Plant Viruses:
Exploiting Agricultural and Natural Ecosystems

11th International Plant Virus Epidemiology Symposium
and
3rd Workshop of the Plant Virus Ecology Network

June 20-24, 2010
Cornell University,
Ithaca, New York
http://www.isppweb.org/ICPVE/
http://bioinfosu.okstate.edu/pve_rcn/PVENWksp10.html

Program Committees
Alberto Fereres, IPVE
Ulrich Melcher, PVEN
Stewart Gray

Virus Epidemiology
Sunny Power, Mike Thresh, Roger Jones, Juan Alvarez

Virus Ecology and Evolution
Carolyn Malmstrom, Marilyn Roossinck, Hanu Pappu

Vector Biology and Transmission
Nilsa Bosque-Perez, Rodrego Almeida, Stephane Blanc, Russ Groves

Disease Management, Diagnosis, and Detection
Jari Volkonen, Ian Barker, Scott Adkins

Local Arrangements
Stewart Gray, Chair
Keith Perry, Sunny Power, Brian Nault, Marc Fuchs

The meeting organizers would like to acknowledge the financial support of the following institutions and companies.

United States Department of Agriculture – Agricultural Research Service

Cornell University

Agdia, Inc – Please visit the poster for more information

Dow Agrosciences, LLC

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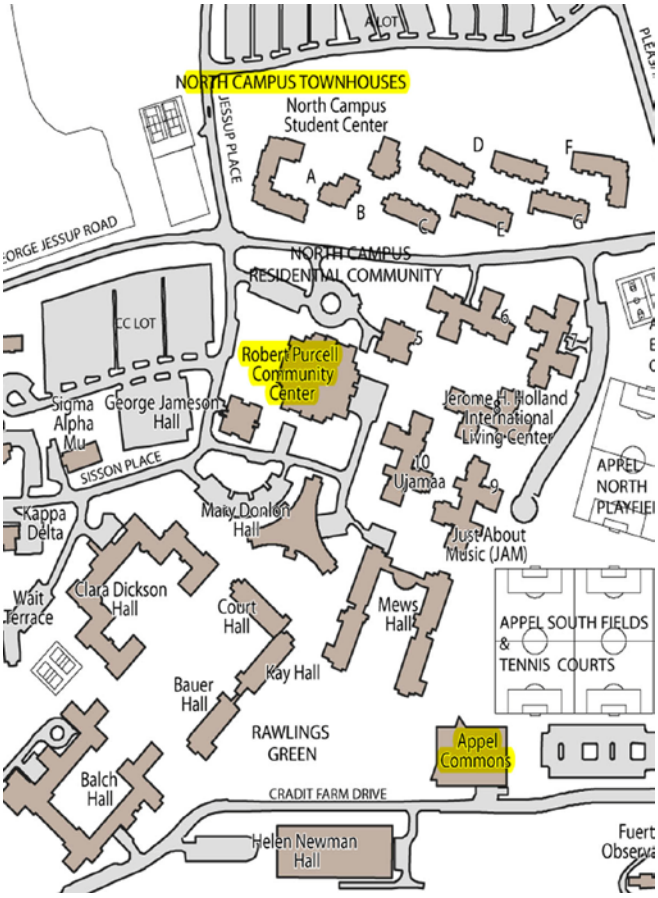
Meeting Locations



The Sunday Reception and Dinner will be held in Room 303, Appel Commons

All of the symposia, the 15 minutes oral presentations and the 5 minute oral poster advertisements will be held in Room 303, Appel Commons.

Lunch is available in the cafeteria on the second floor of Appel Commons – your name tag will be required for entrance into the cafeteria.

The afternoon poster session will be held in Room 218, Robert Purcell Community Center. All posters will be displayed for the entire meeting

The PVEN business meeting will be held Tuesday @ 5:00 in Room 105 Robert Purcell Community Center

The IPVE business meeting will be held Tuesday @ 5:00 PM in Room 303, Appel Commons

Please be sure to stop by the Hospitality Suite at the Townhouses to socialize with conference participants and their guests. This will be open Sunday and Wednesday evenings after the dinners, and Monday, Tuesday and Thursday evenings from 7-11 PM. Complimentary assorted beverages and snacks will be available.

Meeting Schedule at a Glance

Time/Day	Sunday	Monday	Tuesday	Wednesday	Thursday
Theme		**Epidemiology**	**Ecology**	**Vectors**	**Management**
7:00 – 8:00		Breakfast	Breakfast	Breakfast	Breakfast
8:00		Introductions	Announcements	Announcements	Announcements
-:15			Keynote A. Power	Keynote S. Blanc	Keynote I. Barker
-:30		Keynote M. Jeger			
-:45					
9:00			W. Schneider Horizontal trans.	B. Bonning Blocking uptake	J. Valkonen Cryotherapy
-:15		J. Legg Cassava viruses			
-:30			F. Garcia-Arenal Co-evolution	R. Almeida Mealybug trans.	S. Adkins Vegetable viruses
-:45		C. Desbiez Cucurbit viruses			
10:00			Coffee	Coffee	Coffee
-:15		Coffee			
-:30			M. Roossinck Wild plant viruses	P. Trebicki Impact of CO_2	B. Lennefors Rhizomania
-:45		R. Jones ZYMV			
11:00			J. Kreuze Virus discovery	J Ng Whitefly trans.	R. Resende Durable resistance
-:15		A. Culbreath TSWV			
-:30			P. Cronin Host physiology	D. Stenger Phytoreovirus	R. Groves Vector control
-:45		N. Bosque-Perez Aphid response			
12:00	Registration and check-in	Lunch	Lunch	Lunch	Lunch
-:15					
-:30					
-:45					
1:00					
-:15					
-:30		15 minute oral presentations	15 minute oral presentations	15 minute oral presentations	15 minute oral presentations
-:45					
2:00					
-:15					
-:30					
-:45					
3:00					
-:15					
-:30		5 min Poster summaries	5 min Poster summaries	5 min Poster summaries	5 min Poster summaries
-:45					
4:00		Posters and social time	Posters and social time Business meetings 5:00	Posters and social time	Posters and social time
-:15					
-:30					
-:45					
5:00	Welcome reception followed by dinner				
-:15					
-:30					
-:45		Dinner on own	Dinner on own	Banquet at Wager Vineyard	Dinner on own

Sunday – Registration and Welcome reception and dinner

12:00-5:00 Registration and Check in – Lobby of Robert Purcell Community Center
5:00-6:00 Welcome reception – Room 303 Appel Commons
6:00-8:00 Buffet dinner – Room 303 Appel Commons

Monday – Virus Epidemiology and Etiology

8:00-8:30 Welcome and Introductions – Room 303 Appel Commons

Moderator, Sunny Power
8:30-9:15 Keynote Speaker:
Mike Jeger, Imperial College London, UK
Modeling plant virus transmission: from within-plant virus dynamics to epidemic development
***M. Jeger**, G. Powell, L.V. Madden and F. van den Bosch*

Invited Presentations
9:15 - James Legg, IITA-Tanzania
A tale of two epidemics: the contrasting dynamics of cassava mosaic and cassava brown streak diseases in East Africa.
***J.P. Legg**, S.C. Jeremiah, H.M. Obiero, M. N. Maruthi, I. Ndyetabula, G. Okao-Okuja, D.J. Kim, H. Bouwneester, S. Bigirimana, W. Tata-Hangy, G. Gashaka, G. Mkamilo, T. Alicai, and P.L. Kumar*

9:45 - Cecile Desbiez, INRA, France
Molecular epidemiology of potyviruses infecting cucurbits in France: a case study for understanding evolution of plant virus populations.
***C. Desbiez**, B. Joannon, C. Chandeysson, C. Wipf-Scheibel, and H. Lecoq*

10:15 – Coffee break

10:45 - Roger Jones, Dept of Agriculture and Food, Australia
Epidemiology of Z*ucchini yellow mosaic virus*: effectiveness of non-host barriers, spread, resistance breakdown, alternative hosts, and molecular characterization.
*B. Coutts, M. Kehoe, S. Wylie, C. Webster and **R. Jones***

11:15 - Albert Culbreath, Univ. of Georgia, U.S.
Epidemiology of tomato spotted wilt in peanut in the Southeastern United States.
***A. Culbreath** and R. Srinivasan*

11:45 - Nilsa Bosque-Perez, Univ. of Idaho, U.S.
Influence of virus-induced changes in plants on aphid vectors and potential impacts on virus epidemiology.
N. Bosque-Perez

12:15-1:30 – Lunch – North Star Cafeteria, Appel Commons

15 minute Oral Presentations – Room 303 Appel Commons
Moderator – Juan Manuel Alvarez

1:30 – Landscape/pathosystem components that contribute to epidemic risk in plant virus pathosystems: a conceptual model
F. W. Nutter, Jr.

1:45 – Emergence, establishment, and epidemiology of *Cucurbit yellow stunting disorder virus* in California
W. M. Wintermantel*, E. T. Natwick, R. L. Gilbertson and J. D. McCreight*

2:00 – Epidemiology of *Iris yellow spot virus* in eastern North American onion ecosystems
B. Nault*, C. Hsu, E. Smith, A. Shelton, M. Fuchs and C. Hoepting*

2:15 – Search for factors potentially involved in the rapid shift in *Watermelon mosaic virus* (WMV) populations in south-eastern France
H. Lecoq*, F. Fabre, B. Joannon, C. Wipf-Scheibel, C. Chandeysson, A. Schoeny and C. Desbiez*

2:30 – *Pepino mosaic virus*: what do we know so far and how to proceed
R. A. A. van der Vlugt

2:45 – The epidemiology of *Tobacco streak virus* in central Queensland, Australia
M. Sharman, ***J. E. Thomas*** *and D. M. Persley*

3:00 – Alternative hosts of two tospoviruses in Queensland, Australia
D. Persley*, M. Sharman, J. Thomas and C. Gambley*

3:15 – Epidemiological analysis of multi-virus infections of watermelon in experimental fields in southwest Florida
W. W. Turechek*, C. S. Kousik, C. G. Webster, P. A. Stansly, P. D. Roberts and S. Adkins*

5 minute Poster Advertisements – Room 303 Appel Commons
Moderator – Roger Jones

3:30 – Phenology of aphids and their potential as virus vectors in a northern seed potato production area in Finland – Poster # Ep1
S. M. Kirchner*, L. Hiltunen, E. Virtanen and J. P. T. Valkonen*

3:35 – Preliminary disease progress curve of *Potyviruses* and vector interaction in garlic crop in Argentina – Poster # Ep2
M. C. Perotto, S. Lanati, S. Panonto, E. E. Cafrune and ***V. C. Conci***

3:40 – **WITHDRAWN** Occurrence and epidemics of *Wheat dwarf virus* in China - Poster # Ep3
***Xifeng Wang**, B. Wu and G. Zhou*

3:45 – **WITHDRAWN** Temporal and spatial spread of *Chickpea chlorotic dwarf virus* (CpCDV) in chickpea in northern Sudan – Poster # Ep4
Abdelmagid Adlan Hamed

3:50 – The genus *Torradovirus*, a new plant virus genus now harbouring three species all infecting tomato – Poster # Ep5
***Martin Verbeek**, A. Dullemans, P. Maris, H. van den Heuvel and R. van der Vlugt*

3:55 – Evaluation of thrips-*Iris yellow spot virus* interactions through an indicator host, lisianthus (*Eustoma grandiflorum*) – Poster # Ep6
***Rajagopalbabu Srinivasan**, D. Riley, S. Diffie, H. Pappu and R. Gitaitis*

4:00 – 6:00 Posters – Room 218, Robert Purcell Community Center

Coffee, Iced Tea and snacks provided –cash bar also available
AUTHORS – please plan to be at your posters from at least 4:30-5:00

Ep7 Relationships between *Citrus tristeza virus* spread and aphid species (Hemiptera, Aphididae) population composition in different citrus species and geographical areas
*C. Marroquín, **Alfonso Hermoso de Mendoza** and M. Cambra*

Ep8 Dually stressed tobacco plants demonstrate heavier cell pathology
*O. Iutinska, A. Bysov, O. Shevchenko and **V. Polischuk***

Ep9 The incidence and genetic diversity of *Turnip yellows virus* (TuYV) in winter oilseed rape (*Brassica napus*) in England
***Elvis Asare-Bediako**, C. Jenner, M. Stevens and J. Walsh*

Ep10 Occurrence and distribution of viruses in cucurbits from Oklahoma
***Akhtar Ali** and A. Khattab*

Ep11 Detection of *Soil-borne cereal mosaic virus* in Belgium on wheat and barley
*C. Vaïanopoulos, A. Legrève, C. Lorca, V. Moreau, M. Wattiez and **C. Bragard***

Ep12 Epidemiology of *Plum pox virus* in Ukraine
I. Budzanivska, L. Usko, A. Gospodaryk, F. Demyanenko and V. Polischuk

Ep13 Multiplex PCR assay for the simultaneous differentiation *Potato virus Y* strains and the first report of the occurrence of the Eu-PVY^{NTN} strain in Japan
***M. Chikh Ali**, T. Maoka, M. Taniguchi, J. Sasaki and T. Natsuaki*

Ep14 Monitoring and forecasting virus diseases in legumes in the Palouse region of the inland Pacific Northwest, USA
*D. S. Husebye, **Sanford D. Eigenbrode**, E. Bechinksi, A. V. Karasev, S. L. Clement, B. Vemulapati and H. Pappu*

Ep15 Disruption of two defensive signaling pathways by the cucumber mosaic virus 2b RNA silencing suppressor
*M. G. Lewsey, A. M. Murphy, D. MacLean, N. Dalchau, J. H. Westwood, K. Macaulay, M. H. Bennett, M. Moulin, D. E. Hanke, G. Powell, A. G. Smith, **Heiko Ziebell** and J. Carr*

Ep 16 *Cassava brown streak virus* diversity and development of improved virus diagnostics
***M. N. Maruthi**, M. M. Abarshi, I. U. Mohammed, S. E. Seal, R. J. Hillocks, L. Kumar, and J. Legg*

Ep 17 Epidemiology of cassava brown steak disease in Uganda
***T. Alicai**, C. A. Omongo, R. Kawuki, A. Pariyo, Y. Baguma, A. Bua*

Ep 18 Symptomatology and biological characterization of Citrus tristeza virus isolates in Pakistan
***M. Abbas**, M. M. Khan, S. M. Mughal and I. A. Khan*

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There are also a number of restaurants that will deliver to the Townhouses – a list of menus is located in each of the townhouses.

Also, please stop by the Hospitality Suite anytime between 7-11PM.

