studied for more than 20 years. We first provide an overview of epidemiological, experimental and modeling studies within a single metapopulation that reveal significant spatial structure in the distribution of resistance and infectivity. We then describe extensive cross-year epidemiological, infection and genetic studies of multiple wild host and pathogen populations which demonstrate asynchronous phenotypic fluctuations in resistance and infectivity among demes. Changes in allelic frequencies at pathogen infectivity loci, and in host recognition of these genetic variants, correlate with disease prevalence during natural epidemics. These results strongly support reciprocal coevolution maintaining balanced resistance and infectivity polymorphisms and highlight the importance of characterising spatial and temporal dynamics in antagonistic interactions. Finally, at larger regional scales, we discuss evidence for the existence of two genetically distinct pathogen lineages which differ in geographic ranges and mating systems, as well as key life history features that are likely to have a profound impact on coevolutionary outcomes. We conclude with some general comments and highlight some future research directions.

## Plant – virus co-evolution in wild brassicas

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Pathogens are key players in the ecology of higher plants and they have a significant impact on the structure of plant communities. RNA plant viruses provide an excellent model to study co-evolution of pathogens and their natural wild host communities due to their obligate dependency on host cellular machinery, small genomes, relatively high mutation rates and short generation cycles. Surprisingly little information is available on the population dynamics, evolution and ecology of viruses in their natural wild host plant communities.

We have been studying the co-evolution between *Turnip mosaic virus* (TuMV) and wild cabbage (*Brassica oleracea* ssp. *oleracea*) (Raybould *et al.* 2003). TuMV is a member of the *Potyvirus* genus, infects a wide range of cultivated and wild plant species (Walsh and Jenner, 2002). References to wild cabbage cultivation date back many centuries up to the ancient Greek and Roman empires and it has been suggested that the wild populations found in the U.K. were probably introduced at the time of the Roman occupation of Britain, or during Saxon invasions. Several populations exist in the U.K., some with provenance traceable back as far as 1551.

Different wild cabbage communities show big differences in the degree of diversity of the TuMV isolates infecting them. At one site there was very little genetic diversity and no detectable pathotypic diversity, whereas at another, distant site, there was a high degree of genetic diversity and some pathotypic diversity. Competition experiments were carried out between pairs of isolates to determine the relative fitness of the isolates in plants grown from seed collected from two sites.

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## **Abstracts**

## **Short Oral Communications**



## A predictive model for soil seedbank outcomes in the *Pyrenophora semeniperda*-*Bromus tectorum* pathosystem

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*Bromus tectorum* L. is a dominant winter annual weed in cold deserts of western North America. The generalist pathogen *Pyrenophora semeniperda* is abundant in soil seed banks of this grass, where it kills a fraction of seeds throughout the year following their dispersal in early summer. The pathogen engages in a race with host seeds for endosperm resources; pathogen success is negatively correlated with the speed of seed germination. Seed dormancy as well as timing and amount of precipitation inputs are critical variables in determining the level of pathogen success due to their influence on germination speed. We used this knowledge to develop a deterministic model to predict pathosystem outcomes (seed death versus seed escape), using seed bank data from 80 sites collected throughout the western United States over a 13-year period. Data from a subset of collections were used for model development. The response variable (killed seeds present in the spring seed bank) was regressed on multiple predictor variables (pathogen and host densities at seed dispersal, amount and timing of precipitation) to obtain equations for model predictions. Increased seed mortality was associated with high seed rain, high pathogen density and low autumn precipitation (which resulted in high seed bank carryover). Predicted and observed mortality was generally greatest on xeric sites due to lower autumn germination and subsequently greater carryover seed bank. We were able to observe and predict specific scenarios that produced very high mortality of *B. tectorum* seeds (i.e., repeated high pathogen and host densities coupled with high seed bank carryover) on xeric sites (where *P. semeniperda* killed up to 20,000/m or >90% of potential carryover seeds). On mesic sites, rapid germination of non-dormant seeds in autumn resulted in lower pathogen-caused mortality. Year-to-year variation in pathogen inoculum loads, host seed rain and weather patterns resulted in wide variation in this general pattern. These results support our hypothesis that the *P. semeniperda*-*B. tectorum* pathosystem is more significant on xeric sites, where a positive feedback loop between pathogen and host is created by a large carryover seed bank (i.e., containing a level of secondarily dormant seeds that are highly vulnerable to attack by the fungus) and results in higher inoculum loads at seed dispersal the following year.

## Ecological adaptation of the leaf rust fungi attacking *Hordeum spontaneum* in Israel

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Wild barley, *Hordeum spontaneum*, is the direct parent of cultivated barley. It is widespread throughout Israel: north of the 200mm rain line it forms extensive populations and south of it, wild barley occupies the beds of ephemeral streams.

In the more humid areas of north and central Israel, *H. spontaneum* is attacked each year by the barley leaf rust *Puccinia hordei*. The infected barley leaves become densely covered with urediniospore sori in the early winter (the growing season) and telia in the spring (end of the season). This rust is heteroecious and different species of the geophyte *Ornithogalum* (Liliaceae) serve as alternate hosts. When winter rains start, teliospores that have remained dormant on the dry barley leaves and stems during summer, germinate, forming basidia. In each basidium, the 2n nucleus undergo meiosis followed by mitosis, and four basidiospores are formed. The basidiospores (n) infect plants of the alternate host in the neighborhood, and each infection results in a pycnium of a given mating type in which pycniospores (n) multiply. Fertilization between the differently mating pycnial clusters is brought about by insects or rain drops. Aecia containing aeciospores (n+n) are formed, and the wind-blown aeciospores re-infect wild barley seedlings throughout December and January.

In the desert belt, *Puccinia hordei* is rare and the leaf rust that attacks wild barley is another heteroecious fungus, *Uromyces viennot-bourginii*, with *Bellevia eigii* (Liliaceae) as its alternate host. Here, too, teliospores germinate on oversummered dry barley leaves when there are winter rains or water stream, and basidia are formed. After meiosis only two binucleate basidiospores (n+n) are formed that infect leaves of the alternate host and lead directly to binucleate aecia and aeciospores. Absence of the pycnial stage shortens the life cycle of the rust by about three weeks, which is considered an adaptation to brief or single rain events. Moreover, insect or water-drop vectors for fertilizing spores become superfluous.



## Reciprocal virulence pattern among *Blumeria graminis tritici* isolates originating from domesticated wheat and its wild progenitor

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Many biotrophic fungi, such as wheat powdery mildew *Blumeria graminis tritici* (*Bgt*), are highly specialized pathogens and represent an advanced stage in the evolution of host specialization (Eshed and Wahl 1970; Wyand and Brown 2003). The eco-geographical regions of Israel and its vicinity are centers of diversity of the wild form of wheat, emmer wheat (Harlan and Zohary 1966). Emmer wheat is distributed there sympatrically with the domesticated wheat. Phenotypic screening of a wide and diverse collection of wheat germplasm with scores of isolates of *Bgt* demonstrated a pattern of specificity related to the host origin of *Bgt* (Eshed *et al.* 1994, Ben-David *et al.* 2008).

In order to investigate this pattern comprehensively, virulence of 60 *Bgt* isolates sampled from three host species (*T. durum*, *T. aestivum* and *T. dicoccoides*) in four eco-geographic regions within Israel was determined (data included virulence tests on 16 wheat lines: differential lines with *Pm* resistance genes, wild accessions and cultivars). The virulence scores were analysed using the software Virulence Analysis Tool (VAT, Schachtel *et al.* 2012). Isolates sampled from *T. dicoccoides* were significantly different from those collected from *T. aestivum* and *T. durum* (estimates of the Kosman distance were 0.529 and 0.398, respectively), whereas isolates originating from the common and durum wheat were much less distinct (the distance equals 0.205). Virulence complexities were considerably higher for *Bgt* from the domesticated hosts (10.8 and 9.1 for *T. aestivum* and *T. durum* isolates, respectively) compared to *Bgt* from wild emmer wheat (5.4). In addition, clear separation between almost all

isolates from wild (*T. dicoccoides*) and domesticated (*T. aestivum*, *T. durum*) hosts was established using the UPGMA dendrogram with regard to the simple mismatch dissimilarity between virulence patterns of *Bgt* isolates. Nevertheless, a few virulence phenotypes of *Bgt* detected on wild emmer were closely related to those from the domesticated wheats. Existence of such outliers might result from possible reciprocal gene flow of *Bgt* between cultivated wheat fields and natural populations of wild emmer. The eco-geographical factor (*Bgt* isolates sampled from different geographic regions within Israel) was non-significant in explaining the phenotypic variance. Based on the more comprehensive study reported here, we conclude that isolates of the wheat powdery mildew pathogen, belong to two specific and distinct forms, confirming an earlier suggestion (Eshed *et al.*, 1994).

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## New insights into the evolutionary history of geminiviruses derived through the discovery of divergent viruses isolated from wild plants

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During a large scale “*non a priori*” survey in 2010 of South African plant-infecting single stranded DNA viruses, a highly divergent geminivirus genome was isolated from an uncultivated spurge, *Euphorbia caput-medusae*. In addition to being infectious in *E. caput-medusae*, the cloned viral genome was also infectious in the cultivated hosts, tomato and *Nicotiana benthamiana*. The virus, named *Euphorbia caput-medusae* Latent virus (EcmLV) due to the absence of infection symptoms displayed by its natural host, caused severe symptoms in both of the cultivated plant species.

The genome organization of EcmLV is unique amongst geminiviruses and it likely expresses at least two proteins without any detectable homologues within public sequence databases. Although clearly a geminivirus, EcmLV is so divergent that we propose its placement within a new genus that we have tentatively named Capulavirus.