Tuesday – Virus Ecology and Evolution

8:00-8:15 Welcome and Introductions – Room 303 Appel Commons

Moderator – Carolyn Malmstrom
8:15-9:00 Keynote Speaker:
Alison "Sunny" Power, Cornell Univ., U.S.
The community ecology of *Barley/cereal yellow dwarf viruses* in Western US grasslands.
***A. Power**, E.T. Borer, C.E. Mitchell, and E.W. Seabloom*

Invited Presentations

9:00 - William Schneider, USDA-ARS, U.S.
Balancing selection for replication and horizontal transmission by mimicking field conditions
***W. Schneider**, A. L. Stone, B. Tian, F. E. Gildow and V. D. Damsteegt*

9:30 - Fernando Garcia-Arenal, Universidad Politecnica de Madrid, Spain
Arabidopsis thaliana as a model system for plant virus ecology and plant-virus co-evolution
***F. García-Arenal**, I. Pagán, A. Fraile, N. Montes and C. Alonso-Blanco*

10:00 Coffee Break

10:30 - Marilyn Roossinck, Noble Foundation, U.S.
Wild plant viruses and disease ecology
M. Roossinck

11:00 - Jan Kreuze, CIP, Peru
siRNA deep sequencing for the discovery and sequencing of novel viruses
***J. F. Kreuze**, W. Cuellar, G. Müller, R. Kumria, C. Fauquet and I. Barker*

11:30 - J. Patrick Cronin, Univ. North Carolina, U.S.
Host physiological phenotype predicts key epidemiological parameters
***J. Patrick Cronin,** M. E. Welsh, M. G. Dekkers, S.T. Abercrombie and C. E. Mitchell*

12:00-1:30 – Lunch – North Star Cafeteria, Appel Commons

15 minute Oral Presentations – Room 303 Appel Commons
Moderator – Marilyn Roossinck

1:30 – When did the new world and old world begomoviruses diverge?
***Siobain Duffy** and J. A. McConnell*

1:45 – Origin, evolution and molecular epidemiology of *Papaya ringspot virus*
***X. A. Olarte Castillo**, Y. Rojas, P. Tennant, M. Fuchs, R. Sierra, A. J. Bernal, G. Fermin and S. Restrepo*

2:00 – Long-term evolution of the *Luteoviridae*: time-scale and mode of virus speciation
Israel Pagán *and E. C. Holmes*

2:15 – Adaptation of *Soybean dwarf virus* to new host species
Bin Tian*, W. L. Schneider and F. E. Gildow*

2:30 – Towards the description of the plant virus metagenome of the French sub-Antarctic Islands
Armelle Marais, C.Faure, S. Arous, L. Svanella-Dumas, C. Couture, M. Hullé and ***Thierry Candresse***

2:45 – Ecogenomic study of plant viruses reveals widespread infection of wild plants with *Zucchini yellow mosaic virus*
Prasenjit Saha*, F. Chavarría, J. Quan, H. C. Lai, B. A. Roe and M. J. Roossinck*

3:00 – The significance of wild plants in the evolutionary diversification of *Sweet potato feathery mottle virus* in East Africa
A. K. Tugume *, S. B. Mukasa and J. P. T. Valkonen*

3:15 – Plant-virus co-evolution in wild brassicas
J. A. Walsh*, C. Obermeier, P. J. Hunter, R. Machado, K. Ohshima and M. J. Kearsey*

5 minute Poster Advertisements – Room 303 Appel Commons
Moderator – Hanu Pappu

3:30 – The effect of transmission mode on genetic diversity in *Zucchini yellow mosaic virus* (ZYMV). Poster # Ec1
Heather E. Simmons*, E. C. Holmes and A. G. Stephenson*

3:35 – Genetic structure and molecular variability of *Grapevine fanleaf virus* populations within three naturally infected California vineyards. Poster # Ec2
J. E. Oliver*, E. Vigne and M. Fuchs*

3:40 – Next-generation sequencing of plant viruses. Poster # Ec3
Wendy Monger*, R. Glover, I. Adams and N. Boonham*

3:45 – Genetic variability, recombination events and rates of molecular evolution in *Citrus tristeza virus*. Poster # Ec4
G. Silva*, and G. Nolasco*

3:50 – A survey of begomoviruses and associated satellites infecting plants in the cotton-growing areas of Northwestern India. Poster # Ec5
V. Zaffalon, V. S. Reddy, S. K. Mukherjee, M. Tepfer and ***Jeremy R. Thompson***

3:55 – The evolution of cassava brown streak-associated viruses (family *Potyviridae*) in East Africa. Poster # Ec6
***D. R. Mbanzibwa**, Y. P. Tian, A. K. Tugume, S. B. Mukasa, F. Tairo, S. Kyamanywa, A. Kullaya and J. P. T. Valkonen*

4:00 – 6:00 Posters – Room 218, Robert Purcell Community Center

Coffee, Iced Tea and snacks provided –cash bar also available
AUTHORS – please plan to be at your posters from at least 4:30-5:00

Ec7 The diversity of ampeloviruses and badnaviruses in Australian pineapples and their association with mealybug wilt of pineapple (A*ananas comosus*)
***C. F. Gambley**, V. Steele, A. D. W. Geering and J. E. Thomas*

Ec8 Variation in sugarcane cultivar host range of *Sugarcane yellow leaf virus* genotypes in Guadeloupe
***Jean Heinrich Daugrois**, D. Sarah, F. Emmanuel, G. Jean-Claude and R. Philippe*

Ec9 Sequence comparison of different *Cauliflower mosaic virus* isolates infecting canola in Iran
***Nooh Shahraeen**, S. Ghaderi Maryam and F. Rakhshandehroo*

Ec10 Metagenomics and quarantine: searching for the unknown
Philippe Roumagnac

Ec11 Epidemiology and phylogenetic aspects of *Iris yellow spot virus* (Tospovirus) naturally infecting onion plants in Peru
*A. S. Oliveira, R. C. T. Aliaga, T. A. Melgarejo, R. N. Lima and **R. O. Resende***

Ec12 Sequence analysis of *Potato virus M* isolates from Czech Republic
***Helena Plchova**, N. Cerovska, T. Moravec and P. Dedic*

Ec13 Use of plant viruses in non-food agriculture
***Noemi Cerovska**, H. Plchova, T. Moravec, H. Hoffmeisterova, J. Folwarczna, V. Ludvíkova and M. Smahel*

Ec14 Genetic diversity of *Pepino mosaic virus* in the U. S. and identification of a tomato infecting strain capable of inducing disease on potato
Kai-Shu Ling

Ec15 Evolution of resistance-breaking in *Tomato spotted wilt virus*: response to selection by *sw5* mediated resistant tomato
***Jessica L. Houle**, J. W. Moyer and G. G. Kennedy*

Ec16 Molecular characterization of endogenous plant pararetroviruses in wild *Dahlia* sp from natural habitats
S. G. Eid, C. V. Almeyda, K. L. Druffel, D. E. Saar and ***H. R. Pappu***

Ec17 Molecular epidemiology of *African cassava mosaic viruses* in Yangambi, northeastern Democratic Republic of Congo
G. Monde*, J. Walangululu, S. Winter and C. Bragard*

Ec18 Three commonly co-occurring perennial grass species have less herbivore and pathogen attack in their introduced range than in their native range
G. Kai Blaisdell *and Bitty A. Roy*

5:00-6:00PM Business Meetings to be held independently for IPVE and PVEN

The PVEN business meeting will be held Tuesday @ 5:00 in Room 105 Robert Purcell Community Center

The IPVE business meeting will be held Tuesday @ 5:00 PM in Room 303, Appel Commons

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Wednesday – Vector Biology/Virus Transmission

8:00-8:15 Announcements – Room 303 Appel Commons

Moderator – Nilsa Bosque-Perez

8:15-9:00 Keynote Speaker:

Stephane Blanc, INRA, France

The seemingly simple non-circulative transmission of a plant virus is hiding an extremely sophisticated interplay between virus, plant and vector

Invited Presentations

9:00 - Bryony Bonning, Iowa State University, U.S.

An aphid gut binding peptide impedes entry of *Pea enation mosaic virus* into the aphid hemocoel

S. Liu and ***B. Bonning***

9:30 - Rodrigo Almeida, Univ. California Berkley, U.S.

Grapevine leafroll-associated viruses – mealybug transmission biology and ecology

R. P. P. Almeida *and K. M. Daane*

10:00 – Coffee Break

10:30 - Piotr Trebicki, DPI, Biosciences Division, Australia

The impact of elevated CO_2 on wheat, *Cereal yellow dwarf virus* and its aphid vector

P. Trebicki*, A. Freeman, J. Luck, M. Aftab, S. King, M. Spackman, G. Fitzgerald, K. Powell, S. Seneweera and N. A. Bosque-Perez*

11:00 - James Ng, Univ. California Riverside, U.S.

Virus-vector interactions mediating the specific retention and whitefly transmission of criniviruses

J. Ng

11:30 - Drake Stenger, USDA, ARS, U.S.

Sequence polymorphism of a glassy-winged sharpshooter phytoreovirus reveals a bottleneck in the Californian population

D. Stenger

12:00-1:30 – Lunch – North Star Cafeteria, Appel Commons

15 minute Oral Presentations – Room 303 Appel Commons

Moderator – Rodrego Almeida

1:30 – Differential transmission rates of PVY^{O} and PVY^{NTN} from two inoculum sources by three aphid vectors

J.M. Alvarez *and F. Cervantes*

1:45 – Vector fitness on infected plants affects virus epidemiology
***Belén Belliure**, B. Sabater-Muñoz, M. E. Martínez and M. R. Albiach-Marti*

2:00 – Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts
***Kerry Mauck**, C. De Moraes and M. Mescher*

2:15 – Comparative genome analysis of an asymptomatic *Citrus tristeza virus* isolate with its symptomatic aphid transmitted sub-isolates
***Avijit Roy**, N. Choudhary, V. D. Damsteegt and R. H. Brlansky*

2:30 – Alterations of capsid protein amino acid positions internal to the virion disrupt nonpersistent virus transmission by aphids
*C. A. Bricault and **Keith Perry***

2:45 – New insights on the transmission mechanisms of plant viruses by their aphid vectors
*A. Moreno, E. Garzo, G. Fernandez, M. Kassem, M. A. Aranda and **A. Fereres**.*

3:00 – Identification of *Myzus persicae* proteins that interact with PVY HC-Pro *in vitro*
***Ahmad Al-Mrabeh**, A. Ziegler, B. Fenton, G. Cowan and L. Torrance*

3:15 – Biomarkers distinguishing virus transmission competent and refractive insect populations identified by coupling genetics with quantitative intact proteomics
***Michelle Cilia**, C. Tamborendeguy, K. Howe, T. Fish, T. Thannhauser and S. Gray*

5 minute Poster Advertisements – Room 303 Appel Commons
Moderator – Russ Groves

3:30 – Determination of aphid transmission efficiencies for N, NTN and Wilga strains of *Potato virus Y*. Poster # Vb1
***Martin Verbeek**, P. Piron, A. Dullemans, C. Cuperus and R.van der Vlugt*

3:35 - Antagonistic effects of PVY-infected potato plants on aphids. Poster # Vb2
*S. Boquel, P. Giordanengo and **A. Ameline***

3:40 - Variation in transmission of *Tomato spotted wilt tospovirus* among isolates and populations of a vector, *Thrips tabaci.* Poster # Vb3
***Alana Jacobson** and G. Kennedy*

3:45 - Do secondary bacterial endosymbionts of *Sitobion avenae* clones affect vector specificity or transmission efficiency for barely yellow dwarf virus? Poster # Vb4
***Hussein Alkhedir**, S. Vidal, P. Karlovsky, A. Habekuss, and E. Schliephake*

3:50 - What's new in polerovirus transmission? Poster # Vb5
***Sylvaine Boissinot**, B. Bencharki, B. Monsion, S. Revollon, M. Erdinger, C. Reinbold, V. Ziegler-Graff, S. Tanguy, D. Tagu and V. Brault*

4:00 – 6:00 Posters – Room 218, Robert Purcell Community Center

Coffee, Iced Tea and snacks provided –cash bar also available
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Vb6 Transmission of several isolates of *Tomato spotted wilt virus* (TSWV) by *Frankliniella occidentalis*
D. Debreczeni, L. Rubio, J. Aramburu, L. Galipienso, C. López, S. Soler and ***Belén Belliure***

Vb7 Transmission of two isolates of *Broad bean wilt virus*1 (BBWV-1) by several aphid species
I. Ferriol, L. Rubio and ***Belén Belliure***

Vb8 Responses of *Myzus persicae* to headspace volatiles of *Nicotiana benthamiana* infected with artificial mutants of *Potato leaf roll virus*
Sanford D. Eigenbrode*, H. Ding, A. V. Karasev and J. Kuhl*

Vb9 Bird cherry-oat aphid behavior in response to barley yellow dwarf virus disease infection of wheat
L. L. Ingwell, ***Nilsa A. Bosque-Perez****, L. M. Unger, H. Ding, A. V. Karasev and S. D. Eigenbrode*

We will board the buses at 5:30PM for a 45 minute trip to Wagner Vineyard and Micro-Brewery located on the east side of Seneca Lake, the largest and deepest of the Finger Lakes. There will be a reception from 6:30-7:30. Appetizers and a cash bar will be available. The winery tasting room and store will be open at this time if you want to sample the Wagner Wines and Beers or pick up some New York State Wine paraphernalia. A buffet dinner will be served at 7:30. We should be back on campus by 10:30.



Thursday – Virus Disease Management/Detection/Diagnosis

8:00-8:15 Announcements – Room 303 Appel Commons

Moderator – Scott Adkins
8:15-9:00 Keynote Speaker
Ian Barker, CIP, Nairobi
Clean seed programs in Africa

Invited Presentations

9:00 - Jari Valkonen, University of Helsinki, Finland
Cryotherapy of shoot tips as an efficient means for virus and phytoplasma elimination and healthy plant production
J. Valkonen

9:30 - Scott Adkins, USDA, ARS, U.S.
Ecology and management of whitefly-transmitted vegetable viruses in Florida
S. Adkins*,C.G. Webster, C. S. Kousik, S. E. Webb, P. D. Roberts, P. A. Stansly and W. W. Turechek*

10:00 Coffee Break

10:30 - Britt-Louise Lennefors, Syngenta Corp, Sweden
Combination of natural and engineered resistance to rhizomania in sugar beet
B. Lennefors*, E. I. Savenkov,J. Bensefelt, E. Wremerth-Weich, P. van Roggen, S. Tuvesson, J. P. T. Valkonen and J. Gielen*

11:00 - Renato Resende – Universidade de Brasilia, Brazil
Development of broad, stable and durable resistance to monopartite and bipartite begomoviruses in Brazilian tomato lines
R. Resende

11:30 - Russell Groves, University of Wisconsin, U.S.
Modeling vector flights to increase effectiveness of foliar protectant programs
R. L. Groves*, A. Charkowski and A. Crockford*

12:00-1:30 – Lunch – North Star Cafeteria, Appel Commons

15 minute Oral Presentations – Room 303 Appel Commons
Moderator – Jari Valkonen

1:30 – A mutation in the NIB cistron of *Potato virus Y* confers virulence towards the *Pvr4* resistance of pepper and a high competitiveness cost in susceptible cultivar
B. Janzac, J. Montarry, A. Palloix, O. Navaud and ***Benoît Moury***

1:45 – Modulation of virus-host plant interplay in the tomato yellow leaf curl disease by using insect resistance in the tomato host
M. J. Rodriguez-López, E. Garzo, J. P. Bonani, A. Fereres, R.Fernández-Muñoz and ***E. Moriones***

2:00 – Mild and aggressive *Pepino mosaic virus* isolates: tomato transcriptomic responses and the potential of cross-protection as a control strategy
Inge M. Hanssen, *H. P. van Esse, A. Paeleman, I. Gutiérrez-Aguirre, K. Goen, L. Wittemans, B. Lievens, M. Ravnikaran- Bart and P. H. J. Thomma*

2:15 – Controlling the banana bunchy top disease pandemic in sub-Saharan Africa
P. Lava Kumar*, R. Hanna, A. Fotso, M. Soko, S. A. Akinbade, O. J. Alabi, J. Ngeve and R. A. Naidu*

2:30 – Improved virus diagnostics to support seed certification in Australia
B. C. Rodoni*, M. Milinkovic, C. Bottcher, P. Pongsapit and S. Sombat*

2:45 – Epidemiology of *Plum pox virus* (PPV) in nursery blocks and evaluation of the effect of horticultural mineral oil treatments
Eduardo Vidal*, A. Moreno, E. Bertolini and M. Cambra*

3:00 – Biological control of vectors affects virus dispersal
Belén Belliure*, H. R. Amorós, M. A. Marcos-Garcia, I. R. Steba, A. Moreno and A. Fereres*

3:15 – A cucumber mosaic virus mutant that induces resistance to its aphid vector in tobacco
Heiko Ziebell*, A. Murphy, M. G. Lewsey, J. H. Westwood, K. L. Perry, M. Stevens and J. P. Carr*

5 minute Poster Advertisements – Room 303 Appel Commons
Moderator – Ian Barker

3:30 – Rhizomania of sugar beet: Similarities and differences between the Iranian and European situation. Poster # Dm1
Y. Galein, A. Champeil, M. Merhvar and ***C. Bragard***

3:35 – Survival of *Pepino mosaic virus* in aqueous environment reveals the need for efficient detection system suitable not only for plant but also for environmental samples. Poster # Dm2
Maja Ravnikar*, N. Mehle, N. Prezelj, D. Delić, U. Vidic, P. Kramberger and I. Gutieerrez-Aguirre*

3:40 – Viruses in weeds in *Dioscorea* yam fields in Nigeria. Poster # Dm 3
S. Asala*, C. P. Shinggu, R. Asiedu and P. Lava-Kumar*

3:45 – Simultaneous detection of two important bean RNA viruses by multiplex reverse transcription polymerase chain reaction. Poster # Dm4
*A. Saidi, **Nooh Shahraeen** and A. Azizi,*

3:50 – Distribution of PVY strains in susceptible and moderately resistant North American cultivars. Poster # Dm5
***Jonathan Whitworth**, S. Gray, A. Karasev and J. Lorenzen*

3:55 – Detection and molecular epidemiology of *Oat sterile dwarf virus.* Poster # Dm6
*I. Eriksson, J. N. E. Ramsell, A. Linnell, N. Shad, J. Holmblad, B. Ekbom, L. Frykberg, F. Rabenstein and **Anders Kvarnheden***

4:00 – 6:00 Posters – Room 218, Robert Purcell Community Center

Coffee, Iced Tea and snacks provided –cash bar also available
AUTHORS – please plan to be at your posters from at least 4:30-5:00

Dm7 Restricted spread by *Bemisia tabaci* of *Tomato yellow leaf curl virus* in the begomovirus-resistant Brazilian line TX-468
*R. C. Pereira-Carvalho, J. A. Díaz-Pendón, R. Fernández-Muñoz,R. O. Resende, L. S. Boiteux and **E. Moriones***

Dm8 Occurrence, incidence and distribution of viruses infecting yam (*Dioscorea* spp) in Nigeria
***S. Asala**, M. D. Alegbejo, B. D. Kashina, O. O. Banwo, R. Asiedu and P. Lava-Kumar*

Dm9 A *Cucumber mosaic virus* 2b-mutant was not able to establish a systemic infection in bell pepper (*Capsicum annuum* L.)
*J. Masiri, N. V. Velasquez and **John F. Murphy***

Dm10 Incidence and prevalence of *Strawberry mild yellow edge virus* (SMYEV) in Argentina
*A. K. Torrico, F. Fernández, A. Ishikawa, N. G. Meneguzzi, L. R. Conci, M. del Huerto Sordo, A. M. Borquez, R. Pacheco, V. Obregón, D. S. Kirschbaum and **Vilma C. Conci***

Dm11 Plum pox potyvirus
Vahida Seremet

Dm12 A tospovirus new to North America: virus detection and discovery through the use of a macroarray for viruses of solanaceous crops
***Keith L. Perry** and X. Lu*

Dm13 Toward aphid-resistant transgenic plants
*S. Liu, Z. Wang, S. Sivakumar, L. Georgievska, G. F. King, W. A. Miller and **Bryony C. Bonning***

Dm14 Potyviruses of legume weeds and *Passiflora* spp. from Western Australia: Biological properties and phylogenetic placement of coat protein sequences
B. A. Coutts, M. A. Kehoe, C. J. Webster, S. J. Wylie and ***R. A. C. Jones***

Dm15 Incidence and control of cucurbit viruses in NWFP Pakistan
Asad Ali*, A. Hussain, M. Ahmad and T. Natsuaki*

Dm16 Post-transcriptional gene silencing (PTGS) is a mechanism of plant host-defense against viruses
P. Gouveia*, A. Costa and G. Nolasco*

Dm17 Development of several laboratory assays for the detection of *Apricot latent virus*
L. Grimová*, M. Zouhar and P. Ryŝánek*

Dm18 Virus diseases of cereal crops in the Czech Republic
J. Jarošová, S. Gadiou, J. Ripl and ***Jiban Kumar Kundu***

Dm19 *Potato leafroll virus* (PLRV) resistant potatoes
Tomas Moravec*, H. Plchova, P. Dedic and N. Cerovska*

Dm20 Viral infection of wild orchids in Ukraine
V. Polischuk*, I. Budzanivska and G. Koroteeva*

Dm21 Analysis of the temporal and spatial spread of *Plum pox virus* as influenced by USA and Canadian eradication programs
A. Gougherty and ***Forrest W. Nutter, Jr.***

Dm22 *Bean common mosaic virus* and *Bean common mosaic necrosis virus* in Mazandaran province of Iran
Shahraeen Nooh*, S. Asghari and G. Shereen*

Dm23 Precision breeding *Potato virus Y* resistance using a modified potato gene
J. Cavatorta, M. Jahn and ***S. Gray***

Dm24 Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy
Andreas Voloudakis, Thomas Hohn and Maria Holeva (**Jari Valkonen** presenting)

6:00 – The conference is officially adjourned. For those of you staying in Ithaca we will have the hospitality suite open from 7:00 – 11:00PM.

Thank you for attending the conference and have a safe trip home.

Monday, June 21, 2010

Virus Epidemiology and Etiology

Modelling plant virus transmission: from within-plant virus dynamics to epidemic development.

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A full understanding of plant virus epidemiology requires studies at different scales of interaction: from within-plant cell processes to vector population dynamics, behaviour and broader ecological interactions. Viruses multiply and move systemically within plants, sometimes interacting with other viruses and often with evolutionary change as a consequence. Vectors respond to various cues derived from plants (both healthy and virus-infected), natural enemies and other environmental influences, that directly affect the temporal and spatial pattern of disease dynamics. The key element in linking these scales of interaction is the transmission process and the determining factors involved. We use mathematical models to show how: (a) consideration of within-plant virus dynamics can limit the extent to which epidemics develop, and possibly explain reports of few but heavily affected diseased plants in an otherwise healthy plant population; and (b) natural enemies can increase the rate of epidemic development (confirming results obtained in microcosm studies) while at the same time reducing population numbers.

A tale of two epidemics: the contrasting dynamics of cassava mosaic and cassava brown streak diseases in East Africa

[1]Legg JP, [2]Jeremiah SC, [3]Obiero HM, [4]Maruthi MN, [5]Ndyetabula I, [6]Okao-Okuja G, [7]Kim DJ, [1]Bouwmeester H, [8]Bigirimana S, [9]Tata-Hangy W, [10]Gashaka G, [11]Mkamilo G, [12]Alicai T and [13]Kumar PL

[1] – IITA-Tanzania, Dar es Salaam, Tanzania; [2] – IITA-Tanzania and LZARDI, Mwanza, Tanzania; [3] – KARI, Kakamega, Kenya; [4] – NRI, Chatham, UK; [5] – MARI, Bukoba, Tanzania; [6] – IITA-Uganda, Kampala, Uganda; [7] – IITA-Kenya, Nairobi, Kenya; [8] – ISABU, Gitega, Burundi; [9] – INERA, Bukavu, Democratic Republic of Congo; [10] – ISAR, Butare, Rwanda; [11] – NARI, Mtwara, Tanzania; [12] – NARO, Namulonge, Uganda; [13] – IITA-Nigeria, Ibadan, Nigeria

The rapid geographical expansion of the cassava mosaic disease (CMD) pandemic, caused by cassava mosaic geminiviruses, is well documented. Twelve countries of East and Central Africa are now known to be affected. Region-level epidemiological studies continue to reveal a more-or-less regular pattern of annual spread along a contiguous 'front'. More recently, outbreaks of cassava brown streak disease (CBSD) were reported from Uganda and other parts of East Africa that had been hitherto unaffected. Recent survey data reveal several significant contrasts between the regional epidemiology of these two pandemics. 1. Severe CMD spreads in an 'expanding concentric rings'-like manner, whilst CBSD seems to be spreading from independent hot-spots; 2. CMD pandemic spread has been tightly linked with the appearance of super-abundant *B. tabaci* whitefly populations, in contrast to CBSD, where outbreaks have occurred 3-10 years after whitefly population increases; 3. The severe CMD pandemic has arisen from virus recombination and inter-species synergy, whilst current knowledge suggests that the CBSD pandemic is a 'new encounter' situation. Field epidemiology experiments conducted in CBSD hot-spots in southern Uganda and north-western Tanzania confirm associations between the spread of both virus diseases and *B. tabaci* abundance, although the link was weaker for CBSD, suggesting a different mode of transmission. Field data provide no evidence for a synergistic interaction between CMD and CBSD. Moreover, no link is apparent between varietal resistance to CMD and resistance/susceptibility to CBSD. Although some of the varieties being promoted for their CMD resistance are susceptible to CBSD, the inter-regional movement of planting material does not appear to be the primary cause of CBSD spread. The scale and economic impact that is a common feature of both cassava virus diseases highlights the continuing need to conduct research that will facilitate the development of novel and effective ways to manage them.

Molecular epidemiology of potyviruses infecting cucurbits in France: a case study for understanding evolution of plant virus populations

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Two major potyviruses, *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV), infect cucurbits in France, with contrasted epidemiological situations. WMV has a high prevalence every year throughout the growing season, whereas ZYMV epidemics are very irregular both in spatial distribution and timing of infection. Large-scale epidemiological surveys followed by molecular analysis of all collected samples revealed the recent appearance of new, "emerging" (EM) isolates of both viruses in south-eastern France. Four subgroups of EM isolates of WMV were defined, probably resulting from several introductions. They presented a strong geographic structure that remained stable over 5 years, and tended to replace rapidly the original "classic" isolates in areas where both types of strains were present. For ZYMV, new isolates were also detected in the last years, although the geographic structure of infections and evolution of populations was less obvious than for WMV.
Multiple and complementary approaches including multilocal and multiscale epidemiological studies, molecular analyses, and population genetics, were used to characterize the evolution of viral populations, in order to estimate the agronomical risks associated with the emergence of new strains of cucurbit-infecting potyviruses in France.

Epidemiology of *Zucchini yellow mosaic virus*: effectiveness of non-host barriers, spread, resistance breakdown, alternative hosts and molecular characterization

B.A. Coutts1, M.A. Kehoe1, S.J. Wylie2, C.G. Webster2,3 and R.A.C. Jones1,2
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Field experiments with Z*ucchini yellow mosaic virus* (ZYMV) were done in the tropical (Kununurra), sub-tropical (Carnarvon) and Mediterranean (Medina) climatic regions of Western Australia. At Kununurra, the objective of cultural control experiments with cucurbits was to delay spread of ZYMV infection until after flowering and early fruit set, thereby minimising yield and quality losses. Planting upwind, tall non-host barriers (millet) and manipulation of sowing date all helped to delay spread. Manipulation of sowing date was the most effective, decreasing ZYMV incidence by up to 70%. When commercial ZYMV-resistant, pumpkin and zucchini cultivars were exposed to infection in experiments at Kununurra and Medina respectively, a Kununurra isolate overcame resistance in pumpkin and zucchini, although there were still yield and quality benefits from delayed spread. In contrast, when a local isolate was used with pumpkin at Carnarvon, resistance was not overcome. Similarly, resistance was not overcome when ZYMV-resistant cucumber cultivars were exposed to a Kununurra isolate at Medina. When five commercial ZYMV-resistant zucchini cultivars were sap inoculated, systemic infection occurred in 5/5 with a Kununurra isolate, 3/5 with a Carnarvon isolate, and 1/5 with a Victorian isolate, reflecting the different resistances present.

Surveys for alternative hosts among weed and native plants that tested >6600 samples (>30 species) were done at Kununurra and Carnarvon. ZYMV was only detected in three samples of *Mukia maderaspatana*, a native cucurbit. Seed from infected pumpkin and zucchini was germinated and seedlings tested by ELISA, ZYMV was detected in 0.2% zucchini but not in >4800 pumpkin seedlings.

The complete coat protein genes of 35 ZYMV isolates from seven cucurbit growing areas around Australia were sequenced. Phylogenetic analysis of nucleotide sequences revealed that all 25 isolates from Kununurra grouped with Singapore/Reunion Island isolates. In contrast, isolates from the six other areas (including Carnarvon) clustered with isolates from elsewhere.

Epidemiology of Tomato Spotted Wilt in Peanut in the Southeastern United States

Albert K. Culbreath, Dept. of Plant Pathology, and Rajagopalbabu Srinivasan, Dept. of Entomology, The University of Georgia, Tifton, 31793-0748

Tomato spotted wilt, caused by *Tomato spotted wilt tospovirus* was observed in peanut (*Arachis hypogaea*) in the late 1980s and rapidly became a major limiting factor for peanut production areas Alabama, Georgia, and Florida. Tobacco thrips (*Frankliniella fusca*) and western flower thrips (*Frankliniella occidentalis*) both occur on peanut in Georgia, but *F. fusca* is the predominant species that reproduces on peanut, and is thought to be the more important vector. Several noncrop sources of potential primary vectors and TSWV inoculum have been identified, but the relative importance of those has not been concluded. The peanut growing season in Georgia runs from April through November, and volunteer peanut plants are present much of the remainder of the year, so peanut itself has huge potential for perpetuating vector and virus. Symptoms are often evident within a few days after seedling emergence, and apparent disease progress is often rapid within the first 50-60 days after planting. Based on destructive sampling and assay for TSWV, there often is a high incidence of asymptomatic infections even in peanut genotypes that produce few symptoms of infection in the field. Planting date can have a large effect on incidence of spotted wilt within a location. This may be linked to thrips reproductive cycle and environmental effects on the plant and plant-thrips-virus interactions. Severity of spotted wilt epidemics fluctuates significantly from year to year. Explanation for the variability has not been concluded, but lower levels of tomato spotted wilt intensity have been associated with years categorized as “La Niña” in the El Niño-Southern Oscillation. An integrated management program has been successful in minimizing losses to spotted wilt in peanut during the last ten years.