Using the most divergent set of geminivirus genomes ever assembled, we detect strong evidence that recombination has likely been a primary process in the genus-level diversification of geminiviruses. We demonstrate how this insight, taken together with phylogenetic analyses of predicted coat protein and replication associated protein (Rep) amino acid sequences indicate that the most recent common ancestor of the geminiviruses was likely a dicot-infecting virus that, like modern day mastreviruses and becurtoviruses, expressed its Rep from a spliced complementary strand transcript.

## Barley/Cereal yellow dwarf viruses in prairie and grasslands habitats in the US Pacific Northwest

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Barley/Cereal yellow dwarf viruses (B/CYDV) cause an important disease of cereals worldwide, including wheat. B/CYDVs are ubiquitous in *Poaceae* species and infect small-grain crops, rangelands and native grasslands. The wheat-producing region of northern Idaho and eastern Washington in Northwestern USA is characterized by fragmented native Palouse Prairie and Conservation Reserve Program (CRP) habitats embedded in an agricultural matrix. B/CYDVs have the potential to negatively impact native grasses that grow in these habitats and influence ecosystem dynamics. While these habitats harbor a diversity of *Poaceae* that potentially serve as hosts of B/CYDV and/or contribute to disease dynamics in cereal production, no studies have examined virus or insect vector prevalence among them. Our main objectives were to determine in which habitats and grass species B/CYDV occur and what B/CYDV and insect vector species are present, to contribute to a better understanding of wild plant pathosystems in the region. The diversity of *Poaceae* species in five Palouse Prairie remnants and six CRP fields adjacent to wheat fields was surveyed from October 2010 through June 2012. Tissue was collected from each grass species and aphids were sampled along transects in each habitat. A multiplexed PCR designed to detect six species within the B/CYDV complex was used to assess the samples. Collections yielded 28 grass species among the prairie and CRP remnants. Vector species collected include *Metopolophium dirhodum* and *Sitobion avenae*, of which the latter was determined to be infectious. B/CYDVs were detected in a variety of grass species from both prairie and CRP remnants. Potential implications of our findings for disease ecology and epidemiology within the cereal-based agroecosystems of the Palouse and for management of the endangered Palouse Prairie will be discussed.

## Diversity, phylogeny and host range of the *Pythium oligandrum* species complex

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Oomycetes are well-known as pathogens of crop plants, but they also contain several mycoparasites that are of potential use for fighting fungal and oomycete diseases.

*Pythium oligandrum* s.l. of the Peronosporales is known for its ability to parasitise a wide array of fungi and oomycetes, including *Phytophthora infestans*, the causal agent of potato late blight. But so far, there are no detailed investigations into this species complex, with respect to aspects of genetic divergence and host spectra of *P. oligandrum* strains. In order to elucidate the phylogeny, physiology and host range of *P. oligandrum* s.l. several investigations were carried out. Growth trials with different temperatures and on media with different carbon sources as well as infection trials with economically important fungi were carried out. For those trials different *Pythium oligandrum* strains were chosen as well as strains of the close relative, *P. dissotocum*.

The strains of *Pythium oligandrum* fall into distinct groups, based on sequence data. These groups though, did show only minor differences in their overall growth ability, but some strains within these groups showed different growth on some carbon sources. Some colony morphology differences were found between phylogenetic groups. . The infection trials revealed only minor infectivity to the fungi and oomycetes tested for most of the *P. oligandrum* s.l. strains. This provides evidence for facultative mycoparasitism in a poor nutrient situation in some strains and predominant feeding as saprotrophs. This is also supported by the wide spectrum of carbon sources that *P. oligandrum* s.l. strains were able to use.

Although there might be strains within the species complex that are predominantly mycoparasitic, and possibly also some host specialization, as there

were some differences in carbon source usage, the current investigations rather supports *P. oligandrum* as a competitive saprotroph that possibly has a beneficial effect on plant growth by outcompeting potential necrotrophic pathogens in the rhizosphere.

## Exposure to low water potentials and seed dormancy favor the fungus in the *Pyrenophora semeniperda*-*Bromus tectorum* pathosystem

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Water availability strongly influences growth and development of fungal pathogens as well as their plant hosts. In semi-arid regions of the Western United States, the ascomycete seed pathogen *Pyrenophora semeniperda* can kill large fractions of the *in situ* seed bank of *Bromus tectorum*, a winter annual native to Eurasia that has successfully invaded millions of hectares of former shrub-grasslands. In this paper we report on outcomes for this pathosystem under a variety of manipulated water potential and temperature environments, for both dormant and non-dormant seed populations. Water potentials were controlled using solutions of polyethylene glycol (PEG) or controlled humidities above saturated salt solutions. Inoculated dormant seeds were virtually always killed by the fungus, except when seeds were continuously hydrated at low temperature (5°C). With continuous hydration in free water (0 MPa), non-dormant seeds germinated in 2-4 days and escaped death from the fungus. Visible stromata (evidence of seed death) required at least 14 days under optimum conditions (20°C, 0 MPa). Exposure to negative water potentials achieved continuously through PEG or intermittently through hydration-dehydration-rehydration treatments, rendered non-dormant seeds much more likely to be killed by the fungus. Treatments that progressively lengthened the time required for germination (e.g., imbibition followed by drying at – 4 MPa followed by rehydration) resulted in a corresponding increase in seed mortality (e.g., up to 90% killed seeds). Both pathogen and host remained tolerant of desiccation following imbibition for up to 24 hours. However, when comparing disease development and germination progression at low water potentials, the pathogen made more progress. Thus, exposure to low water potentials favors the pathogen.

Based on our results, we have proposed the range of likely outcomes for seeds that become infected at different times during the year. With summer infection, seeds are still in a state of primary dormancy and are killed across a range of water potentials. With autumn infection, seeds escape death with rapid germination or are killed if radicle emergence is delayed by intermittent

hydration. In winter/spring, secondarily dormant seeds are killed by the fungus. Thus, the only likely scenarios where seeds escape death include absence of infection (autumn, spring, or following autumn, germination) or infection in autumn when seeds germinate rapidly.

## Population structure and biology of the grape powdery mildew fungus, *Erysiphe necator*, at its center of diversity

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Eastern North America is considered the center of diversity for many *Vitis* species and for the grape powdery mildew pathogen, *Erysiphe necator*. However, little is known about populations of *E. necator* from wild and domesticated *Vitis* species in the eastern USA. We determined the phenotypic variation in pathogenicity, and aggressiveness among 65 *E. necator* isolates from five wild and domesticated *Vitis* species from diverse geographic regions. To test pathogenicity, we inoculated four wild *Vitis* species, and the European wine grape, *V. vinifera*, with the collection of *E. necator* isolates. *Vitis rotundifolia* (muscadine grapes) was the only host species on which complete host specialization was evident; it was only susceptible to isolates collected from *V. rotundifolia*. All isolates, regardless of source host, were pathogenic on the other *Vitis* species. We found no differences in the aggressiveness components among isolates from different source hosts when inoculated on *V. vinifera*, which is highly susceptible to powdery mildew. However significant variation was found among isolates on the more resistant *V. labrusca* hybrid variety ‘Niagara’. Isolates from the wild species *V. aestivalis* were the most aggressive, while isolates from *V. vinifera* were not more aggressive than isolates from other source hosts. These variations in aggressiveness were also associated with high genetic diversity of the fungal populations in eastern USA which was much greater than in Europe. In addition, genetic differentiation was detected between a) isolates from *V. rotundifolia* and isolates from other *Vitis* hosts and b) populations from the northeastern vs. southeastern USA. The results imply that breeders who use wild American *Vitis* spp. in their breeding programs should also use *E. necator* isolates from wild species to screen for resistance.

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## Secondary dormancy of seeds in relation to the *Bromus tectorum*-*Pyrenophora semeniperda* pathosystem

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*Bromus tectorum* is a facultative winter annual that has invaded millions of hectares in western North America. The fungal pathogen *Pyrenophora semeniperda* can kill a large fraction of *B. tectorum* seeds in wildland ecosystems. In this pathosystem the outcome is often determined by the speed of seed germination. Fast-germinating seeds generally escape death by the pathogen, while slow-germinating seeds are killed. In this paper we extend previous efforts to describe the pathosystem by characterizing the secondary dormancy acquisition of *B. tectorum* seeds (i.e., the process whereby non-dormant seeds return to a dormant state resulting in increased vulnerability to the fungus). Laboratory and field experiments were conducted to assess the induction of secondary dormancy in *B. tectorum* seeds. In the laboratory, seeds were exposed to low temperatures and a range of water potentials. In the field, seeds were placed in the seed bank in autumn, retrieved monthly and dormancy status determined. Seeds incubated in water at 5°C germinated within 7 days. However, approximately 75% of seeds incubated at -1.0 MPa became dormant. In the field, seeds were placed in the seed bank in late autumn, retrieved monthly and dormancy status determined. The field study confirmed the laboratory results; ungerminated seeds became increasingly dormant through the winter. These data suggest that secondary dormancy is much more likely to occur at xeric sites, due to unpredictable autumn moisture. Also, efforts to use *P. semeniperda* for biological control will likely be more successful where precipitation is often insufficient for complete seed germination.



## Knowledge of plant virus biodiversity and ecology is required for prediction of virus disease epidemics: an antipodean perspective.

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Knowledge of the virosphere is required to predict new virus pathogens and to potentially maximize the benefits of virus infections. It is thought that plant viruses have co-evolved with their wild hosts prior to the domestication of crops (Lovisolo *et al.* 2003), possibly providing some benefit to the host; but when new hosts are encountered may become pests. Viruses may also evolve to become pathogenic on a previously asymptomatic host plant or a new host plant, possibly facilitated through a change in vector interactions or cultural practice.

New encounters of viruses and hosts can occur at crop, weed and native plant boundaries, especially when grown in close proximity, potentially causing damage in the new host. For example, Luo *et al.* (2011) reported that, in the South-western (SW) Australian floristic region, the potyvirus *Hardenbergia* mosaic virus was transmitted to *Lupinus angustifolius* when this crop was planted in proximity to the native legume host *Hardenbergia comptoniana*. The reverse has also been observed in SW Australia, where *Bean yellow mosaic virus* has been naturally transmitted from crop legumes to the native *Kennedia prostrata* (Webster *et al.* 2007). Such infections are of concern, especially if the native plant is an endangered species. For example, *Turnip mosaic virus*, *Cauliflower mosaic virus* and *Turnip yellows virus* have been found infecting the endangered New Zealand species, Cook's scurvy grass (*Lepidium oleraceum* agg.) (Van Vianen

*et al.* 2013). The introduction of exotic viruses can clearly lead to infection of native plants, and a potential decline in biodiversity.

The process of domestication can reveal new virus pathogens. Ecological changes that arise when plants are shifted from a native ecosystem to an agrosystem, such as monoculture, may lead to viruses becoming pathogenic where previously they were not. Identification in New Zealand of five kiwifruit adapted viruses appears only to have occurred since the crop's recent domestication in monocultures (Blouin *et al.* 2013). As yet, most of these viruses do not cause symptoms and no virus vectors have been identified in New Zealand. In South Australia, thrips species responded differently (e.g. relative abundance) to plant taxa, their proximity to crops and the season (Schellhorn *et al.* 2010). Thus, virus spread may only occur when the insect vector occurs at the optimal density, at the preferred time of year. Without these conditions, virus spread may not occur.

Knowledge of the biodiversity of viruses within a region, and an understanding of virus ecology is needed to determine the relationship of new viruses with existing and potential new plant hosts and vectors. Viruses should be recognized as part of the wider ecosystem and, while they may not cause disease, may result in host adaption that would otherwise be impossible, e.g. drought tolerance (Xu *et al.* 2008). It is also conceivable that climate change will aggravate disease epidemics and hasten the emergence of new diseases (Webster *et al.* 2007). Notwithstanding the many scientific barriers, once a virus pathosystem is identified and its epidemiology understood, models can be used to predict virus disease epidemics (Jones *et al.* 2010).

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## Macroevolutionary strategy of downy mildew: A case study of *Bremia* and Asteraceae

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Oomycete plant pathogens include necrotrophic, hemi-biotrophic, and obligate biotrophic species causing devastating diseases on a wide variety of economic and wild plants. Having the highest species diversity and host-specificity in Oomycetes downy mildews are useful as model systems for understanding the coevolution between obligate pathogen and host plant. An increasing number of genome sequences and molecular interaction studies are revealing more details of the oomycete–plant interactions at molecular level, but there is still hardly any knowledge of how downy mildews evolve with their host plants from a macro-evolutionary viewpoint.

The genus *Bremia*, a well-known downy mildew with *B. lactucae* which causes one of the most important diseases of lettuce (*Lactuca sativa*) contains species parasitizing three subfamilies of the largest flowering plant family Asteraceae (Compositae), Asteroideae, Carduoideae, and Cichorioideae. To uncover the degree of evolutionary congruence between the parasite *Bremia* and the host Asteraceae, 181 sequences of mitochondrial (cox2, cox1, cox2-cox1 spacer) and nuclear (18S, 28S, ITS1, RxLR11) for *Bremia* and of matK and ITS genes for Asteraceae were obtained. All cophylogenetic analyses using different models and algorithms agree that phylogenies of the *Bremia* and the Asteraceae are significantly congruent. It also suggests that there are two dominant strategies in the evolutionary history of *Bremia*: host-jumping and co-divergence. The strong congruence between *Bremia* and Asteraceae extremely biased towards terminal branches is most likely due to pseudo-cospeciation rather than cospeciation, resulting from parasite radiation and speciation following host-jumping, which have occurred between phylogenetically close or distant plants within Asteraceae, long after the radiation of the host plants. This contradicts an earlier hypothesis that *Bremia* diverged and evolved contemporarily according to host phylogeny. Instead, it raises a new hypothesis that at the first stage *Bremia* may have jumped to and diversified on the subfamily Cichorioideae but failed



to colonize on Asteroideae and Carduoideae, and long after the radiation of *Bremia* on the Cichorioideae there was a host-jumping from Cichorioideae onto Carduoideae. The second jumping from Carduoideae from Asteroideae occurred recently, and did not yet begin a dynamic coevolutionary adaptation toward host plant specificity. The present study provides not only obvious evidence of coevolution of downy mildews and host plants at the macro-evolutionary level, but also that the evolutionary strategy used by the biotroph Oomycete for successful survival and diversification on changing host plants is also much more complex and dynamic than previously thought.

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## Matrix models of plant infected by systemic pathogen show low impact of the disease and high influence of the environment

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The diseases of wild plant species are thoroughly researched but their long-term effect at the population level is often unknown. To acquire such information one can use matrix models that allow for determining population characteristics like population growth rate, stable stage distributions and importance of the stages. I studied populations of common plant *Falcaria vulgaris* infected by systemic rust fungus *Puccinia sii-falcariae*. I followed healthy and systemically infected plants in four populations over 4-5 years to calculate population characteristics for both whole populations and for only the healthy plants and their transitions. *Falcaria* has a broad range of living conditions so I chose two types of populations to describe the differences in life cycle of steppe and ruderal populations. Prevalences, sizes and other factors of 40 populations were also surveyed to find effects of environment on prevalence of the disease.

There was considerable variation both within and among populations. Deterministic growth rates differed between population types with ruderal populations growing in size and steppe populations either around equilibrium or dropping in size. The growth rate of healthy part of the population was significantly larger than the growth rate of the whole population only in one year and locality. However, overall stochastic growth rate of all populations was higher for healthy matrices. The stable stage distributions changed substantially among individual years suggesting changes in conditions favourable for various transitions. The steppe populations had balanced amounts of plants projected in healthy and in infected stages while ruderal populations had most plants projected in the healthy stages. The model was also able to predict pathogen prevalences comparable to values surveyed in the field. The results suggest that although only minor effect of the disease on population growth was found, matrix modelling can still provide insights into the disease effects using stage distributions and other tools. The landscape survey confirmed effect of environment on pathogen prevalence.

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## **Smut fungi are model systems for investigating the evolution of pathogenicity– insights from the genome of *Melanopsichium pennsylvanicum***

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Smut fungi are well-suited model organisms for understanding the ecology and evolution of plant pathogens, as they are strictly biotrophic, yet cultivable. Here we report the genome sequence of *Melanopsichium pennsylvanicum*, responsible for gall smut on *Persicaria* species. It is closely related to other smut fungi like *Ustilago maydis* and *Sporisorium reilianum*, which are model systems for molecular plant-fungal interactions. To explore the evolutionary events that may have occurred during host adaptation, the assembled genome of *M. pennsylvanicum* was compared with another three available Ustilaginales genomes, which are *Ustilago maydis*, *Sporisorium reilianum*, and *Ustilago hordei*. Annotated 6280 protein-coding genes identified and 5277 orthologs found for all four genomes. Genes gained and lost during the evolution were estimated and we found 301 genes were lost in *M. pennsylvanicum* and 361 genes has been gained. In the *M. pennsylvanicum* genome, we could find 418 putative secreted effector proteins, 545 in *U. hordei*, 629 in *U. maydis* and 633 in *S. reilianum*. On average 20% of the pathogenicity clusters of all four genomes were conserved among all the species. *Melanopsichium pennsylvanicum* showed a lower conservation of pathogenicity clusters, and 12 were lost. Whole genome screening of pseudogenes revealed highest number of pseudogenes in the genome of *U. hordei*, which was followed by *Ustilago maydis*, *Melanopsichium pennsylvanicum* and *Sporisorium reilianum*. To investigate adaptation and selection pressure, genome-wide identification of positively selected genes were performed comparing all the four Ustilaginales genomes. It was revealed that the *M. pennsylvanicum* genome has the highest number and proportion of genes under positive selection. In general, putative effectors showed a higher proportion of positively selected genes than non-effector genes. This is in line

with the fact that *M. pennsylvanicum* evolved as the result of a host jump across angiosperm subclasses and had to adapt to a completely new host environment. Our results demonstrate that the smut fungi might be a useful group for not only investigating molecular, but also evolutionary mechanisms with respect to pathogenicity and host-jumps.

## Evolution of *Hyaloperonospora* effectors: ATR1-effector genes in sister species of *H. arabidopsidis*

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The species of *Hyaloperonospora* belong to the class of the oomycetes, a group of eukaryotic organisms with a broad distribution, including a number of significant plant pathogens of economically important crop plants. Like other plant pathogens, oomycetes manipulate their hosts by secreting an arsenal of proteins, collectively known as effectors. Within the context of host–pathogen interactions, effectors are molecules, typically proteins, secreted by pathogens that target plant molecules and alter plant processes (1).