Influence of virus-induced changes in plants on aphid vectors and potential impacts on virus epidemiology

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Plant virus infection can alter the suitability of host plants for their aphid vectors. Most reports indicate that virus-infected plants are superior hosts for vectors compared to noninfected plants with respect to vector growth rates, fecundity and longevity. Some aphid vectors preferentially respond to virus-infected plants compared to noninfected ones, while others avoid infected plants that are inferior hosts. Thus, it appears vectors can exploit changes in host plant quality associated with viral infection. Enhanced vector performance and preference for virus-infected plants might also be advantageous for viruses by promoting their spread and possibly enhancing their fitness. Our research and that of our colleagues has focused on two of the most important luteoviruses that infect wheat, [*Barley yellow dwarf virus* (BYDV)], or potato, [*Potato leafroll virus* (PLRV)], and their respective aphid vectors, the bird-cherry oat aphid, *Rhopalosiphum padi*, and the green peach aphid, *Myzus persicae.* Our work has demonstrated that virus infection alters the concentration and relative composition of volatile organic compounds in host plants. We have also shown that non-alates of each vector species settle preferentially on virus-infected plants and that such responses are mediated by volatile organic compounds. Furthermore our findings indicate that plants respond heterogeneously to viral infection and as a result different plant parts change in attractiveness to vectors during infection and vector responses to virus-infected plants are dynamic. Such dynamic responses could enhance or reduce the probability of virus acquisition by individual aphids searching within plants. Finally, our work indicates that compared to non-viruliferous aphids, viruliferous ones are less or not responsive to virus-induced host plant volatiles. Changes in vector responsiveness to plants after vectors acquire virus could affect virus field spread. Recent findings from our research will be presented and potential impacts on virus disease epidemiology will be highlighted.

Landscape/Pathosystem Components that Contribute to Epidemic Risk in Plant Virus Pathosystems: A Conceptual Model

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The flow of virus inoculum from alternative hosts to a susceptible crop and back to alternative hosts is a dynamic, cyclical process that often involves multiple plant and insect species. Alternative host and insect vector species can vary in relative seasonality and abundance, as well as their inherent epidemiological risk. In the *Tobacco etch virus* (TEV)/bell pepper/aphid pathosystem, six solanaceous weed hosts are known to serve as alternative hosts for TEV, as well as for several aphid species. It cannot be assumed, however, that all six alternative weed hosts represent the same level of epidemiological risk. Two perennial weed host species emerge in early March (before peppers are transplanted), whereas four annual, alternative weed host species emerge primarily after peppers are transplanted. Moreover, aphids may not acquire TEV from TEV-infected weed hosts with equal efficiencies. For example, the green peach aphid (*Myzus persicae*; GPA) was found to have a mean acquisition efficiency of 19% after feeding on TEV-infected pepper source plants, whereas mean acquisition efficiency from TEV-infected annual ground-cherry source plants was 36.8% (two times higher). Similarly, not all alternative weed hosts are as receptive to TEV transmission from TEV-infected pepper plants: mean receptivity efficiency from pepper-to-pepper (by GPA) was found to be 38%, compared to 80% receptivity for pepper-to-jimsonweed. With regards to aphid seasonality, the potato aphid (*Macrosiphum euphorbiae*) was present when peppers were transplanted (GPA arrived several weeks later). Also, acquisition and transmission efficiencies for *M. euphorbiae* were 3 to 4 times higher than transmission efficiencies for GPA. Thus, early-season transport of TEV from TEV-infected weed hosts to the pepper crop is limited not only by the presence or absence (seasonality) of specific weed hosts, but also by seasonality and abundance of aphid species within the local landscape. By quantifying landscape, vector, alternative weed host and cropping system components, conceptual models describing landscape and pathosystem component interactions can be synthesized, tested, and refined.

Emergence, Establishment, and Epidemiology of *Cucurbit yellow stunting disorder virus* in California

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Cucurbit yellow stunting disorder virus (CYSDV) emerged in southwestern US cucurbit production areas in the fall of 2006, infecting virtually the entire fall melon crop and causing severe yield losses. Reemergence of CYSDV in the spring following an extended freeze suggested the presence of weed or alternate crop hosts, although previous studies indicated a host range restricted to members of the *Cucurbitaceae*. Weed and crop plants were collected from throughout California's Imperial Valley over a period of three years, and tested by RT-PCR using CYSDV HSP70h- and coat protein gene-specific primers. Many non-cucurbits collected from infected melon fields and nearby areas were symptomless and virus free; however, CYSDV was detected in plants of seven non-cucurbit families common in the region including alfalfa (*Medicago sativa*), lettuce (*Lactuca sativa*), and snap bean (*Phaseolus vulgaris*), and several widely prevalent weed species. Most non-cucurbit hosts were symptomless; however, transmission tests demonstrated that several asymptomatic hosts were effective reservoirs for transmission to melon. Efforts are in progress to identify the most significant reservoirs for overwintering and transmission to spring melons. The entire fall crop has been infected with CYSDV annually since 2006, resulting in reduced acreage planted. CYSDV incidence in spring melons has increased each year; in 2009 63% of spring fields were infected and significant portions of several fields exhibited symptoms by late May. A new source of resistance, PI313970, exhibited resistance to CYSDV in field trials in the region, and studies are in progress to introgress this new resistance into preferred melon varieties and combine the resistance with the better known resistance source from TGR-1551. Recent studies have illustrated the potential to dramatically reduce symptom development when resistance is combined with an active insecticide treatment program, suggesting the potential to return to fall melon production with development of effective resistance in melon.

Epidemiology of *Iris yellow spot virus* in eastern North American onion ecosystems

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Iris yellow spot virus (IYSV) (*Bunyaviridae: Tospovirus*) is a major pathogen of onion and other *Allium* spp. IYSV was first detected in the US in 1989, but was not discovered in northeastern North America until 2006. IYSV can cause significant economic loss because infected onion plants often produce undersized bulbs. Onion thrips, *Thrips tabaci*, is the principal vector of IYSV and indirectly reduces onion bulb yield by feeding on leaves. Managing IYSV is difficult because there are no onion varieties that are resistant to either the virus or the vector that can be grown successfully in the eastern US. Knowledge of the epidemiology of IYSV and its relationship with onion thrips is needed so that alternative strategies to mitigate the spread and severity of IYSV in onion fields can be developed. In 2007 and 2008, sources of IYSV in New York's onion ecosystem were identified and temporal patterns of IYSV-infected plants were described for dozens of commercial onion fields. Sources of IYSV included onion transplants imported from Arizona, volunteer onion plants in non-rotated fields and cull piles, and several perennial weed species. Weeds may be the most abundant source of IYSV in the onion ecosystem and also would allow the virus to bridge seasons. In 2007 and 2008, IYSV was first detected in onion fields in June and July, but levels remained very low (<12% samples infected) until mid to late August when some fields reached near 100%. Adult onion thrips densities late in the season were positively correlated with final IYSV levels. The time onions were harvested was likely important in determining final IYSV levels because viruliferous thrips emigrated from harvested fields into late-harvested fields. Managing thrips populations before harvest, and manipulating the spatial arrangement of fields based on harvest date could mitigate the spread of IYSV.

Search for factors potentially involved in the rapid shift in *Watermelon mosaic virus* (WMV) populations in south-eastern France

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Watermelon mosaic virus (WMV, *Potyvirus*) has been first reported in France 35 years ago, and it is now the most prevalent virus in cucurbit crops. WMV has a relatively wide host range –including several weeds- and is non-persistently transmitted by more than 35 aphid species. In 2000, new strains (referred as 'emerging' (EM) strains) were detected in south-eastern France. EM strains are generally more severe and phylogenetically distinct from those previously reported in France (referred as 'classic' (CL) strains). EM strains are divided in four phylogenetic subgroups. Since 2000, EM strains have been progressively replacing CL strains in cucurbit crops in south-eastern France. In order to look for factors that may explain this rapid change in virus populations, properties such as host range, symptomatology in susceptible hosts and aphid transmissibility were investigated for a set of 26 WMV isolates including isolates from the 3 phylogenetic groups and CL-EM or EM-EM recombinant isolates. A modelling approach, based on a 3-year observation of the spatio-temporal spread of EM and CL WMV strains in zucchini squash fields at Montfavet suggested that a dissymmetrical partial cross-protection between EM and CL strains could be a major factor in the population shifts that are observed. This hypothesis will be discussed in the light of the results of the biological tests.

Pepino mosaic virus: What do we know sofar and how to proceed

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Pepino mosaic virus is a typical example of how a 'new' plant virus can cause major problems worldwide in a very short period of time. After its first scientific description in 1980 it was not reported until it suddenly appeared in the beginning of 1999 in indoor tomato crops in the UK and the Netherlands. Since then it has spread very rapidly and is causing sometimes severe problems in tomato production.
Reports on symptom severity and economic effects of the virus were conflicting and initially believed to be related to particular virus strains. More detailed research has now learned that there are mild and severe isolates within each PepMV strain.
Within Europe the virus quickly led to a debate about the possible economic impact of the virus and the necessity of a quarantine (Q) status. A Q-status on tomato seeds was issued but many questions on the precise dangers of the virus remained. A detailed Pest Risk analysis (PRA) was needed and in 2007 The European Commission financed an EU-wide research project (PEPEIRA) to produce the scientific data needed for such a PRA.
In January 2010 this project ended and it has indeed produced a significant amount of scientific data on PepMV. Detailed knowledge on seed transmission, biological properties on various Solaneceous crops and optimized detection protocols as well as detailed data on the biological and economic effects of different virus strains on tomato crops. These will be discussed.
PepMV is an example of how a relatively simple plant virus can not only lead to direct economic damage but also to considerable confusion on the regulatory level. Which lessons can we learn from this virus that can help us to better deal with future plant virus problems?

The epidemiology of *Tobacco streak virus* in central Queensland, Australia

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Tobacco streak virus (TSV) is transmitted by thrips via infected pollen, and has recently caused serious losses in sunflower and grain legume crops, especially mungbeans, in central Queensland. The virus was detected in 20 weed species in the region, including parthenium (*Parthenium hysterophorus*) and crownbeard (*Verbesina encelioides*). Isolates of TSV from affected crops had near-identical genome sequences to those from parthenium, a symptomless host which is commonly infected in central Queensland, independent of any association with crops. Seed transmission rates up to 48% were demonstrated in parthenium and these rates did not decline in seed stored at ambient temperature for over two years. The virus was transmitted by the commonly collected thrips species, *Frankliniella schultzei* and *Microcephalothrips abdominalis* using TSV-infected parthenium pollen. TSV is also frequently found in crownbeard, whose distribution overlaps the southern range of parthenium. The virus was also symptomless in crownbeard and seed transmitted at rates of up to 37%. Interestingly, isolates of TSV from crownbeard and parthenium are genetically distinct (85.4% identity for coat protein amino acid sequence) and appear to be restricted to their respective hosts in nature, even when infected plants of both species are growing together. Epidemics of TSV in crops are linked to the presence of parthenium, a host which produces prolific quantities of pollen, and can harbour the virus in infected seed through adverse climatic conditions including drought and cold winters. The crownbeard strain had a coat protein nt identity of 97% with an archived sample of *Xanthium occidentale* from 1975, suugesting a long presence of this strain in Australia. The parthenium strain is likely to be a more recent incursion, as the disease in sunflower was not apparent prior to 2000, and the genetically most similar isolate known is from Brazil (GenBank AY354406, 98% coat protein nt identity).

Alternative hosts of two Tospoviruses in Queensland, Australia

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The tospoviruses (Genus: *Tospovirus*, Family: *Bunyaviridae*), *Tomato spotted wilt virus* (TSWV) and *Capsicum chlorosis virus* (CaCV) cause important diseases in capsicum, tomato and peanut in Queensland. As part of investigations into the management of these viruses, the alternative hosts of the two viruses have been examined.

TSWV infects a range of annual weed species including *Bidens pilosa*, *Sonchus oleraceus, Tagetes minuta* and several *Solanum spp.*. While these and other species can be locally important sources of virus during the cropping season they are often not well adapted to survival during harsh conditions of winter or summer. However, the introduced perennial species *Stachytarpheta jamaicensis* (Jamaican snakeweed; *Verbenaceae*) is commonly infected with TSWV in overgrazed pastures and disturbed areas, particularly in north and eastern Queensland. Surveys over five years indicate this species is likely to have an important role in the survival of TSWV, providing a virus source for thrips transmission into nearby susceptible vegetable crops. Recently, TSWV isolates from Jamaican snakeweed at several geographically separate locations have been virulent when inoculated onto capsicum cultivars having TSWV resistance conferred by the *Tsw* gene. This virulence appears to have occurred in the absence of selection pressure imposed by the *Tsw* gene.

Survey data indicates that CaCV has fewer weed hosts than TSWV. However, *Ageratum conyzoides* (Billygoat weed; *Asteraceae*) is a common and symptomless host of CaCV, widely distributed throughout some 1000 km of coastal Queensland.

Infection levels exceeding 50% have been found in random samples and high infection levels in tomato and capsicum crops are linked to the presence of infected *Ageratum*.

Epidemiological Analysis of Multi-Virus Infections of Watermelon in Experimental Fields in Southwest Florida

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Whitefly-transmitted *Squash vein yellowing virus* (SqVYV), *Cucurbit leaf crumple virus* (CuLCrV), and *Cucurbit yellow stunting disorder virus* (CYSDV) have had serious impact on watermelon production in southwest and west-central Florida in recent years. We monitored the progress of all three viruses and the density of whiteflies in a 2.5 acre experimental field of 'Fiesta' in which whiteflies were not controlled, located at SWFREC in Immokalee, FL over the course of 5 growing seasons. Symptoms of CuLCrV were generally found before SqVYV and were present as soon as 5 weeks after planting (wap). Symptoms of SqVYV appeared about 7 wap in nearly all seasons and the plants fully collapsed from SqVYV infection by 12 wap. Both viruses appeared in 4 of the 5 seasons but were conspicuously absent in the spring 2009 season. Weather-related phenomena are suspected as the cause of the absence of viruses in this season. In our experiment, CYSDV was first found 6 wap in the 2009 fall season although its presence in the region has been known since 2008. The largest number of whiteflies was typically found midway through the season, just prior to the rapid collapse of plants. Preliminary analyses indicated that the degree of association between SqVYV and CuLCrV was not greater than would be expected from random arrangement of the two viruses, and that SqVYV was distributed randomly at low incidences, but became more aggregated as disease increased. These results are an indication that the viruses are being introduced independently by whiteflies, although the whiteflies may be emigrating from the same source, with secondary spread being dominated by within-field populations of whiteflies. This pattern is supported by other results showing that the two viruses were generally spatially separated within individual watermelon plants. Additional studies are in progress to verify and extend these findings.

Phenology of aphids and their potential as virus vectors in a northern seed potato production area in Finland

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The Tyrnävä-Liminka area (64°46′N, 25°38′E) in northern Finland is one of the five European High Grade (HG) seed potato production zones which are free from the dangerous plant pests and pathogens recognized by the plant protection legistlation of the European Union. The area is characterized by overall low aphid abundance and a short growing season. The aphid-transmissible *Potato virus Y* (PVY) is, however, the most common single factor causing seed class reductions in the official seed potato certification in Finland. To understand epidemiology of PVY in the HG area, aphid phenology data were recorded in this study.

The aphid flight activity was monitored over the growing season (mid-June to the end of August) with a 12.2 m Rothamsted suction trap (ST) and with yellow pan traps (YPT) placed on bare soil at the edge of eight seed potato fields in 2007-2009. Morphological identification of aphid species was supplemented by DNA barcoding using mitochondrial COI gene region sequence.

Ninety-seven aphid taxa were identified (84 taxa in the YPTs, and 51 in the ST). In 2007, the ST catch was low in comparison to 2008 and 2009 (66, 579 and 880 individuals, respectively). *Rhopalosiphum padi* (L.) was the dominating species in the ST in all three years (30.3 %, 56.2 % and 40.6 %, respectively) and caused a pronounced peak at the end of August each year. It also dominated the YPT catch in 2008. *Hayhurstia atriplicis* (L.) was the most dominant species in YPTs in 2007 and *Chaitophorus* spp. in 2009. Very few *Myzus persicae* (Sulzer) were found in the total catch.

PVY is known to be vectored by more than 50 aphid species. Transfer of the aphid phenology data obtained in the HG region into a statistical PVY forecasting model by using different sets of relative vector efficiency factors and further explanatory variables will be discussed.

Preliminary disease progress curve of *Potyviruses* and vector interaction in garlic crop in Argentina

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Garlic is vegetatively propagated, resulting in widespread infection by viruses. All the materials currently in use have varying levels of virus infection, which affect their development and limit productivity. Plants are mainly infected by *Potyviruses. Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) have been detected worldwide. These are the most important viruses in terms of the damage, with garlic bulb weight reduced between 24 and 60% for OYDV and between 17 and 54% for LYSV. It is clearly important for the control of these pathogens to obtain virus-free material, and to have programs to produce certified virus-free stocks of popular garlic cultivars. At field, the virus-free material is natural infected by vectors. The present studied aimed to evaluate the epidemiology of *Potyviruses* in virus free plants exposed at normal conditions. *Potyviruses* epidemics were monitored in the main garlic growing area. OYDV and LYSV incidence were evaluated during a crop cycle, determined with nitrocellulose-enzyme-linked immunosorbent assay (NC-ELISA). Temporal analysis of epidemics was compared with monomolecular, logistic, Gompertz and exponential models for goodness of fit. Of all the models, Exponential model described well the epidemics, R^2=0.97 for OYDV and R^2=0.90 for LYSV. Area under the disease progress curve was higher for LYSV 687.64 than OYDV 372.23. Aphid activity was assessed with water traps (Moerike type). Weekly estimation was performed to determinate number and specie. There were 22 different species with a range 403 to 2. Correlation between total number of vectors and potyviruses incidence, were high and consistent (0.95, $p<0.000035$ for OYDV and 0.85, $p<0.00174$ for LYSV).

Occurrence and Epidemics of *Wheat dwarf virus* in China

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Wheat dwarf virus (WDV), transmitted by leafhopper (*Psammotettix striatus* L.), is a member of the genus *Mastrevirus*, *Geminiviridae*. In 2007, we reported firstly its occurrence in China. Since then, several diseased wheat and barley plants showing extreme dwarfing, various types of yellowing and reduced or no heading were found and collected from 13 provinces throughout China. Most of these samples were identified as WDV positive by PCR and ELISA. The results suggested a broad distribution of WDV in China. Also, WDV was epidemic extensively in Hancheng, Shaanxi province, northwestern China in 2008 and 2009. Plant susceptible varieties (Xiaoyan 22 and Xiaoyan 6) and high density of vector *P. striatus* (above 600 insets/ m^2) play a critical role in WDV epidemics in this area. In other hand, the weather during both winter and spring could be related with the epidemics of WDV. From our investigations it seems that a mild winter (not very dry and not very cold) followed a warm and dry spring will cause a high level of WDV infection of wheat cereals. In this area, very susceptible winter wheat varieties may die in the following spring, and, in many case, the yield loss was 50% ~ 80%. We have evaluated more than 200 wheat varieties or breedlines for WDV resistance, in order to find resistance. In our opinion, the main control method for WDV will be using resistant varieties and chemical control for vector insects.

Temporal and spatial spread of chickpea chlorotic dwarf virus (CpCDV) in chickpea in northern Sudan

Abdelmagid Adlan Hamed

Chickpea chlorotic dwarf gemininvirus (CpCDV) has present in chickpea fields in northern Sudan since early 90 ’s . The seasonal, spatial and temporal spread of the disease were studied for the first time in experiments conducted at Hudeiba Research Station Farm, northern Sudan. The highest levels of disease incidence were found to occur during summer in year 2003 and 2004, while low incidence was noticed during winter months. Low spread of the disease was consistently associated with low numbers of leafhopper vector, *Orieus orienalis*. and high spread with higher numbers. Ordinary runs analysis indicated that the arrangement of infected chickpea plants within rows was random throughout the life cycle of the crop regardless of sowing dates. Disease progress and rate curves (*dy/dt*) indicated that logistic, monomolecular, logistic and monomolecular growth models would best describe disease progress in chickpea crop grown in may, June, November and December 2004, respectively. However, the monomolecular was chosen for the purpose of comparing epidemics. Estimated rates of infection were 0.214, 0.081, 0.012 and 0.001 with respect to the day of the year for the chickpea that grown in May, June, November and December 2004, respectively. For the year 2005 the rates of disease progress were 0.09, 0.079, 0.01 and 0.0019 in the crop that grown in May, June, November and December 2005, respectively.

The genus *Torradovirus*, a new plant virus genus now harbouring three species all infecting tomato

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In 2007 a new virus was reported in tomato crops in the South-East of Spain. This virus causes a severe disease in tomato, inducing heavy necrosis in leaves and fruits. The local farmers called this disease 'Torrado', which means roasted or burned. The virus has spherical particles of approximately 28 nm in diameter which are composed of three coat proteins and harbour two ssRNA's of approximately 8 and 5 kb. Analysis of the full length sequence revealed that the virus could not be placed in any known plant virus genus. The new virus was named *Tomato torrado virus* (ToTV), and placed in the newly created, and recently ICTV ratified, genus *Torradovirus*. One year later a second species of the genus *Torradovirus* was identified in tomato crops in Mexico and named *Tomato marchitez virus* (ToMarV) (marchitez means withered).
Very recently a third torradovirus species was identified from a tomato plant from Guatemala showing necrotic spots on the bases of the leaves and chocolate-brown patches on the fruits. Structural and molecular analysis showed the virus to be clearly related to ToTV and ToMarV. Overall sequence comparisons, but notably in the ORF1 on RNA2 and the 3'untranslated regions, revealed low levels of identity to ToTV and ToMarV. This new virus, for which the name tomato chocolàte virus is proposed is now the third virus species for the new genus torradovirus, all of which infect tomato.

Evaluation of thrips-*Iris yellow spot virus* interactions through an indicator host, lisianthus (*Eustoma grandiflorum*)

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Iris yellow spot virus (IYSV) is a relatively newly described *Tospovirus* capable of causing serious losses to bulb and seed onion production worldwide. IYSV is transmitted by the onion thrips [*Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae)] in a persistent, circulative, and propagative manner. Information available on vector-virus interactions is very limited due to the fact that onion has been less than an ideal host for mechanical inoculation with IYSV. Attempts were made to characterize thrips-IYSV interactions by using lisianthus [*Eustoma grandiflorum* (Salisb.)] as an indicator host. Lisianthus plants upon infection with IYSV produced sunken round chlorotic spots which later coalesced, and caused systemic necrosis and death. Lisianthus plants produced the same symptoms when subjected to mechanical and thrips inoculation procedures. IYSV mechanical inoculations were attempted from onion to lisianthus as well as from lisianthus to lisianthus. Both inoculations produced the aforementioned symptoms within 5 days post inoculation, and the transmission efficiency ranged from 68-79%. We also attempted to develop plant tissue-based bioassays for IYSV detection. Mechanical inoculation of various plant parts, such as shoots, leaves, and leaf discs produced round chlorotic spots upon IYSV-infection. Inoculations with *T. tabaci* also produced the same symptoms, both larvae and adults transmitted IYSV at 63% and 66% respectively. Further, IYSV symptom expression and percentage of infection was monitored at three alternating night and day temperature regimes 18-23℃, 25-30℃, and 30-37℃. Results indicate that symptom expression and IYSV-infection was affected at 30-37℃, at 30-37℃ IYSV-infection was lower than the other two temperature ranges, and infected plants produced concentric ring spots. Lisianthus plants when inoculated with another commonly occurring *Tospovirus* (*Tomato spotted wilt virus*) exhibited yellowing and chlorosis on leaves, these symptoms were different from the symptoms associated with IYSV.

Relationships between *Citrus tristeza virus* spread and aphid species (Hemiptera, Aphididae) population composition in different citrus species and geographical areas.

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Three different aphid species monitoring systems (yellow water traps, sticky shoot and sampling of colonies on leaves) were used from 1997 to 1999 in citrus orchards of three different species (sweet orange, satsuma and clementine) to identify and to estimate the relative percentage of aphid species and their population dynamics. The study was conducted in two Mediterranean counties (La Plana and L'Horta) in Valencia region in Spain, where *Citrus tristeza virus* (CTV) spreads differently.

Aphis spiraecola and *Aphis gossypii* were the predominant caught and observed aphid species. In La Plana, *A. spiraecola* (87.2 %) was the predominant species in front of *A. gossypii* (12.8 %). In L'Horta, both species showed similarly high population rates (52.8 % *A. spiraecola* and 47.2 % *A. gossypii*). This situation could explain why CTV is spreading faster in L'Horta than in La Plana, since *A. gossypii* is the most efficient CTV vector in Mediterranean areas.

Preferences of both aphid species for citrus species showed *A. gossypii* preferred clementine trees, and *A. spiraecola* sweet orange and satsuma citrus species. These results agree with the faster calculated ratio of CTV spread in citrus clementine orchards compared with other citrus species orchards as sweet orange.

Aphid monitoring and species identification is a useful tool to predict CTV outbreaks in Mediterranean areas, where *Toxoptera citricida* is absent, when the relative abundance of different citrus species is known.

Dually stressed tobacco plants demonstrate heavier cell pathology

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Well characterized model system '*Tobacco mosaic tobamovirus* (TMV) – *Nicotiana tabacum* cv. Samsun' was used for studying the development of virus infection at the cellular level in presence of elevated concentration of heavy metals zinc (Zn) or lead (Pb). Ultrathin sections of young leaf tissue were analyzed using transmission electronic microscopy. Heavy metals had multiple and mainly similar effects on the progress of systemic TMV infection in tobacco cells. Dually stressed cells demonstrated higher degree of cytoplasm vacuolization, degeneration and destruction of nucleus. Chloroplasts of such cells were significantly more damaged and developed many large starch grains which were not typical for cells subjected to a single stressor. Atypical alterations of cell walls and expanded intercellular spaces were characteristic for plants treated with Pb. Both heavy metals induced prominent visual increment in virus content in tobacco cells which was in accordance with quantitative ELISA data. TMV particles have been detected virtually in every cell organelle including the nucleus and chloroplasts, and in general the heavy metals favoured the formation/compartmentalization of virus-induced inclusion bodies in the cytoplasm. In addition, TMV inclusion bodies containing discernible virus particles have been identified in peroxisomes for the first time. Altogether, obtained results obviously indicate that combined effect of virus infection and heavy metals has synergistic result in terms of more pronounced cytopathology in tobacco, and that abiotic stressor clearly potentiates the development and the influence of biotic one.

The incidence and genetic diversity of *Turnip yellows virus* (TuYV) in winter oilseed rape (*Brassica napus*) in England.

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Oilseed rape (*Brassica napus*) is an important oilseed crop in the UK. *Turnip yellows virus* (TuYV) is the most common virus infecting oilseed rape in the UK and causes significant reduction in both seed and oil yield. Research has been undertaken to determine the incidence and genetic diversity of the TuYV infecting oilseed rape in three regions of England.

Leaf samples were collected from three fields of winter oilseed rape in each of three growing regions, Lincolnshire, Warwickshire and Yorkshire in autumn and spring of the 2007-08, 2008-09 and 2009-10 crop seasons. The leaf samples were tested for the presence of TuYV using TAS-ELISA and virus incidences compared with Rothamsted suction trap aphid data. TuYV infection was widespread in the three regions but there were big differences in the incidences of the virus in the various fields within and between regions sampled. Incidences of infection ranged from 0 to 100%. Highest levels of infection were recorded in the 2009-10 crop season, followed by 2007-08 crop season whilst 2008-09 crop season recorded the lowest. The level of infection of oilseed rape crops by TuYV appeared to be related to the flight activity of *Myzus persicae*.

The P3 (coat protein) and P0 genes of up to 10 isolates of TuYV from each of the fields are being sequenced to study genetic variation between them. Preliminary phylogenetic analysis of both nucleotide and amino acid sequences obtained so far suggest the existence of more than one distinct cluster. Viral isolates from each region occurred in each cluster. The P3 amino acid sequences were more conserved (homologies of 93.1 to 100%) than those of P0 gene (homologies of 87.6 to 100%).

Occurrence and distribution of viruses in cucurbits from Oklahoma

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Virus diseases are a major constraint for the production of cucurbits which are economically important cash crops cultivated worldwide. During the growing season of 2008, surveys were conducted to detect and determine the incidence of viruses in major cucurbit growing areas of Oklahoma. A total of 588 symptomatic leaf samples from 36 fields in 3 counties (Atoka, Blaine and Tulsa) were collected from five cucurbit crops (cantaloupe, cucumber, pumpkin, squash and watermelon). All samples were tested by dot-immuno-binding assay (DIBA) against the antisera of seven viruses, including *cucumber mosaic virus* (CMV), *cucumber green mottle mosaic virus* (CGMMV), *melon necrotic spot virus* (MNSV), *papaya ringspot virus*-watermelon strain (PRSV-W, formerly known as *watermelon mosaic virus*-1), *squash mosaic virus* (SqMV), *watermelon mosaic virus*-2 (WMV-2) and *zucchini yellow mosaic virus* (ZYMV). The highest incidence was recorded for PRSV-W, followed by WMV-2, and ZYMV, which were contained in 67%, 17% and 15% respectively of the collected samples. MNSV, SqMV, and CMV were detected in 6.0%, 5.2% and 1.2% of the samples, respectively. None of the samples reacted positively against the antiserum of CGMMV. Mixed virus infections were common involving two or three viruses in various combinations. Triple and double infections were found in 6.8% and 5.6% of samples, respectively. Some symptomatic samples of watermelon, squash and pumpkin did not react with the antiserum of the above tested viruses, indicating that other unknown viruses may be infecting cucurbit crops.

Detection of *Soil-borne cereal mosaic virus* in Belgium on wheat and barley

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Soil-borne cereal mosaic virus (SBCMV) had been reported in countries neighbouring Belgium, such as France and Germany, but until 2004 this virus had not been detected in Belgium. In order to assess the occurrence of SBCMV in Belgium, a broad-spectrum reverse transcription-polymerase chain reaction (RT-PCR) protocol was previously developed, using primers targeting the conserved untranslated 3' region of RNA-1 and RNA-2 of SBCMV causing the mosaic diseases in Europe. The application of this detection method enabled to identify SBCMV in wheat plants with mosaic symptoms that had been collected in Belgian fields. The presence of the virus in Belgium was evaluated using a bioassay with the wheat cvs. Cezanne and Savannah, and the rye cv. Halo, grown in 104 Belgian soils. The SBCMV was detected in 30%, demonstrating the widespread occurrence of SBCMV in Belgium. The RT-PCR method developed for the study, combined with extensive sampling, allowed the SBCMV detection for the first time in Belgium. WSSMV was found to be associated with SBCMV in the same samples in about half soils infested by mosaic diseases, as stated in previous studies.

The broad-spectrum detection method allowed also to identify SBCMV in barley plants grown in Belgian and French soils. The SBCMV, however, was detected most frequently on wheat, confirming earlier reports in the literature. The detection of SBCMV on barley showed that the SBCMV host range is larger than reported in the literature, where it refers only to SBCMV on wheat and rye. The simultaneous and frequent presence in a single plant of the SBCMV together with the *Wheat spindle streak mosaic virus*, a virus also previously reported in Belgium, raises questions about possible recombinations, synergism and/or between viruses or complementation for the transmission by *Polymyxa*.