Secreted by the pathogen, effector proteins modulate plant innate immunity and enable parasitic colonisation and reproduction (1-3). Although effectors function primarily in virulence, they can also elicit innate immunity in plants. In such cases, effectors have an avirulence (Avr) activity. Effectors with avirulence activity trigger resistance of *A. thaliana* mediated by RPP-proteins (RPP stands for Recognition of Peronospora parasitica, a name previously used for *Hyaloperonospora arabidopsidis*).

Deciphering the virulence and avirulence activities of effectors is crucial for understanding how pathogens interact and co-evolve with their host plants and has become an emerging topic in the field of oomycete and fungal pathology.

Despite of intense research efforts devoted to the *A. thaliana*/*H. arabidopsidis* pathosystem, there are currently no studies related to the immediate sister species of *H. arabidopsidis*. Six diverse clades could be recognized within the genus *Hyaloperonospora* (4). Clade 3, which includes *H. arabidopsidis*, comprises closely related pathogens of a rather diverse assemblage of hosts within the Brassicaceae (e.g. *Microthlaspi perfoliatum*, *Arabidopsis thaliana*, *Erophila verna*) but also from the Resedaceae (e.g. *Reseda lutea*) (5).

Infection trials were done to confirm that these species are separate. No sporulation on any *A. thaliana* ecotype, even on the immune-deprived Ws-eds1 mutant, could be observed for *H. thlaspeos-perfoliati* or *H. crispula*.

In this work we report the identification of ATR1-homologous effectors in 2 sister species of *H. arabidopsidis*: *H. thlaspeos-perfoliati* and *H. crispula*.

Using a transient *Agrobacterium tumefaciens* mediated protein expression system in tobacco we tested the capability of *A. thaliana* RPP1 to recognize the ATR1s from different isolates of *H. thlaspeos-perfoliati* and *H. crispula*. Co-infiltration of these effectors with RPP1-WsB in tobacco showed that these effectors are not recognised by the resistance protein from *Arabidopsis*.

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## The effect of co-infection to pathogen epidemiology in *Plantago lanceolata* – *Podosphaera plantaginis* –interaction

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Pathogens occur often as co-infection where several genetically different strains infect same host. Co-infection may lead to competition when the hosts' resources are limited. Under competition the optimal reproduction rate can be altered compared to the optimal rate in situations without competition. However, the effect of co-infection on realized epidemiological dynamics is poorly understood.

Here, our aim is to understand how competition affects the evolution of virulence and epidemiology of diseases. We surveyed natural populations of *Plantago lanceolata* infected by *Podosphaera plantaginis* in the Åland Islands, southwest of Finland. The pathogen populations were genotyped to test the prevalence of co-infection and potential links epidemiological consequences were studied.

We also used an experimental approach to pinpoint the effect of co-infection in genetically controlled hosts that vary in their resistance levels. We studied how *Po. plantaginis* performance differs in co-infection and single infection using host populations with qualitative, quantitative and susceptible resistance strategies in semi-natural conditions. We followed the epidemics throughout the growing season and genotyped the pathogen populations at the end of the season.

The experimental populations supporting co-infection had always higher disease prevalence than single strain pathogen populations, demonstrating that within host dynamics can be linked to the spread of the disease. Host resistance strategy affected epidemics, and the outcome of competition. We observed high levels of co-infection in the natural populations, as well as variation in the prevalence of co-infection between host populations, plants and within plant scales. The patterns observed in the natural populations showed increased levels of disease in co-infected plants and populations, suggesting that results obtained under semi-natural conditions are also important determinants of disease dynamics in the natural metapopulation.

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## Diversity of *Arabidopsis thaliana* in Italy as inferred from next-generation genotyping in relation to biotic and abiotic factors

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As a model plant *Arabidopsis thaliana* is increasingly used in functional, evolutionary and ecological studies. It is thus important to understand patterns of intraspecific genetic diversity and identify environmental factors that shape them, including the presence and absence of pathogens. Although several studies address the genetic diversity of *Arabidopsis thaliana* throughout its distribution range, biotic and abiotic factors and their role in generating diversity patterns on regional or even local scales are poorly characterized. Using next-generation sequencing of restriction-site-associated DNA (RAD) tags, we genotyped 338 *Arabidopsis thaliana* individuals sampled in 40 populations from the Italian Peninsula. Analysis of 3509 SNP markers showed significant population structure across Italian Peninsula, with a north-south diversity gradient. Analysis of these 40 populations with different model-based clustering algorithms inferred four genetic clusters. North and Central Italy share a single genotype, which is also commonly occurring in North Europe, Turkey and Armenia. South Italy and Sicily have three genotype groups each, with one genotype group present in one or the other. A rural-urban gradient of genetic diversity is observed in South Italy and Sicily, with rural populations significantly more heterogeneous than urban populations. Infected and non-infected populations differed in their genetic diversity, with healthy populations being more diverse than infested ones. However, possibly due to the small sample size, this trend did not show high significance values. Our results suggest no isolation by distance among populations either over entire Italian population, or three sub regions. Pattern of genetic variation is not observed to be associated with altitudinal and temperature gradient. Monthly precipitation during early growing season was found to be significantly correlated with genetic diversity, a factor that probably also influences the presence of oomycete pathogens.

## **Molecular tools for the study of the interaction between *Podosphaera plantaginis* and *Plantago lanceolata* in the Åland metapopulation**

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The interaction between the host plant *Plantago lanceolata* and its obligate fungal pathogen *Podosphaera plantaginis* has been intensively surveyed in the Åland archipelago, with 12 years occurrence data in more than 3 000 host populations. Such long-term data show that the pathogen persists as a metapopulation with local alternation of colonization and extinction events allowing regional persistence.

The aim of my post-doc project was the design and use of molecular tools for the study of *Po. plantaginis* population dynamics and evolution. Firstly, transcriptome sequencing of a pooled sample allowed the design of a set of 27 SNP (*Single Nucleotide Polymorphism*). These molecular markers were used to estimate within-population levels of mixed-genotype infection, revealed as high as 40% of the collected infected leaves. At the spatial scale of the archipelago, genetic diversity was found to increase with the age of the local population, an expected pattern under metapopulation theory. At the small spatial scale of the host population, genetic markers were used to distinguish the different strains circulating during one seasonal epidemic. The relative contribution of the different strains to the disease progression was assessed from field data and then compared to the pathogen performance estimated from experimental infections performed in controlled conditions. Finally, a molecular detection test of a fungal hyperparasite (*Ampelomyces* spp.) of the focal powdery mildew species was designed. Application of this test to field samples collected throughout the archipelago revealed the potential role of the hyperparasite on the population dynamics of its mycohost.

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## **Abstracts**

## **Posters**



## Population genetic diversity and structure of *Podosphaera plantaginis* in Åland Islands

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Numerous aspects of a fungal life cycle can affect the evolutionary trajectory of a pathogen species. Amongst them, in a fragmented landscape with various host resistances, ability to recombination and capacity of dispersion are two of the most important characteristics that can influence the coevolution between the two interacting species in a variable spatiotemporal framework. The *Podosphaera plantaginis* – *Plantago lanceolata* population dynamic has been studied for 10 years in the Åland archipelago, in South-Western Finland. It has been well established that this natural pathosystem undergo a metapopulation dynamic. Evidences of local adaptation of the pathogen at the population and metapopulation levels have been detected and it has been recently demonstrated that *Po. Plantaginis* is a homothallic species. These information indicate that some dispersion occurs in the system and that the powdery mildew has the ability of reproduce sexually. Nevertheless, the extent of spore dispersion and commonness of sexual outcrossing remain unclear.

Using molecular biology tools and an extensive sampling strategy of Åland metapopulation, we have studied the genetic diversity and structure of this pathogen species during two consecutive years. High level of coinfection, change in the multilocus genotype composition of *Po Plantaginis* populations between years and low level of linkage disequilibrium between loci support frequent sexual recombination across the metapopulation. The occurrence of identical multilocus genotypes in distant *Pl. lanceolata* patches and the absence of a strong genetic structure in the archipelago are the landmark of important gene flow at the investigated spatial scale during the epidemic season.

## *Phytophthora* spp. invasions in post-communist economies – the example of the Czech Republic

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*Phytophthora* spp. belong among most important and well known pathogens of ornamentals and forest trees in Europe. Many of them are considered to be alien or cryptogenic and recently invading in the area. The number of introductions depends on the globalization and level of imports of goods (Desprez-Loustau 2009, Santini et al. 2013). Libely, the isolation of Central- and East European economies partially protected them from the invasion. The diversity and distribution of *Phytophthora* spp. in the Czech Republic support this claim.

The intensive investigation of *Phytophthora* spp. diversity in different environments (from ornamental nurseries, garden centres to riparian and forest stands) started in the area in 2006. Hundreds of *Phytophthora* isolates have been collected and deposited in the culture collection yet (the list of selected strains is accessible at [www.vukoz.cz](http://www.vukoz.cz)). The distribution of the collected strains was analysed.

The 21 taxa have been found in the area since 2006. The 15 taxa from this amount (including *P. multivora*, i.e. *P. citricola* p.p.) are listed in the database of European invasive forest pathogens (Santini et al. 2013) – and only one species of them (*P. polonica*) is considered to be native to Europe. *P. alni alni*, and *P. plurivora* can be considered as the most frequent and important in the area of the Czech Republic. The other presented species are *P. palmivora* (alien to Europe), *P. roseacearum* and *P. gregata* (both probably cryptogenic) and *P. gallica*, *Phytophthora lacustris*, *P. taxon* oaksoil. The last three species were found only in natural environments – forest and riparian stands and they are undoubtedly native. Finally, it can be concluded that 4 taxa are native (19%) and 17 (81%) are probably alien or cryptogenic (!).