The RNA-2 coat protein sequence of the Belgian and French SBCMV isolates in the barley and wheat plants confirmed their identification, despite the small variations in the nucleotide sequences compared with the sequences reported for British, French, German and Italian isolates of SBCMV.

EPIDEMILOLOGY OF *PLUM POX VIRUS* IN UKRAINE

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Screening of stone fruit cultures for PPV was carried in 7 regions of Ukraine. PPV was shown to be most spread in Transcarpathian region at the west of Ukraine.

ELISA testing demonstrated that plum trees (*P. domestica*) were most infected, when peach trees (*P. persicae*) were less infected representatives of *Prunus* genus in this region. Submountain district of Transcarpathian region was characterized with the highest percentage of PPV-infected trees. Significant spread of the disease shown for plantings in Transcarpathian region is due to the cultivation of PPV-susceptible plum cultivars: Ugorka zvychayna, Ugorka italiyska and Ugorka moldavska.

Comparison of different agroclimatic zones for PPV spread on stone fruit cultures showed that Sharka disease is most significantly spread and pronounced in the submountain agroclimatic zone. This is obviously because the climate of this zone does not favour cultivation of other more thermophyte stone fruit cultures, like apricot and peach which are less susceptible to this virus. In addition, the majority of plum cultivars grown in the submountain agroclimatic zone are particularly susceptible to PPV.

We have also identified four aphid species which are most efficient vectors for *Plum pox virus* – *Hyalopterus pruni*, *Phorodon humuli*, *Brachycaudus helichrysi* and *B. cardui*. *Hyalopterus pruni* and *Brachycaudus helichrysi* were regularly detected on cherry-plum and plum trees.

ELISA testing of various plant parts (generative and vegetative buds, flowers, young and mature fruits, bark) proved virus presence in all organs studied, however significant dependence of virus load and localization on time of the year has been shown. Maximum content of *Plum pox virus* was noted for flowers, young leaves and mature fruits. In young fruits and bark of one-year-old shoots PPV was difficult to detect from May to August.

The PPV coat protein region has been sequenced; data comparison with Genbank inputs showed 99% homology with PPV strain D.

Multiplex PCR assay for the simultaneous differentiation *Potato virus Y* strains and the first report of the occurrence of the Eu-PVY^{NTN} strain in Japan

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A multiplex polymerase chain reaction (PCR) was developed for the simultaneous detection and identification of single or mixed infections of the main *Potato virus Y* (PVY) strains, PVY^{O}, PVY^{N}, PVY^{NTN}, PVY^{N}W and the recently described strain PVY^{NTN-NW}. In the last two years, PVY incidence has significantly increased in Hokkaido region, Northern Japan, therefore, this multiplex PCR assay was applied on samples collected in 2007-2009 to reveal the strain type of PVY infection. In the results, the European recombinant type of PVY^{NTN} (Eu-PVY^{NTN}), which had never been found in Japan, was the most frequent strain detected. The incidence of the Eu-PVY^{NTN} was dramatically increased starting from 2008 which indicated a possible relation between the introduction of this strain and the increased incidence of PVY in that region. A Japanese isolate of Eu-PVY^{NTN} shared highest sequence similarity with Eu-PVY^{NTN} isolates collected from Idaho, USA, during a PVY outbreak in 2007 suggesting a common origin.

MONITORING AND FORECASTING VIRUS DISEASES IN LEGUMES IN THE PALOUSE REGION OF THE INLAND PACIFIC NORTHWEST, USA

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Intermittently, aphid-borne viruses cause widespread and severe injury to legume crops in the Palouse region of eastern Washington State and northern Idaho, USA. These outbreaks are sporadic and unpredictable based on a 26-year record. The most important viruses, *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus* (BLRV), are persistently transmitted and potentially manageable by suppressing the vector, the pea aphid, *Acyrthosiphon pisum*. Whether it is economical for producers to treat aphids aggressively with insecticides, however, depends upon the risk of virus outbreak, which is currently impossible to determine. As part of our effort to address this problem, during every growing season since 2007, we have been monitoring the incidence of aphids and viruses virus across the approximately 1800-km2 region. A network of pan traps captures arriving alate pea aphids, which are then sampled twice weekly for abundance and the presence of viruses in the aphids using a multiplex PCR-based method. Patterns of aphid arrival and presence of virus in these aphids and subsequently in the crop are being used to generate a temporal and spatial model of risk of virus infection that can be used by producers. Three year's data indicate that virus risk is greater in the south and west of the study region, which may be consistent with a hypothesized source of aphids and viruses in the Columbia basin of central Washington State. BLRV and PEMV have different patterns of occurrence consistent with their likely different sources.

Disruption of two defensive signaling pathways by the cucumber mosaic virus 2b RNA silencing suppressor

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The cucumber mosaic virus (CMV) 2b counter-defense protein disrupts plant antiviral mechanisms mediated by RNA silencing and salicylic acid (SA). We used microarrays to investigate defensive gene expression in *2b*-transgenic *Arabidopsis thaliana* plants. Surprisingly, 2b inhibited expression of few SA-regulated genes and in some instances enhanced the effect of SA on certain genes. Strikingly, the 2b protein inhibited changes in the expression of 90% of genes regulated by jasmonic acid (JA). Consistent with this, infection of plants with CMV, but not the *2b* gene deletion mutant CMVΔ2b, strongly inhibited JA inducible gene expression. JA levels were unaffected by infection with either CMV or CMVΔ2b. Although the CMV-*Arabidopsis* interaction is a compatible one, SA accumulation, usually considered to be an indicator of plant resistance, was increased in CMV-infected plants but not in CMVΔ2b-infected plants. Thus, the 2b protein inhibits JA signaling at a step downstream of JA biosynthesis but it primes induction of SA biosynthesis by another CMV gene product or by the process of infection itself. JA is important in plant defense against insects and CMV, like many plant viruses, is aphid-transmitted. This raises the possibility that disruption of JA-mediated gene expression by the 2b protein may influence CMV transmission.

Cassava brown streak virus diversity and development of improved virus diagnostics

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Cassava production in Africa is constrained by two important viral diseases; cassava mosaic disease (CMD) and cassava brown streak virus disease (CBSD). CMD has received the most research input due to being the most widespread and most economically important. In contrast, until recently little research has been carried out on CBSD. In the last few years, however, CBSD has been spreading to new countries (eg., Uganda, DR Congo, Burundi) and the reasons for the spread are unknown. To determine whether or not a severe form of CBSD is spreading into new countries, *Cassava brown streak virus* (CBSV) (*Ipomovirus*, *Potyviridae*) isolates from Uganda, Tanzania, Mozambique, Kenya and Zanzibar islands were collected and their symptom severity compared. Significant differences were observed in symptom severity on five cassava varieties, and also on *Nicotiana benthamiana*, *N. clevelandii* and *N. glutinosa.* Isolates from Tanzania and Mozambique produced the most severe symptoms and caused dieback of *Nicotiana* plants in 2-3 weeks following virus inoculation, while the Ugandan isolate produced relatively mild symptoms. To further investigate the genetic differences between the virus isolates, total RNA was extracted from infected cassava leaves. Coat protein (CP) genes were amplified by RT-PCR, cloned and sequenced. Based on CP sequences, CBSV isolates clustered into two groups with those of the previously reported isolates from respective countries.

In order to optimize sampling and virus detection protocols various experiments were carried out. Total RNA extractions from cassava leaves with and without CBSD symptoms were subjected to RT-PCR to determine the association of the virus with symptoms. The distribution of CBSV in a cassava plant was investigated by RNA extractions from top, middle and bottom leaves, stems, roots and tubers. It was evident that CBSV was distributed throughout the plant in parts with or without CBSD symptoms, except in newly formed secondary and tertiary roots where the virus was absent. Virus was present in both symptomatic and non-symptomatic parts of tuberous roots, stems and leaves. A dilution series experiment to determine virus load in the newly-formed and old leaves revealed equal virus distribution in them. However, both young and old leaves appear to be less suitable for the purpose of virus detection because of the high amounts of inhibitors present in them, and the third or fourth fully expanded leaf was most suited for sampling. CBSV diversity and the various parameters that affect its detection will be discussed.

Epidemiology of cassava brown streak disease in Uganda

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Over the last six years, cassava brown streak disease (CBSD) has emerged as an epidemic and is now the most serious threat to cassava production in the entire East and Central Africa. Previously, CBSD was restricted in occurrence to the coastal lowlands of East Africa along the Indian Ocean. The new CBSD epidemic is widespread in areas surrounding Lake Victoria in Uganda, western Kenya and northern Tanzania. The disease is caused by viruses with single stranded RNA genomes. Two virus species associated with CBSD have been identified to date, tentatively referred to as cassava brown streak virus (CBSV) and cassava brown streak Uganda virus (CBSUV) (genus: *Ipomovirus*, Familiy: *Potyviridae*). The latter (CBSUV) is the virus most prevalent species at high altitudes around Lake Victoria where the new epidemic of CBSD is affecting cassava. The viruses are transmitted by the whitefly, *Bemisia tabaci*. Since the outbreak of CBSD in Uganda in 2004, surveys have been conducted which confirm that the disease is present in at least 30 districts. Incidence and yield losses of up to 100% on individual farmers' fields have been reported. Since 2008, spread of CBSD has been studied among several clones at different locations. In one study, no disease spread was observed at Abi and Ngetta in northern Uganda, but there was high rate of spread at Namulonge in central Uganda. Final CBSD incidence was highest in the cultivar TME 204, attaining disease saturation (100%) by 7 months after planting, while no spread was observed on MM 96/4271 and TMS 30572. In an assessment of the effect of CBSD on yield of cassava, the percentage of unusable roots spoilt by necrotic rot due to CBSD ranged from 28% in TME 14 to 95% in TME 204. Clearly, CBSD is spreading fast in Uganda, significantly affecting yields and undermining economic importance of cassava.

Symptomatology and biological characterization of *Citrus Tristeza Closterovirus* isolates in Pakistan

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Pakistan is the major citrus producing country but citrus trees have had shorter productive life. The citrus species are susceptible to large number of virus and virus-like diseases and among viral pathogens *Citrus tristeza closterovirus* (CTV) most destructive in nature.

The present study was initiated to record the symptoms associated with CTV and biologically identify the nature of CTV strains prevalent in the citrus orchards. The citrus trees in the Punjab were found to be CTV infected regardless of scion-rootstock combination. The CTV symptoms commonly present in infected trees were honey combing at or below bud union, bark cracking, stunted growth and decline. The declining symptoms were higher in sweet oranges than mandarins. The biological characterization suggested that variant mild types are prevalent in the citrus trees. All the infected cultivars reacted positively on lime showing only mild vein clearing and leaf flecking but produced no symptom when grafted on sour orange rootstock. No symptoms of seedling yellow and stem pitting were encountered in the indicators. The symptomatology and biological characterization of CTV isolates suggested their mild nature.

Tuesday, June 22, 2010

Virus Ecology and Evolution

The community ecology of barley/cereal yellow dwarf viruses in Western US grasslands

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Understanding disease dynamics requires considering the community context in which the host and pathogen are embedded. Host traits play an important role in pathogen dynamics, but pathogens exist within complex food webs of interacting hosts, vectors, competitors, and predators. We carried out a series of field experiments that examine the importance of local community factors and regional patterns in shaping the prevalence of the aphid-vectored barley/cereal yellow dwarf viruses (B/CYDVs) in grass hosts across a range of sites in Pacific Coast grasslands. We examined the effect of long-term exclusion of large vertebrate herbivores on the prevalence of B/CYDVs and found that prevalence was almost 4-fold higher in the presence of consumers than where they were excluded, due to changes in the relative abundance of highly competent hosts in the grassland community. In a second experiment, we quantified drivers of infection at hierarchically nested spatial scales (10^5 to 1m) in five grassland sites spanning 7 degrees of latitude in a factorial design including applications of nitrogen and phosphorus. Infection increased with cover of long-lived hosts and phosphorus, but not nitrogen, fertilization. Finally, we examined patterns of co-infection by multiple B/CYDVs, measuring the prevalence and diversity of four B/CYDV's in three host species in 22 natural grasslands along a 2000 km latitudinal gradient in the western United States. In this study, vector community was a stronger determinant of patterns of coinfection than within-host processes. We found few differences in viral prevalence or diversity among hosts at a single site, but pathogen diversity within a host increased with increasing latitude and associated increases in precipitation and host abundance. Taken together, these studies suggest that local community factors largely shape the dynamics of B/CYDV's in western grasslands, but regional factors can also play an important role.

Balancing selection for replication and horizontal transmission by mimicking field conditions

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Multiple factors drive the evolution of RNA viruses, each with their own particular forms of selection. Plant viruses are selected for their ability to replicate efficiently and move systemically through their hosts. However, on a ecological scale each single plant population is competing against other viral populations for efficient host to host horizontal transmission, frequently by insect vectors. Luteoviruses are transmitted in a circulative fashion, which leads to very specific vector virus relationships. Soybean dwarf virus (SbDV), a luteovirus with two distinct strains, the D (dwarfing strain) and the Y strain (yellowing strain), is efficiently transmitted by several aphids. However, none of these aphid species colonize soybeans, limiting the potential for outbreaks. The only aphid that does colonize soybeans is *Aphis glycines*, but this aphid species is not a vector for SbDV. We identified an *A. glycines* transmissible SbDV isolate that is a mixed infection of D and Y strains. Both the D and Y strains fare well in the original source host, clover, but the Y strain is considerably less fit than the D strain in soybeans. However, the Y strain is absolutely required for transmission by *A.glycines*. Passaging experiments using *A.glycines* were designed to determine if the selection for aphid transmissibility was sufficient enough to preserve the Y component of the mixed infection in soybeans. In normal transmission experiments the D component came to dominate the population no matter the transmission frequency, suggesting the host selection would always trump vector selection. However, this changed when aphids were constantly present, replicating conditions in the field. With the constant presence of *A. glycines* both the D and Y components were maintained, suggesting that the aphid was affecting viral evolution when allowed to provide constant selection pressure

Arabidopsis thaliana as a model for the study of plant-virus ecology and plant-virus co-evolution

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The ecology of virus infection in wild plant ecosystems is an under-explored subject, which is highly relevant to understand plant-virus interactions. Specifically, understanding plant-virus co-evolution requires the study of wild systems in which, at odds with agricultural systems, there is no human manipulation of either host or virus. To develop such a system, we analysed virus infection in six wild populations of *Arabidopsis thaliana* in Central Spain. The incidence of five virus species with different life-styles was monitored during four years, and this was analysed in relation to the demography of the host populations. Total virus incidence reached 70%, which suggests a role of virus infection in the population structure and dynamics of the host, under the assumption of a host fitness cost caused by the infection. Maximum incidence occurred at early growth stages, and co-infection with different viruses was frequent, two factors often resulting in increased virulence. Experimental infections under controlled conditions with two isolates of the most prevalent viruses, *Cauliflower mosaic virus* and *Cucumber mosaic virus*, showed that there is genetic variation for virus accumulation, although this depended on the interaction between host and virus genotypes. Comparison of Q_{ST}-based genetic differentiations between both host populations with F_{ST} genetic differentiation based on putatively neutral markers, suggests different selection dynamics for resistance against different virus species or genotypes. Together, these results are compatible with a hypothesis of plant-virus co-evolution.

Wild Plant Viruses and Disease Ecology

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Plant viruses were surveyed from wild plants in a low-diversity wildland (Tallgrass Prairie Preserve, Oklahoma) and a high-diversity wildland (the Area Conservación Guanacaste, Costa Rica) using double-stranded RNA isolation followed by random-primed amplification and multiplexing and sequence analysis with 2nd generation methods. Sampling was done so that the identity of the host was maintained. While most viruses found were asymptomatic and persistent, a few viruses appear to be acute. Some viruses that cause disease in nearby crops were found in wildlands, but they were evolving away from pathogenicity, and were asymptomatic in the wild plants. In addition, some new plant viruses were identified from virus families traditionally thought to be of fungal origin. Data analysis includes phylogenetic studies to assess origins of these viruses and probably spread within wildlands, and between wildlands and crops.

siRNA deep sequencing for the discovery and sequencing of novel viruses

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Vegetative propagated crops are prone to build up of virus infections and new viral diseases continue to appear. Early detection of the appearance of new viruses followed by rapid and accurate identification of these agents is essential if correct control measures are to be deployed. This is particularly true for entirely new diseases where novel control strategies may have to be developed alongside characterization of novel agents. Plants defend themselves against viruses by RNA silencing which involves the generation and use of small interfering RNA (siRNA): short RNA sequences of 20-25 nt derived from the viral genomic or sub-genomic RNA. Recently we developed a new technique based on deep sequencing and assembly of plant derived small RNAs, to rapidly identify viral infections in plants. The technique could not only identify known viral pathogens, occurring at extremely low titers, but also novel viruses, without the necessity of any prior knowledge. We are currently assessing the applicability of the technique to be used in routine indexing of plants using representative samples infected with various known as well as unknown viruses. Results from potato, sweetpotato, cassava and various indicator plants will be presented, revealing the power and limitations of the technique.

Host physiological phenotype predicts key epidemiological parameters

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A major scientific goal is to predict a host's risk of infection by generalist vectored pathogens (i.e., infection risk) and a host's potential to increase infection risk to other hosts (i.e., reservoir potential). We hypothesize that a single axis describing variation in host physiological phenotype explains why plant hosts differ in three epidemiological parameters (susceptibility to infection, competence to infect vectors, and ability to support vector populations) that determine disease risk and reservoir potential. This hypothesis predicts that "quick return" (QR) hosts have a physiological phenotype (i.e., short-lived, poorly defended, nutrient rich tissue and high metabolism) that causes them to be more susceptible, competent, and able to support vector populations compared to "slow return" (SR) hosts. We further predicted the SR-QR continuum would provide a better explanation compared to conventional explanations, namely phenotypic plasticity, phylogeny, lifespan, and geographic provenance.

We conducted greenhouse experiments using six phylogenetically paired invasive annual and native perennial California grass species and an aphid-vectored generalist viral pathogen (*Barley yellow dwarf virus-PAV*; BYDV-PAV). We quantified host phenotypes and conducted experimental inoculations using aphid vectors (*Rhopalosiphum padi*). We show that "quick return" phenotypes supported greater vector populations, were more likely to become infected when inoculated (i.e., were more susceptible), and produced more infected vectors (i.e., were more competent). Moreover, the single axis of physiological phenotype provided a more consistent explanation compared to the four conventional explanations. These results show that host physiological phenotype can predict key epidemiological parameters. Thus, infection risk, transmission, and prevalence may be greater in QR-dominated communities.

When did the New World and Old World begomoviruses diverge?

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Begomoviruses, the whitefly-transmitted geminiviruses, are differentiated into several clades, the largest of which are the “Old World” and “New World” groups. With few exceptions, including recent introductions of viruses beyond their native range, these geographic clades are supported in phylogenetic analyses of the DNA-A. This biogeographic pattern has historically been associated with the breakup of Gondwanaland, which occurred 510-570 million years ago. Several papers have suggested that this is the likely cause for the split between the Old World and New World begomoviruses. However, recent studies that demonstrate high rates of begomovirus evolution imply that 500 million years is far too ancient of a date of divergence for the extant begomoviruses, which still show substantial amounts of sequence similarity. We undertook a BEAST analysis of the two major ORFs on the begomovirus DNA-A: the coat protein and replication-associated protein genes. We conducted some analysis enforcing a divergence date of ~500 million years ago, and others to have the rate of evolution that begomoviruses demonstrably have in modern times. Our results suggest more recent divergence, perhaps corresponding to the migration of humans across Beringia.

ORIGIN, EVOLUTION AND MOLECULAR EPIDEMIOLOGY OF PAPAYA RINGSPOT VIRUS

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Papaya ringspot virus (PRSV) is the most important virus affecting papaya and cucurbit plants in tropical and subtropical areas. PRSV isolates are divided into two types: the P type (PRSV-P), infects papaya (Carica papaya) and plants belonging to the Cucurbitaceae family, and isolates of the W type (PRSV-W), which only infects plants in the Cucurbitaceae family. Studying the genetic variability of PRSV is of great interest to better understand the diversity, evolution and epidemiology of this virus. For this purpose, phylogenetic relationships have been examined using sequences of the Capsid Protein (CP) gene. However it is essential to include a comprehensive collection of isolates, especially from South America for which limited sequences have been obtained. To address these issues the sequences of the CP gene of PRSV isolates from Colombia were characterized and analyzed alongside sequences reported previously from different parts of the world by using two distinct approaches. The first approach used a relaxed molecular clock based on dates of isolate collection for an accurate calculation of divergence times and a better estimate of virus origin. The second approach was based on phylogeographic analysis for which the historical biogeography is reconstructed by using the distribution areas of the virus. Finally, the phylogeny of the CP gene was compared to that of the HC-Pro gene in order to determine which viral gene resolves better the history of PRSV. Our studies showed that movement of the virus between countries contributes to the observed population variation and suggested that the hypothesis of the origin of PRSV-P type from PRSV-W should be revised, because evidence for the opposite process is found in several instances.

Long-term evolution of the *Luteoviridae*: time-scale and mode of virus speciation

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Despite their importance as agents of emerging disease, the time-scale and evolutionary processes that shape the appearance of new viral species are largely unknown. To address these issues we analyzed intra- and inter-specific evolutionary processes in the *Luteoviridae* family of plant RNA viruses. Using the coat protein gene for 12 members of the family, we determined their phylogenetic relationships, rates of nucleotide substitution, times to common ancestry, and patterns of speciation. An associated multi-gene analysis enabled us to infer the nature of selection pressures and the distribution of recombination events. Although rates of evolutionary change and selection pressures varied among genes and species, and were lower in some overlapping gene regions, all fell within the range of those seen in animal RNA viruses. Recombination break-points were commonly observed at gene boundaries, but less so within genes. Our molecular clock analysis suggested that the origin of the currently circulating *Luteoviridae* species occurred within the last four millennia, with intra-specific genetic diversity arising within the last few hundred years. As a consequence, speciation within the *Luteoviridae* therefore seems to be associated with the rise and expansion of agricultural systems. Notably, speciation events also tended to occur within the same plant host species and country of origin, as expected if speciation is largely sympatric, rather than allopatric, in nature.

Adaptation of Soybean Dwarf Virus to New Host Species

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Soybean Dwarf Virus (SbDV) is an important plant pathogen infecting soybean, and Asian strains of SbDV cause high economic losses. In North America, indigenous strains of SbDV commonly occur in clover, with recently outbreaks occurring in soybean in Virginia, Wisconsin and Illinois. Thus, there is a critical need for evaluating the risk of SbDV host adaptation and increased incidence in U.S. soybean fields. Preliminary studies indicated that a clover-infecting strain of SbDV (SbDV-MD6) infected commercial legume crops, including peanuts, fava beans and peas. To evaluate the host adaptability of SbDV, SbDV-MD6 was serially passaged on pea and soybean using the aphid vectors, *Acyrthosipon pisum* and *Nearctaphis bakeri*, respectively. When SbDV was transmitted from pea to pea sequentially 8 times, transmission efficiency varied from 66-100%. During each sequential transmission, the symptom severity and virus titer increased gradually. When SbDV was transmitted from soybean to soybean, transmission efficiency decreased gradually from 33% to 20%. Although transmissibility in soybean was lost after 6 sequential transmissions, virus titers increased significantly with each transmission. Sequence analysis MD6 from both pea and soybean passages identified 11 non-synonymous mutations in soybean, and 5 mutations in pea. The d_N/d_S analysis indicated that SbDV was under strong selective pressures in soybean, but not in peas. The significant increases in MD6 titers in soybean passage lines support this analysis, but the loss of transmission efficiency seems counterintuitive. Based on these observations, we believe that the clover strain of SbDV-MD6 rapidly adapts to the soybean host. However, the improved replication appears to have trade-off effects resulting in decreased vector transmission, eventually selecting against viral populations with efficient horizontal transmission.

Towards the description of the plant virus metagenome of the French Sub-Antarctic Islands

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Although the characterization of viral metagenomes in sea water and a few other environments has made significant progress, plant virus metagenomic studies are still largely in their infancy. At the same time, knowledge on the diversity of phytopathogenic viruses in ecosystems would have important implications in taxonomy, in viral ecology and, potentially, in our understanding of viral evolution or of the emergence of viral diseases.

The French sub-antarctic islands represent an original and constrained ecosystem characterized by a paucispecific flora rich in endemic species and already impacted significantly by introduced plant and animal species. This ecosystem is perceived as particularly at risk, both through the effect of global climate change and through further introductions, including those of plant viruses and their vectors. At the same time, very little is known on plant virus biodiversity or even presence in such ecosystems, except for the description of a new Badnavirus in the Macquarie islands.

We have used two approaches to try to identify phytopathogenic viruses in this ecosystem: the analysis through classical sequencing or pyrosequencing of double stranded RNAs extracted from symptomatic or asymptomatic plants and the use of a range of polyvalent, genus-specific PCR assays.

Our results indicate the presence of both well known viruses such as *Cherry leaf roll virus*, *Barley yellow dwarf virus* and *Cucumber mosaic virus* and of novel agents. The well known viruses likely represent introductions and have been observed in introduced host plants but also, in the case of BYDV, in the native *Poa cookii*. The novel agents were mostly observed in endogenous plant species. Together with the introduction a few years ago of several aphid species, these findings suggest the possibility of epidemic spread of introduced viruses and raise the question of the potential impact of this phenomenon on these vulnerable plant communities.

Ecogenomic study of plant viruses reveals widespread infection of wild plants with *Zucchini yellow mosaic virus.*

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Little is known about plant viruses in natural ecosystems, but the advent of next-generation sequencing technology has allowed us to study viruses of wild plants in depth in the Area de Conservación Guanacaste (ACG), northwestern Costa Rica, a well-known 'hot spot' for global biodiversity. This survey revealed widespread infection of wild plants by *Zucchini yellow mosaic virus* (ZYMV; family *Potyviridae*), a devastating pathogen of cucurbits, in a unique interface between a natural ecosystem and a recent agroecosystem. Bioinformatic analysis of pyrosequenced cDNA libraries converted from double-stranded RNA (dsRNA), a hallmark for RNA virus infection, showed infection of ZYMV in 60 different species of 39 genera belonging to 10 plant families. Nucleotide sequence comparison showed that most of the ZYMV sequences are conserved among the various plant species, with minor transitions and insertions, suggesting a recent widespread dissemination. In addition to symptomatic infection in the agricultural host melon (*Cucumis melo*) from the family Cucurbitaceae, ZYMV causes asymptomtic infections in the non-cucurbitaceous wild plants belonging to the families Acanthaceae, Fabaceae (subfamilies Caesalpinaceae, Mimosaceae, Papilionaceae), Gesneriaceae, Melastomataceae, Poaceae, Rubiaceae and Solanaceae that have not previously been reported as hosts for ZYMV. A preliminary study using a phylogenetically informative region of the genome suggested that ZYMV has migrated from cultivated melons (primary host) to wild plants. The emergence of ZYMV in melons and its relationship to the viruses isolated from wild plants will be presented.

The significance of wild plants in the evolutionary diversification of *Sweet potato feathery mottle virus* in East Africa

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Sweet potato feathery mottle virus (SPFMV; genus *Potyvirus*; *Potyviridae*) is globally the most common and harmful pathogen of cultivated sweetpotato (*Ipomoea batatas*; Convolvulaceae). While >100 isolates of SPFMV from cultivated sweetpotato have been partially sequence-characterized, nothing is known about isolates from wild hosts. Additionally, evolutionary forces shaping SPFMV population structures are largely unknown. In this study, 46 SPFMV isolates from 14 wild species of genera *Ipomoea*, *Hewittia*, and *Lepistemon* (Convolvulaceae) and 13 isolates from cultivated sweetpotatoes were detected in Uganda and the′3 -proximal half of the genome was characterized. Wild plants were infected with the EA, C, or O strain, or co-infected with the EA and C strains of SPFMV. Phylogenetic clustering of isolates from wild species and sweetpotatoes was incongruent with the host species, suggesting inter-host transmission of SPFMV. Globally, spatial diversification of the 178 isolates analyzed was observed; strain EA being largely geographically restricted to East Africa. In the EA strain, 74% of the isolates were predicted to be recombinants. Recombination was frequently detected in the 6K2-VPg-NIaPro region, demonstrating a recombination 'hotspot'. In contrast, only 29% of the isolates in the globally distributed strain C were found to be recombinants. Non-homologous recombination between EA and C was rare, despite frequent co-infections of these strains. Strong purifying selection was implicated on evolution of the five proteins encoded by the analyzed part of SPFMV genome. However positive selection was predicted on 17 amino acids distributed over the entire coat protein in the globally distributed strain C, as compared to only four amino acids in the coat protein N-terminus of the EA strain, implying possibly a more recent introduction of the C strain and a higher adaptation of the EA strain to the local ecosystem. Thus, East Africa appears as a hotspot for evolution and diversification of SPFMV.

Plant – virus co-evolution in wild brassicas.

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Pathogens are key players in the ecology of higher plants; 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 UK were probably introduced at the time of the Roman occupation of Britain, or during Saxon invasions. Several populations exist in the UK, 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.

The effect of transmission mode on genetic diversity in Zucchini Yellow Mosaic Virus (ZYMV)

Heather E. Simmons, Edward C. Holmes & Andrew G. Stephenson

As a result of selective pressures and population bottlenecks, viral genetic diversity is modulated by the manner in which transmission occurs. By sequencing the coat protein (CP) gene of ZYMV, we generated clone data from three distinct populations, two horizontally transmitted populations (one via aphid, the other without), and one vertically transmitted population (via seed). As individual transmission events are at least partially random, both selection and drift may shape the evolutionary trajectories of RNA viruses. Thus our clone data will provide a unique opportunity to look at how the interplay between transmission mode and genetic diversity is reflected in clonal populations of ZYMV. As our data encompasses the epidemiological spread of the virus this has also enabled a determination of the intra-host RNA viral diversity. This is important for understanding how RNA viruses, such as ZYMV, emerge, overcome host resistance, switch hosts, and adapt to rapidly changing environments. In addition our data indicate that the ZYMV seed transmission rate is approximately three orders of magnitude higher than the most commonly cited rate. Correctly accounting for seed transmission will permit the refinement of management strategies for this devastating crop pathogen that annually costs millions in agricultural losses.