The analyse of the distribution of 17 alien or cryptogenic taxa shows that 6 taxa (28.6% of total number of taxa) are more or less regularly distributed in natural stands: *P. a. alni* and *P. a. uniformis* (hybrids; *P. a. uniformis* is probably alien), *P. plurivora*, *P. multivora* (*P. multivora* is probably alien), *P. gonapodyides* and *P. cambivora*. Their introductions are probably older (*P. plurivora*) or their natural spread is extraordinarily effective (*Paa*). The 6 other species (28.6%) are regularly distributed in anthropogenic environments and only occasionally in invisable riparian stands. The distribution of the last 5 pathogens (23.8 %): *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. palmivora* and *P. ramorum* is scarce and limited to nurseries, gardening centres and ornamental plantings. Their introductions are apparently of recent, post-revolutional origin.



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## Landscape typology of Vltava River Basin and impact of *Phytophthora alni* subsp. *alni* invasion

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*Phytophthora alni* subsp. *alni* (*Paa*) belongs to the most serious invasive *Phytophthora* pathogens damaging European native trees. Its invasion started in western Europe in the 80s in the last century and than the pathogen has spreaded in important part of Europe. *Paa* caused also heavy losses in alder riparian stands, mixed ash-alder alluvial forests and alder carrs in western part of the area in the Czech Republic and killed tens of thousands black and grey alder trees. Especially the Vltava River basin (VRb) is highly affected by the pathogen, because alders create ca 42% of trees in its riparian stands.

However the pathogen is heterogenously dispersed in the landscape – some landscape types, environments and stands are highly affected by the pathogen whereas others are less affected or not in the last. It would be very beneficial to predict the potential impact of the pathogen in the different landscape types in the area.

The information about pathogen distribution in the Czech Republic and in other invaded European areas were summarised and evaluated. The important environmental factors influencing the disease impact were selected. The following independent factors were used in statistical model: 1. density of river system (95% findings of *Paa* in the Czech Republic were related to water; Gibbs et al. 1999 etc.); 2. density of forest alder plantings (see *Paa* invasion in forests in neighbouring Bavaria – Jung et Blaschke 2004); 3. vertical heterogeneity (relation of *Paa* to lower, slow reaches in broad valleys and plains – Thoirain et al. 2007, etc.) and 4. average January temperature (relation to temperature – Aguayo et al. 2012, Černý et Strnadová 2012, Černý et al. 2012, Chandelier et al. 2006; strong correlation to altitude).

The VRb area was divided by rectangular grid (2.5 × 2.5 km), the average values of four selected environmental variables were computed for all quadrates using GIS and standardized (density of river system was weighted; Statistica 10.0). The quadrates were clustered into 6 groups according to their environmental similarity, the average values of all four variables were computed for each cluster and rank transformed. The ranks were averaged and the ranking of clusters hypothetically corresponding to the *Paa* impact was assembled.

The landscape of VRb was divided into the six different types. The most potentially affected was landscape of South Bohemian flat basins with high

density of water courses. The two other potentially highly affected landscape types were flatlands, depressions and valleys of smaller rivers with low altitudinal heterogeneity in middle altitudes and warm valleys and plains of broad rivers in low altitudes. Potentially the less affected landscapes were uplands with higher vertical heterogeneity and sparse water systems. The least affected were mountain landscapes with high vertical heterogeneity and usually cold climate (characteristically Šumava Mts.) and vice versa dry, warm and usually flat landscapes with low frequency of water courses and alder plantations (typically Pražská plošina plateau). It can be stated that more than ½ of the Czech landscape is highly susceptible to *Paa* invasion. The outcomes will be verified in field study during following years.

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## Phylogenetic analysis, fumonisin production and pathogenicity of *Fusarium fujikuroi* strains isolated from rice in the Philippines

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*Fusarium fujikuroi* Nirenberg is a maize and rice pathogen causing important agricultural losses and produces fumonisins, mycotoxins which pose health risk to humans and farm animals. However, little information is available about the phylogenetics of this species and its ability to produce fumonisins in rice. We studied 32 strains isolated from rice in the Philippines and performed a phylogenetic analysis using the partial sequence of the Elongation Factor 1 alpha (EF-1 $\alpha$ ) including isolates belonging to closely related species. Fumonisin B<sub>1</sub> (FB<sub>1</sub>) production was analyzed in seven-day-old cultures grown in fumonisin-inducing medium by an ELISA-based method and by Real Time RT-PCR using primers for *FUM1* gene, a key gene in fumonisin biosynthesis.

Nucleotide diversities per site ( $\pi$ ) were  $0.00024 \pm 0.00022$  (standard deviation) for the 32 *F. fujikuroi* from the Philippines and  $0.00189 \pm 0.00143$  for all the 34 *F. fujikuroi* strains, respectively. *F. fujikuroi* isolates grouped in one cluster separated from the rest of isolates belonging to the closely related *F. proliferatum* and showed very low variability, irrespectively their geographic origin. The cluster containing strains of *F. proliferatum* showed higher intraspecific variability than *F. fujikuroi*. Thirteen out of the 32 strains analyzed were FB<sub>1</sub> producers (40.62%) with production ranged from 0.386 ppm to 223.83 ppm. All isolates analyzed showed *FUM1* gene expression above 1, and higher than the C<sub>T</sub> value of the non-template control sample. Both seedling stunting and elongation were induced by the isolates in comparison with the control. *F. fujikuroi* are distinct from *F. proliferatum* isolates based on phylogenetic analysis and are potential fumonisin producers because all are positive for *FUM1* gene expression. No relationships between fumonisin production and pathogenicity could be observed.

### Acknowledgements

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## Environmental factors influencing the distribution of beet cyst nematodes in the wild

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*Heterodera schachtii* and *Heterodera betae* are two cysts nematodes causing severe damages in sugar beet (*Beta vulgaris*) crop every year. Several control methods exist but resistant varieties are often the most cost-effective alternative to pesticides. New varieties are currently developed with resistances coming from a wild relative species of beet, *Beta maritima*. Such resistance is probably derived from co-evolved gene for gene interactions that must still remain effective in natural plant nematodes communities. As a result, the analysis of the genetics and ecology of these original interactions may illustrate features that can be used to predict durability of resistance, in a context of possible ongoing gene flow between wild and field populations. Indeed, *Heterodera betae* and *Heterodera schachtii* can develop on the wild beet. Wild populations are thus supposed to have evolved historically on this wild host and have recently been transferred in cultivated fields. However, there is no data about occurrence and characteristics of populations of both species in wild ecosystems.

We carried out a hierarchical sampling of the three species along the Atlantic coastline, which the wild beet recolonized after the Last Glacial Maximum (Villain 2007), to give a first description of the occurrence of both nematodes in the wild and identify some environmental factors influencing their distributions on *B. maritima*. *Heterodera betae* is preferentially distributed in connected and warm environments of southern part of Europe. Those observations do not support the hypothesis of a recent appearance of this species in The Netherlands. *Heterodera schachtii* mainly occur in colder environments of the northern part of Europe, contrasting with its biological preferences (Maas and Heijbroek 1982). Habitats of this nematode are also characterized by crop field proximity. Finally, this study indicates that wild and field nematode populations can potentially interact together (gene flow) highlighting the necessity to characterize genetically and phenotypically those populations to evaluate the impact of wild populations on resistance management. The genetic characterization of wild populations of *H. betae*, *H. schachtii* and *B. maritima* are currently in progress and results will be developed in further studies. Phylogeographic analyses on these three species will be carried out to make inferences and reconstruct their evolutionary histories, which should bring elements to improve predictions of durability of resistances.

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## Leaf- and stem rust resistance of wild grasses and bread wheat relatives

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Novel sources of disease resistance are needed to cope with the spread of new races of pathogens that overcome resistance genes in the grown cultivars. Wild grasses and bread wheat relatives are important sources of new resistance genes for bread wheat. The Gene Bank of the Crop Research Institute, Praha-Ruzyně contains a large collection of wild grasses and bread wheat relatives. The aim of our work was screening of that collection for resistance to leaf- and stem rust of wheat.

Tests were carried out in the greenhouse at temperature 20±2°C. Plants (4-6 from each accession) were inoculated at the stage of 2-4 leaves with water suspension of rust urediospores. Inoculated plants were kept in closed glass cylinders for 24 hours to provide high air humidity. Infection types were evaluated two weeks after inoculation according to Stakman et al. (1962). Three different leaf rust races and three different stem rust races were applied in the tests. Tested were accessions of the following *Triticum* species: *T. urartu*, *T. monococcum*, *T. dicoccum*, *T. boeoticum* and the following *Aegilops* species, *Ae. tauschii*, *Ae. cylindrica*, *Ae. speltoides*, *Ae. umbellulata*, *Ae. mutica*, *Ae. geniculata*, *Ae. triuncialis*, *Ae. columnaris*, *Ae. lorentii*, *Ae. markgrafii*, *Ae. ventricosa*, *Ae. searsii*, *Ae. crassa*, *Ae. comosa*, *Ae. juvenalis*, *Ae. uniaristata*, *Ae. peregrina*, *Ae. bicornis*, *Ae. kotschyii*, *Ae. neglecta* and *Ae. longissima*. Percentage of resistant accessions was evaluated only in the species of which at least 20 accessions were tested.

The highest proportion of accessions resistant at least to one leaf rust race was in the following species: *Ae. speltoides* (100%), *T. monococcum* (95%), *Ae. lorentii* (91%) and *Ae. triuncialis* (88%). To stem rust it was in the following species: *Ae. speltoides* (100%), *Ae. cylindrica* (97%), *Ae. triuncialis* (97%), *Ae. neglecta* (95%) and *Ae. geniculata* (95%). Different reaction pattern to different rust races indicate that several different specific resistant genes are carried by the majority of the tested species. Many accessions possessed both stem- and leaf rust resistance genes. Proportion of accessions resistant to one, two and all three applied rust races and infection types were also evaluated.

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## Genetic variability of *Lactuca aculeata* germplasm expressed by AFLP and SSR markers, and by resistance variation against *Bremia lactucae*

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The wild lettuce, *Lactuca aculeata* Boiss, is a robust and very prickly annual plant which is closely related and fully interfertile with cultivated lettuce (*Lactuca sativa* L.) and *L. serriola* L. (Lebeda et al. 2012). This species is distributed in a relatively limited geographical area of Near East and the Anatolian plateau (Lebeda et al. 2004). Preliminary studies showed that *L. aculeata* is rather homogenous species from the viewpoint of morphology and isozyme polymorphism (Lebeda et al. 2012). The recent study is focused on molecular polymorphism and resistance to *Bremia lactucae*, i.e. two not known features of *L. aculeata* variation.

In total, seventy six *L. aculeata* accessions (accs.) originating from natural populations in Israel, Turkey and Jordan (Beharav et al. 2010) were analysed by amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs). Furthermore, the morphological observation and screening for lettuce downy mildew (*Bremia lactucae*) resistance have been performed.

Finally, 287 AFLP and 8 microsatellite markers were analysed using classical „tree-based“ and Bayesian clustering methods confirming morphological re-determination of four *L. serriola* and three hybrid accessions. Molecular data reflect the geographical origin of samples, which grouped according to the country of their origin. Similar genetic characters were observed among accessions from neighboring parts of Jordan and Israel where migration or artificial introduction of plant material is more possible.

Forty-one *Lactuca aculeata* accessions (from the same trial) were tested for the resistance against *Bremia lactucae*. Altogether 5 isolates (BI races – BI 17, BI 18, BI 24, BI 27 and BI 28) were used for resistance screening. In the studied set of *L. aculeata* accessions was found frequent occurrence of race-specific reaction patterns. These patterns are showing that in *L. aculeata* could be expected different race-specific resistance factors/genes. However, susceptible reactions were prevailing what is showing that this species cannot be considered as a very useful source of resistance for lettuce breeding. As the most virulent races were recorded BI 18, BI 24, BI 27 and BI 28, in opposite the race BI 17 was less virulent.

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## Isolation in time explains host-related genetic differentiation in a group of widespread mycoparasitic fungi

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Temporal isolation is probably the least studied speciation mechanism, in particular in fungi. We studied whether temporal isolation could be responsible for the maintenance of genetic differentiation among sympatric populations of *Ampelomyces*, widespread intracellular mycoparasites of powdery mildew (PM) fungi, whose life cycle is tied to that of their host PM species. Both ITS sequence and microsatellite analyses showed that European *Ampelomyces* populations collected from apple PM (*Podosphaera leucotricha*) are genetically highly differentiated from *Ampelomyces* populations sampled from many other PM species infecting plant hosts other than apple. While *P. leucotricha* starts its life cycle in spring, and apple PM epidemics mainly occur before mid-summer, the mycohosts of the other *Ampelomyces* strains cause epidemics essentially in autumn. When we experimentally exposed two PM species to *Ampelomyces* strains naturally occurring in apple PM in spring and to strains naturally present in other host PM species in autumn, cross infections always occurred, even with PM species *Ampelomyces* is unlikely to encounter in nature. Thus, the host-related genetic differentiation in *Ampelomyces* cannot be explained by a narrow mycohost specialization, but could instead result from temporal isolation.



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## Effect of extreme temperatures on powdery mildew development and Hsp70 induction in tomato and wild *Solanum* spp.

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Development of *Oidium neolycopersici*, the cause of tomato powdery mildew, on susceptible *Solanum lycopersicum* cv. Amateur, and moderately resistant *S. chmielewskii* was compared on plant leaves and leaf discs with regards to influence of temperature (4, 20, and 40.5 °C). Pathogen growth was more intensive on leaf discs kept in optimal temperature of 20 °C. Heat and especially cold pre-treatment (2h prior to inoculation) inhibited conidia germination, decreased the number and in general also the length of germ tubes.

Powdery mildew pathogenesis has been shown to alter host's physiology. 70 kDa heat shock proteins (Hsp70) as constitutively expressed members of the Hsp families help the cell to cope with a variety of stresses, both abiotic and biotic. Changes in Hsp70 expression and protein level were studied in the two genotypes mentioned above complemented by *S. habrochaites* f. *glabratum* (resistant to powdery mildew) in response to short-term high and low temperatures and to pathogen infection. *Solanum* spp. plants with different degree of resistance to powdery mildew were exposed to 4, 10, 20 and 40.5 °C up to 1 day and to *O. neolycopersici* pathogenesis up to 3 days. Quantitative real-time PCR and immunoblot analyses demonstrated that the heat stress increased Hsp70 gene expression and protein level in all three *Solanum* spp., whereas cold caused significant changes only in *S. lycopersicum* cv. Amateur following 24 h. Similarly, exposition to infection increased Hsp70 level mainly in susceptible *S. lycopersicum* cv. Amateur where chaperons may be needed to cope with disturbance of metabolic pathways triggered by pathogen infection.

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## Complex research of structure, variation and spatio-temporal dynamics in wild plant pathosystem *Lactuca* spp. – *Bremia lactucae*

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During the last more than thirty years (since the end of 1970th) extensive research on the wild-plant pathosystem, *Lactuca* spp. (lettuce) - *Bremia lactucae* (lettuce downy mildew), has been conducted in the Czech Republic. Studies were focused on pathogen incidence and distribution, host range, variation in symptom expression and disease severity, interactions of *B. lactucae* with different wild *Lactuca* spp., variation in resistance within and between natural populations of *Lactuca* spp., location of sources of resistance for lettuce breeding, the structure and dynamics of virulence within populations of *B. lactucae*, sexual reproduction of *B. lactucae*, and on a comparison of virulence structure and changes in *B. lactucae* populations occurring in wild (*L. serriola*) and crop (*L. sativa*) pathosystems. Migration and gene flow between both pathosystems and the potential risk of wild *B. lactucae* populations for cultivated lettuce were studied and discussed. From the results of our long-lasting research it is evident that *Lactuca* spp. – *Bremia lactucae* pathosystem is not only exciting model for studies on interactions between weedy growing plants and oomycete biotrophic parasites, but it is also extremely important background for lettuce resistance breeding. Examples of some our papers published about this topic are listed below.

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## The dynamics of the distribution of a powdery mildew in natural populations of *Impatiens noli-tangere*: the impact of metapopulation structure, environmental factors and resistance structure of the host population

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Many studies deal with only one side of a pathological system. The main objective of this study is to investigate the dynamics of the host-pathogen system from all sides and to include a large number of variables. The object of this study is the pathosystem of *Impatiens noli-tangere* - *Podosphaera balsaminae* in the Czech Republic near the town of Sázava. Work started in the summer of 2012. 36 populations were monitored and 10 of them were non-infected. There are three parts to this work.

- Pathogen dynamics depends on the structure of the host subpopulation (size of population, population density, and height of the individual organisms studied) and on the location of the subpopulations (specifically, the distance between them and their spatial arrangement). Our results show that the prevalence of the pathogen is correlated with the size of the population.
- Environmental factors can also influence the spread of the disease. Investigated climatic factors include light, pH, moisture, the presence of nutrients and temperature obtained from vegetation relevés using Ellenberg indicator values. At this time, no reliable results are available.
- The genetic resistance structures of both the infected and non-infected populations are the main factors when considering pathogen dynamics. Further laboratory experiments will take place in the fall of 2013. We expect that the less infected populations will be more resistant to the pathogen.

The result of the study will be a model that will include all of the factors mentioned above. This model will help to understand what the relative influence of the individual variables is on the prevalence of the pathogen.

## Occurrence of lettuce downy mildew (*Bremia lactucae*) and powdery mildew (*Golovinomyces cichoracearum*) in natural populations of prickly lettuce (*Lactuca serriola*)

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Wild pathosystem involves a wild plant, the pathogen that attacks and feeds on it, and the environment that affects both the plant and the pathogen. There are several plant pathosystems, where both main groups of biotrophic fungi (powdery and downy mildew) cause the serious infections.

Our study was focused on wild pathosystem *Lactuca serriola* - lettuce downy mildew (*Bremia lactucae*) and lettuce powdery mildew (*Golovinomyces cichoracearum*). Observations were focused mainly on recording of natural infection of powdery mildew and downy mildew on prickly lettuce (*Lactuca serriola*) in localities in East Bohemia and Moravia in the May-September period of 2007-2011 and on influence of environmental conditions on their occurrence.

Only the data for July and August were used for detailed comparisons of the occurrence of both pathogens, because in this period there was the highest intensity of disease. Data were linked with average monthly temperature and rainfall rate in the Czech Republic. Localities with natural populations of *L. serriola* were divided into five categories according to occurrence of both pathogens: 1. localities with only *B. lactucae*; 2. localities with only *G. cichoracearum*; 3. localities with both pathogens but a prevalence of *B. lactucae*; 4. localities with both pathogens but a prevalence of *G. cichoracearum*; 5. localities with both pathogens at the same severity.

In August 2007 there was recorded significantly higher incidence of *G. cichoracearum*. This pathogen prefers rather low levels of precipitation and temperatures around 20°C. On the other hand in July 2009 and 2010, when the intensity of precipitation was higher, prevailed occurrence of *Bremia lactucae*. In August 2010 and 2011 there were occurrence of both pathogens at all locations more or less balanced.

According to the above-mentioned data it was shown that climatic conditions have influence on the occurrence of both pathogens.

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## Using wild plant pathogen systems related to model systems to infer functional evolution in effector genes

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The basal immune system of plants fights pathogens by recognising conserved pathogen associated molecular patterns. To bypass this basal immune system of the plants, pathogens secrete effectors into the host plant. Plants produce resistance proteins to recognise the secreted effectors leading resistance. This is again suppressed by secreted pathogenicity effectors in successful pathogenic interaction.

Plant pathogens diversify by host jumping and co-speciation, while the former is the seed from which diversity evolves through specialisation. Little is known about the functional adaptations of effectors after host jumps, although these constitute the key to understanding effector plasticity and function.

In this study, 48 coding sequences of the ATR1 effector from three different Oomycetes, *Hyaloperonospora thlaspeos-perfoliati* (*Htp*), *H. crispula* (*Hc*) and *H. arabidopsidis* (*Hpa*), have been analysed, using PAML. The availability of crystal structure of *Hpa* ATR1, facilitated homology modelling of ATR1 from *Htp* and *Hc*. A high number of positively selected sites (34) sites were identified, when sequences from all the organisms were analysed together.

In *Hpa*, more sites are under positive selection in comparison to the others, ranging from the N-terminal to the C - terminal, which is responsible for effector function and stability of the protein.

In all species, more than 90 % of the positively selected residues are surface residues.

The huge accumulation, of positively selected sites in ATR1 of *Hpa* that goes far beyond the residues known to be important for recognition and are probably a sign of a long-lasting coevolutionary arms race between *Hpa* and its host, *Arabidopsis thaliana*. The variability has not increased significantly after host jumps in case of *Htp* and *Hc*, suggesting, that ATR1 is not recognised by the corresponding hosts and that only few mutations were necessary to adapt to the new targets in the respective hosts.

## Quantifying virulence variation in the generalist seed bank pathogen *Pyrenophora semeniperda*

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The ascomycete pathogen *Pyrenophora semeniperda* is abundant in the seed banks of *Bromus tectorum* (cheatgrass) and *Bromus rubens* (red brome) in the semi-arid western United States. This fungus can kill tens of thousands of seeds per square meter each year. *Pyrenophora semeniperda*, a generalist seed pathogen, offers potential as an inundative biocontrol to eliminate weeds in persistent seed banks. Quantification of among-strain virulence variation of this pathogen will provide a screening tool for predicting field biocontrol efficacy. Results indicate that favorable conditions allow most non-dormant seeds in the field to escape the pathogen through rapid germination. Laboratory experiments using seed bed microcosms showed that *B. tectorum* mortality ranged from 19 to 94% when inoculated with high loads of the pathogen, whereas mortality of *B. rubens* seeds ranged from 16 to 70%. Mortality of *B. tectorum* and *B. rubens* did not differ substantially; however, seed mortality differed at low inoculum loads. Four highly virulent strains of *P. semeniperda* were selected for use in field inoculation trials and applied as bulk inoculum at Pakoon Valley, Arizona, USA. Suboptimal field inoculum loads were used to facilitate detection of differences among treatments. *P. semeniperda* strains performed equally well, eliminating 74% of the carryover seed bank as compared to the 40% eliminated by the resident pathogen alone. These results demonstrate the existence of considerable among-strain variation in aggressiveness, as measured by the ability to cause host seed mortality by *P. semeniperda*. Furthermore, laboratory and seed bed microcosm evaluations of virulence can predict pathogen strain performance as a biocontrol agent.



## A natural woody plant, *Mallotus japonicus*, as an ecological partner to transfer different pathotypic conidia of *Oidium neolycopersici* to greenhouse tomatoes

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Our greenhouse tomatoes (*Solanum lycopersicum* cv. Moneymaker) have suffered from infection with tomato powdery mildew for 15 years. During this period, we have obtained some isolates of *Oidium neolycopersici*: KTP-01 in 1998 (Kashimoto et al., 2003), KTP-02 in 2009 (Seifi et al., 2012), and KTP-03 and -04 in 2012 (Nonomura et al., 2013). Most recently, we detected a new pathogen (KTP-05) that formed pseudochains consisting of 12 mature conidia, which were distinguishable from those of previous powdery mildew pathogens: 4 conidia in KTP-01 and KTP-02 (Oichi et al., 2006; Seifi et al., 2012) and 8 conidia in KTP-03 and KTP-04. To identify the original partner plant that dispersed the new pathogen, powdery mildew-infected wild plants on our campus were screened, as powdery mildew is an obligate parasite, and therefore partner plants are also susceptible to the pathogen. From a total of 852 wild and cultivated woody and herbaceous plants surveyed, the pathogen on JM produced a similar type of pseudochain (12-conidia pseudochain). The conidia (KMP-01) isolated from infected JM leaves were infectious to tomato, and inversely KTP-05 conidia infected JM. To further characterise these pathogens, infectivity assays and ITS-based phylogenetic analyses were conducted. Two pathogens showed the same infectivity to five wild tomato species (*S. chilense* LA0468, *S. hirsutum* LA0386 and LA1738, *S. peruvianum* LA2172 and *S. parviflorum* G1.1601); tester plants were used to distinguish between each pathogen. In addition, their ITS sequences were completely identical, and therefore were positioned in the same clade of *Oidium neolycopersici* in the phylogenetic tree. These results suggest that the conidia on JM were transmitted to our greenhouse tomatoes. An additional aim of the work was to prove that JM conidia could be transferred by wind. For this purpose, we traced the spreading of the powdery mildew disease among JM plants on campus. It appeared that the disease expanded in the direction of the mountain wind. JM plants surrounding the greenhouse were ultimately infected with the pathogens. This may suggest that wind transports KMP-01 to our greenhouse tomatoes. Thus, the present work provides experimental bases for analysing the ecological transmission of conidia between wild plants and greenhouse tomatoes.

## Comparative study of polymorphism within genomes of cucumber green mottle mosaic virus isolates revealed the remarkable repertoire of the viral genome in wild host plant

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Cucumber Green Mottle Mosaic Virus (CGMMV) is a member of the genus *Tobamovirus*. In Israel it was first discovered at the beginning of the 1990s while infecting mainly the wild plant *Ecballium elaterium*. Only at the late 2000s the virus was spread to different areas of the country, severely affecting cucurbit cultured crops. Viral symptoms including mottling and mosaic on leaves: mottling on cucumber fruits, distortion of melon and watermelon flesh and pulp which cause a severe damage to fruit quality and prevent marketing.

The present study was directed to compare the genetic variation in the full genomes of four CGMMV isolates, three of which were isolated from cultured crops while one isolated from the wild plant *E. elaterium*. The purpose of this comparison was to understand the preservation of CGMMV in the wild plant and its role as a source for the virus in cultured plants.

Viral particles were purified from each of the following isolates: CGMMV-Ah which induces severe symptoms on cucumber fruit; CGMMV-TY which causes mild symptoms on cucumber fruit; CGMMV-Rd causes yellowing and distortion of watermelon flesh and pulp; CGMMV-Ec was isolated from its wild host, *E. elaterium* while causing symptomless infection in cucurbits. Later, viral RNA was extracted from purified virions and subjected to SOLiD Next-Generation Sequencing. SOLiD reads were aligned with the available CGMMV sequences from GenBank which served as a reference sequences permitting the acquisition of the complete genomic sequences.

The results indicate a high level of polymorphism within each viral isolate as well as between isolates. The differences observed allowed the construction of a SNP map for each isolate. The SNPs observed were divided into synonymous and non-synonymous SNP categories. A possible correlation was suggested between the non-synonymous SNPs and the biological differences found among the compared isolates. Significantly more SNP sites were observed in the genes of the CGMMV-Ec (wild host). Construction of a phylogenetic tree for the isolates including potential SNPs, places CGMMV-Ec closely to the potential evolutionary ancestral strain of the Israeli isolates. High potential variation detected in the wild plant, indicating that it may serve as an environment with low evolutionary pressures for the virus, enabling more genetic variation.

Hypothetically, the plasticity of the viral genome as reflected in the SNPs maps reflect the genetic capability enabling adaptation to the environmental changes and new hosts.

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## Natural occurrence of *Ampelomyces quisqualis* in mycelia of cucurbit powdery mildew species in the Czech Republic

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*Ampelomyces quisqualis* Ces. (*Aq*) is a naturally occurring intracellular pycnidial hyperparasite not only on anamorphs as well as on teleomorphs on many hosts within Erysiphaceae (Bélanger and Labbé, 2002; Jarvis et al., 2002; Kiss, 1998; Pérez-García et al., 2009). This wide host range and tolerance to number of fungicides used in powdery mildew control makes *Aq* highly desirable candidate for biocontrol (Angeli et al., 2012; Gilardi et al., 2008).

From 2001 to 2010, in the Czech Republic (CR), 1127 leaf samples with powdery mildew symptoms collected from cucurbits (mainly from *Cucurbita pepo*, *Cucurbita maxima*, *Cucumis sativus*) were microscopically examined for presence of hyperparasitic fungus *Ampelomyces quisqualis* Ces. (*Aq*). Identification of cucurbit powdery mildew (CPM) species (*Golovinomyces orontii* /*Go*/, *Podosphaera fusca* /*Pf*/) was carried out by microscopic examination of the morphological characters of dry conidia in a 3% KOH solution. In total of 777 CPM samples (69%) was determined as *Go*, 258 CPM samples (23%) as mixed infection (*Go*, *Pf*) and 92 CPM samples (8%) as *Pf*. *Aq* was detected on 15% (164 samples) of collected samples from the CR. However, frequency of its occurrence varied among the years. In 2001-2002, 2006-2007 and 2009, *Aq* was more frequent (18-24%) as compared with the years 2003-2005 and 2008, 2010 (only 4-12%). Pycnidia of *Aq* were recorded mainly on *Go* hyphae and basal cells of conidiophores (15%) or on mixed infection (*Go*, *Pf*) (15%). Occurrence of *Aq* on *Pf* was only sporadic (9%). Its distribution was recorded in 96 locations of the CR, it was not restricted to limited areas, nevertheless the most of *Aq* locations originated from the South Moravia. In 31 locations its presence was recorded repeatedly, in some others it was detected only once. The majority of CPM samples with presence of *Aq* were collected in August.

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## Genotype and spatial structure shape pathogen dispersal and disease dynamics at small spatial scales

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Many devastating pathogens are passively dispersed, and their epidemics are characterized by variation that is typically attributed to environmental factors. Here, by combining laboratory inoculations with wind tunnel and field trials using the wind-dispersed pathogen *Podosphaera plantaginis*, we demonstrate striking genetic variation affecting the unexplored microscale (<2m) of epidemics. Recipient and source host genotypes, as well as pathogen strain explain 6-33% of variation of the three key dispersal phases: departure, movement and settlement. Moreover, we find genotypic variation affecting group size of the pathogen dispersal unit, ultimately resulting in increased disease development on hosts nearby the infection source. Together, our results show that the genotypic variation may generate considerable variation in the rate of disease spread through space and time with disease hotspots evolving around initial foci. Furthermore, the extent of genetic variation affecting the entire dispersal process confirms that these traits may be targeted by selection.

## Natural virus infection of Hedge Bindweed (*Calystegia sepium*)

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Virus symptoms were observed on Hedge bindweed (*Calystegia sepium*) a well known plant in Hungary. In the literature there is no record of virus infection on Hedge bindweed, therefore, investigations were carried out to determine the causal agent. Sap from leaves showing virus-like symptoms was inoculated onto test plants inducing systemic infection on *Nicotiana clevelandii*, *N. benthamiana*, local lesions on *Chenopodium quinoa* and no infection on *Datura stramonium* and *Cucumis sativus*. Sap of *N. clevelandii* was examined by electron microscopy, showed the presence of long flexuous particles. The biological and other properties of the virus has also been studied. Properties of particles in sap were as follows: TIP: 78 °C, LIV: 26 days and DEP: log 10 minus 5. The size of coat protein is 36 kDa, and the genome consists of 7-8000 nt RNA. Double-stranded cDNA were produced using random hexanucleotide primers, cloned and sequenced. BLAST search of sequence databases revealed nucleotide sequence identity with carlaviruses. Further investigations are needed to decide whether the virus isolated from hedge bindweed is a new carlavirus or a new strain of an existing carlavirus.

## Interactions between bacterial and viral pathogen species of rice (*Oryza spp.*), experimental co-infections and epidemiological consequences

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Most studies in ecology and evolution of host-pathogen interactions and disease epidemiology are considering a pair of “one host / one pathogen” species while host individuals are likely to be simultaneously or successively be infected by various different pathogen species. In such cases, pathogen species may interact directly (interference competition or mechanical facilitation) or indirectly through host resources or the host defence mechanisms (crossed immunity or immune suppression). These pathogen interactions likely determine overall virulence at the individual host level, but they were also shown to have critical consequences on the disease spread at the host population level and to influence pathogen evolutionary trajectories.

We aim at investigating interactions between pathogen species, focusing of rice diseases in West Africa in order to (1) better understand of the role of pathogen interactions on epidemiology and pathogen evolution and (2) apply our results into recommendations for integrative disease management. First, we present here some results on experimental co-infections of rice by the Rice Yellow Mottle Virus (RYMV) and two different pathovars of the bacterial pathogen *Xanthomonas oryzae*. We then discuss the perspectives on the study of disease interactions at the field and landscape levels in Burkina Faso.

## How gene flow between wild and cultivated lettuce can influence the dynamic of adaptation of *Bremia lactucae*

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Host resistance may be considered as the main driving force of plant pathogens evolution, with pathogens evolving to overcome host resistance strategies. In the model system, *Bremia lactucae*, the causal agent of downy mildew on *Lactuca sativa*, several specific resistance genes are used to counter this pathogen. In turn, pathogens show a rapid adaptation to host resistances, and characterization of population genetic structure could be an important step to identify evolutionary strategies used by the pathogen to overcome host resistance.

Microsatellite markers were developed to study the genetic diversity of *B. lactucae*. More than 400 isolates were recovered in France from 23 regions; on different cultivars (carrying different resistance genes) of *L. sativa* and on the wild host *L. serriola*. Genotypic diversity and gene diversity were low in all the populations sampled; 14 multilocus genotypes (MLG) were identified in France and among them, two were predominant. Within the eight MLG observed on *L. serriola*, six MLG were specific to this host plant and two MLGs were shared by isolates of the two host plants, suggesting the occurrence of gene flow between the wild and the cultivated pathosystems.

Virulence phenotypes of *B. lactucae* were characterized on a set of 19 differential hosts. The two pathotypes the most frequent matched respectively the two most frequent MLGs. Furthermore, clustering analyses revealed that *B. lactucae* populations could be clustered into three genetically distinct groups with good correlations with resistance genes. Each cluster corresponds mainly to different pathotypes. Isolates of MLG 1, found only in cluster 3, were not genetically closely related with the other isolates and pathotypes were very different. Moreover, MLG 1 isolates were also found on wild *L. serriola*. The hypothesis that new virulence may have emerged through migration from the wild host plant is discussed.

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## Identification of host targets of oomycete effectors from *Hyaloperonospora arabidopsidis* and related oomycetes

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Phytopathogenic oomycetes can cause diverse plant diseases by secreting a suite of effector proteins which suppress plant immune response. Downy mildew oomycetes deliver effectors into host cells, thereby eliciting plant defense reactions or controlling plant cellular behavior. A number of resistance proteins from their host plants have been studied, but the primary host targets of effectors are still elusive. The identification of effector host targets is, however, a prerequisite for understanding by what mechanisms effectors promote virulence. In this study we applied yeast two hybrid screenings using home-made libraries from *Arabidopsis thaliana* infected with *Hyaloperonospora arabidopsidis* and *Microthlaspi perfoliatum* infected with *Hyaloperonospora thlaspeos-perfoliati*. Several potential interactors were obtained in these screenings including defense-associated proteins and proteins involved in primary metabolism. Interactions were found to be highly specific in the ATR13 gene family of *H. thlaspeos-perfoliati*.

## Somatic embryogenesis and molecular analysis for conformity test of date palm vitroplants (*Phoenix dactylifera* L.) and their relationship to bayoud disease

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Somatic embryogenesis is the fastest way for date palm propagation at large scale. In addition the plantlets issues from somatic embryos are viable and vigorous. The objective of this work is the regeneration of cultivars, the conservation of rare cultivars, appreciated for the quality, or resistance to bayoud disease.

Three different media of MS basis (1962) modified and added of growth substances of which the concentration in 2-4D varies between 25 mg.l<sup>-1</sup> and 100 mg.l<sup>-1</sup>, and Picloram to 12,5 mg.l<sup>-1</sup>, have been used for the induction of callus embryogenesis: M100, M25, P12.5. The most important callogenesis has been observed in presence of 2.4-D and picloram to concentrations of 100mg.l<sup>-1</sup> and 12mg.l<sup>-1</sup> with 1 to 3mg.l<sup>-1</sup>d'IPA. The embryogenic callus was transferred to multiplication medium for callus maintenance. The maturation of the somatic embryos and their germination is obtained on the medium of GMN200 without growth substance containing 1mg.l<sup>-1</sup> of Thiamine, 100mg.l<sup>-1</sup> of KH<sub>2</sub>PO<sub>4</sub>, 170mg.l<sup>-1</sup> of Na<sub>2</sub>HPO<sub>4</sub>, 200mg.l<sup>-1</sup> of ammonium Nitrate. The elongation and the rooting of the plantlets have been obtained on GMP medium containing 100mg.l<sup>-1</sup> of Thiamine hydrochlorate, 170mg.l<sup>-1</sup> of NH<sub>2</sub>PO<sub>4</sub>, 200mg.l<sup>-1</sup> of ammonium Nitrate, 40 mg.l<sup>-1</sup> of Adenine, 50mg.l<sup>-1</sup> of Acid nicotinique, 50mg.l<sup>-1</sup> of pyridoxine. A better elongation of the aerial part and big number plantlets are obtained. On the whole, 81% vitroplants survival was established in soil.

Molecular markers analysis was examined by comparing the electrophoretic profiles of the plantlets regenerated by somatic embryogenesis to those of the mother plant by RAPD and AFLP markers. Eight primers selected by the RAPD technique, reveal a high percent of common bands that varies 93% to 97%. It also shows the systematic presence of specific bands to the plant mother and to the vitroplants but their percent is low between of 3% to 7%. The 17 primers combination selected by the AFLP technique showed a percent of conformity is between 91% to 98% and a variation between 2% to 9%. The establishment of genetic control test using those markers will allow the date palm micropropagation of date palm to move towards industry multiplication and have an early conformity test before planting. This way of multiplication is a top priority for the preservation and ex situ conservation of our phenicicole heritage.

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