Genetic Structure and Molecular Variability of *Grapevine fanleaf virus* populations within three naturally infected California vineyards

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Grapevine fanleaf virus (GFLV) from the genus *Nepovirus*, family *Secoviridae* causes fanleaf degeneration, one of the most important viral diseases of grapevines worldwide. It is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*. GFLV has a bipartite (+)ssRNA genome, which is expressed into two polyproteins that are cleaved into at least eight individual proteins. Due to its error-prone replication and quasi-species nature, GFLV possesses great potential for genetic variation. While the variability within the coat protein and movement protein genes is well characterized for numerous GFLV isolates, information on the variability of other genomic regions - particularly within the RNA1 - is scarce. Similarly, little is known on the diversity of GFLV isolates from the U.S. To gain insights into the evolutionary mechanisms of GFLV, the sequences of the RNA2-encoded genes $2A^{HP}$, $2B^{MP}$ and $2C^{CP}$, and partial sequence information from the RNA1-encoded gene $1E^{Pol}$ of 14 GFLV isolates from three naturally infected California vineyards were characterized. Phylogenetic analyses suggested two evolutionarily divergent lineages that did not reflect the vineyard origin of the isolates or an association with rootstock genotype or scion cultivar. Examination of the genetic variability of the California isolates alongside isolates worldwide revealed similar patterns of molecular evolution for the different regions within the GFLV genome, but distinct selection constraints with the strongest pressure exerted on genes $2C^{CP}$ and $2B^{MP}$, an intermediate level of pressure exerted on gene $1E^{Pol}$, and the weakest pressure exerted on gene $2A^{HP}$. Some of the California isolates resulted from interspecies recombination events between GFLV and *Arabis mosaic virus* with crossover sites suspected in gene $1E^{Pol}$ and identified in genes $2A^{HP}$ and $2B^{MP}$; and intraspecies recombination events most frequently observed within gene $2C^{CP}$. Our study suggested that purifying selection and recombination are important evolutionary mechanisms in the genetic diversification of GFLV.

Next-generation sequencing of plant viruses

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In recent years next-generation sequencing (NGS) has emerged as a powerful tool in genome sequencing. The applications for the technology are enormous and the use for pathogen identification is one. NGS coupled with metagenomic analysis is being used at FERA to rapidly identify the probable cause of diseases of unknown aetiology in plants. The method requires no prior knowledge of the plant pathogen and can be used in an unbiased approach to identify all possible pathogens. A simple nucleic acid extraction from infected plant material has resulted in complete viral genome construction. Problems of genome construction related to new viruses and tools to increase viral sequences prior to NGS are discussed.

Genetic variability, recombination events and rates of molecular evolution in citrus tristeza virus

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Citrus tristeza virus (CTV) isolates of diverse origin and biological properties were used to study the genetic variability of the 3' terminal region of CTV genome in order to obtain a better knowledge about the epidemiology and evolutionary aspects of CTV. The analysis of the 3' terminal region, comprising the genomic region between p25 and p23 (an extension of about 3000 nucleotides), is of paramount importance since it encodes genes that modulate the interaction with the host. Phylogenetic analysis of the sequences obtained for this region showed the existence of seven well defined clusters or phylogenetic groups. Moreover, for three of the phylogenetic groups it was for the first time obtained a complete sequence for this region, since the available sequences in GenBank only correspond to individual genes.
The sequences obtained in this work were also analysed to determine the extent of recombination events in the 3' terminal region. Our results suggest a relative low recombination rate between CTV isolates even in isolates harbouring a mixture of haplotipes and co-habiting the same host for more than 12 years. However, when detected it is always on the same region, the intergenic region between p13 and p20, suggesting that this is a hot-spot of recombination. These results suggest that CTV isolates maintain a high level of stability over time. This was further studied through estimating the rate of evolution using sequence data of the coat protein gene collected over a period of 20 years. The mean genomic substitution rate was estimated to be 8.57×10^{-5} nucleotide substitutions per site per year. This rate is lower than the values obtained for other plant RNA virus for which data is available.

A Survey of Begomoviruses and associated satellites infecting plants in the cotton-growing areas of Northwestern India

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Since 2001, a new epidemic of cotton leaf curl disease has been affecting cotton crops in Northwestern India and Pakistan, causing economic losses of billions of US dollars. This new epidemic is probably the result of recombination events between viruses of the family *Geminiviridae*, which has led to a new geminivirus species: *Cotton leaf curl Burewala virus* (CLCuBV). In this study, geminivirus populations infecting a variety of plants in the cotton-growing regions of Northwestern India have been molecularly characterized by both Rolling rolling circle amplification (RCA) and PCR. In total, three different CLCuBV DNA-A molecules were identified which appear to encode a truncated transcriptional activator protein. Additionally, the sequences of twelve different full-length DNA-As, along with eight different alphasatellites and one betasatellite were obtained. A new satellite-like molecule, similar in structure to the alphasatellites, was identified. Viruses that infected cotton plants, were not detected in non-cotton plants grown nearby. In okra plants, co-infection by two geminiviruses was observed, and a genome resulting from a recombination event between two geminiviruses was detected, showing that plants growing next to cotton fields could act as reservoirs for recombinant geminiviruses.

The evolution of cassava brown streak-associated viruses (family *Potyviridae*) in East Africa

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Cassava brown streak virus (CBSV; *Ipomovirus*; *Potyviridae*) causes a disease of economic importance in East Africa by making the storage roots of cassava unusable. Previously, CBSV was known at the Indian Ocean coastal lowland areas, but recently, a similar disease appeared also at higher altitudes, notably in the Lake Victoria region. The complete, single stranded RNA genomes of 12 CBSV isolates have been characterized by several laboratories. Comparison between the isolates shows a wide genetic variability and identities of 68.9 to 99.3% and 73.6 to 99.4% at nucleotide and amino acid sequences levels, respectively, and the isolates are placed to two distinct phylogenetic clusters based on analysis of the polyprotein amino acid sequences. The genetic diversity between the two groups of isolates is high (0.323 ± 0.021). Moreover, the CI, VPg and CP protein encoding sequences in the isolates of the two groups differ in size. The terminal parts of the polyprotein are evolutionary intriguing. Atypical of the family *Potyviridae*, CBSV polyprotein contains a HAM1-like protein located between the replicase and coat protein at the C-proximal part. The encoding sequence for this novel protein has the lowest aa identity (<55%) between the two phylogenetic groups of isolates. While other characterized members of genus *Ipomovirus* contain a single P1 protein and HC-Pro or two P1 proteins and no HC-Pro at the polyprotein N-terminus, CBSV contains a single P1 protein that functions as an RNA silencing suppressor but no HC-Pro. According to the current molecular criteria for the taxonomy of viruses in the family *Potyviridae*, these arguably suggest occurrence of two distinct viruses associated with the cassava brown streak disease. The originally described virus occurs still predominantly in the lowland areas and should retain the name CBSV, whereas the name Cassava brown streak highland virus (CBSHV) is proposed for the newly emerged virus.

The diversity of ampeloviruses and badnaviruses in Australian pineapples and their association with mealybug wilt of pineapple (*Ananas comosus*)

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To investigate the aetiology of mealybug wilt disease (MWD) in Australian pineapples, a virus diversity study focusing on mealybug-transmitted viruses was completed.
Pineapple mealybug wilt-associated virus 1 (PMWaV-1), -*2* (PMWaV-2) and -*3* (PMWaV-3) were detected in commercial pineapple crops and shown to be transmitted by the mealybug species *Dysmicoccus brevipes*. A new ampelovirus was also identified, for which the name Pineapple mealybug wilt-associated virus 5 (PMWaV-5) is proposed. In surveys of four MWD-affected crops, none of these viruses was clearly associated with the disease at all sites. At one site, PMWaV-2 was associated with disease symptoms and PMWaV-1 and -3 equally distributed between symptomless and MWD-affected plants. However, at the remaining three sites PMWaV-2 was rare, but either PMWaV-1 and/or -3 were associated with MWD. PMWaV-5 was found at all sites but at low incidences.
The previously published partial sequence for Pineapple bacilliform virus was shown to be from a new *Ty3-gypsy* retrotransposon for which the name Ananas metavirus (AMtV) is proposed and not from a badnavirus as previously thought. Two unique *Badnavirus* species were detected from pineapple, for which the names Pineapple bacilliform comosus virus (PBCoV) and Pineapple bacilliform erectifolius virus (PBErV) are proposed. Both viruses were transmitted by *D. brevipes*, and PBCoV also by *Planococcus citri.* Neither virus was associated with MWD at any of the survey sites. Furthermore, PBErV was only detected in a few commercial field plants whereas the incidence of PBCoV varied from uniform to rare depending on the site.
The presence of a putative endogenous caulimovirid sequence in pineapple was detected, for which the name endogenous Pineapple pararetrovirus-1 (ePPRV-1) is proposed.A. In phylogenetic analyses this sequence clusters with caulimovirids, but does not appear to belong to any of the existing genera within the *Caulimoviridae* family. The role ePPRV has in MWD, if any, is unknown.

Variation in sugarcane cultivar host range of *Sugarcane yellow leaf virus* genotypes in Guadeloupe

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Yellow leaf is a widely spread disease of sugarcane that can cause yield losses in susceptible cultivars. This disease is caused by *Sugarcane yellow leaf virus* (SCYLV) which belongs to the genus *Polerovirus* (*Luteoviridae* family). Five different SCYLV genotypes have been described and three of these were found in Guadeloupe (genotypes BRA-PER, CUB and REU). Additionally, variation in infection capacity and virulence exists among SCYLV genotypes. In order to investigate the cultivar host range of SCYLV genotypes occurring in Guadeloupe, we explored the capacity of genotypes BRA-PER, CUB and REU to infect different sets of sugarcane cultivars. A first experiment was performed with 30 cultivars from a breeding plot (non-replicated design). Four leaves were sampled per cultivar, bulked and processed for identification of virus genotype by RT-PCR. A second experiment was performed with 25 cultivars from a core collection of 200 accessions (experimental design with three replications). Ten leaves were sampled per accession and per replication, bulked and processed for identification of virus genotype by RT-PCR. The breeding plot and the core collection were both located at CIRAD's research station in Guadeloupe where disease pressure was high. In both experiments, SCYLV genotypes CUB and REU were found in a larger number of cultivars than genotype BRA-PER. In the second experiment, genotype CUB was present in all SCYLV-infected cultivars and was therefore the virus genotype with the largest cultivar host range. This genotype appears to have the propensity to infect all sugarcane cultivars infected by genotypes BRA-PER and REU. Additionally, cultivar host range varied to some extent between genotypes BRA-PER and REU, suggesting the occurrence of host-ranges specific to these SCYLV genotypes. Variation in sugarcane cultivar host range between SCYLV genotypes may explain, at least in part, the variations in SCYLV incidence and epidemics reported in different sugarcane growing locations.

Sequence comparison of different *Cauliflower mosaic virus* isolates infecting canola in Iran

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During 2007-2008, different canola fields in different part of country were surveyed and, a number of 560 leaf samples showing various virus-like symptoms were collected from Varamin (Tehran province), Ghazvin and Takestan (Ghazvin province), Shahrekord (Chaharmahal-e-Bakhtiari province), Sari (Mazandaran province), Moghan (Ardabil province), Naghadeh (Azabayejan-e-gharbi province) and Shiraz (Fars province). Using DAS-ELISA method, samples were tested by polyclonal antiseras (DSMZ,Germany) for the presence of *Cauliflower mosaic virus* (CaMV). According to the results obtained by DAS-ELISA 43.03% of samples were infected with CaMV. Results also showed that CaMV is an abundant virus infecting oilseed rape in all the above regions with different frequency. Biological diversity of the selected eight isolates were evaluated on the basis of the reactions on turnip (*Brassica rapa*), Kohlrabi (*B. oleracea var. gongylodes*) and datura (*Datura stramonium*) plants. Using PCR molecular method and specific primers designed for the initial sequence of the ORFV of the viral genome sequence, presence of the CaMV confirmed for the selected ELISA positive samples. All infected tested samples amplified a 840-bp fragment in PCR reaction. The amplified fragments of all the eight isolates have first been sequenced ,aligned with the corresponding data available for CaMV isolates in NCBI. GenBank accession numbers for Iranian CaMV isolates nucleotide sequences assigned (GU361758, GU361759, GU361764, GU361763, GU361762, GU361760, GU361761 and GU361765 .Phylogenetic analysis revealed that all these Iranian isolates together with two NCBI isolate (Cabb-D/H,Xingjiang) were categorized in one cluster The primers designed for this study based on a conserved region in the ORFV of the caulimoviruses which facilitate rapid detection and further determination of the isolates.

Metagenomics and quarantine: searching for the unknown

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A broad cataloging and study of viruses is fundamental for conducting safe quarantines in order to prevent disease from entering a country. Disease detection is one of the main quarantine operations; disease detection tools need to be able to identify very small quantities of pathogen and, during the same test, to detect all the variants of the same pathogen. However, a major challenge remains by using the classical detection tools: identifying latent viruses and detect new and unknown diseases that could emerge in a near future.
For scientific and sanitary reasons, we have chosen plant viruses and the CIRAD's sugarcane quarantine as a test case. Plants are an ideal starting point for studies on virus diversity and ecology since they are immobile and can be readily resampled. In addition, CIRAD's sugarcane quarantine in Montpellier gathers worldwide plants originating from approximately 40 countries. Hence, the overall worldwide diversity of sugarcane viruses is likely to land in the CIRAD's sugarcane quarantine.
We aim at using a metagenomic approach that will combine tagged random RT-PCR and high-throughput pyrosequencing. This system could be useful to discover at a very early stage, potentially emerging viruses and to assess the rate of viral co-infections and the spatial distributions of quarantine viruses detected in the quarantine greenhouse.

Epidemiology and phylogenetic aspects of *Iris yellow spot virus* (Tospovirus) naturally infecting onion plants in Peru

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Iris Yellow spot virus (IYSV) is a *Tospovirus* (Family *Bunyaviridae*) reported worldwide, mainly infecting onion plants. It is responsible for significant yield reductions of this vegetable. In South America, IYSV was already reported in Brazil, Chile and Peru. In the latter, the export market of fresh and dries onions was increased over the past years. In this work, onion plants with typical symptoms of IYSV infection were collected in three onion-producing provinces in Peru. Several samples were collected and the viruses were transferred onto *Nicotiana benthamiana* by mechanical inoculation. Total RNA from each sample was extracted and used as template for amplification of the nucleocapsid gene by RT-PCR with the primers J13 and IYSV-NBH-R. The expected amplified fragments of approximately 0.9 Kb were cloned into pGEM-Teasy and sequenced. Sequences of four Peruvian-IYSV isolates were compared with those available in the GeneBank and multiple alignments of nucleoprotein amino acid sequences were used as input for phylogenetic analysis. These chosen isolates originated from the more important onion-producing areas in the country as Lima, (Barranca), Ica (San Jacinto) and Arequipa (Congata and Alata). The results showed that IYSV is widely spread in all sampled regions in Peru. The amino acid sequence identity when compared among the four isolates ranged from 96% to 99%. The phylogenetic tree based on the amino acid sequences showed that, although variability can be seen in the N-amino acid sequences, the Peruvian isolates were placed in the same cluster. The four new isolates sequenced in this work also clustered with USA isolates and other Peruvian isolates previously characterized. However, they differ from other isolates characterized in South America (eg. Brazil, Chile). The genetic similarity between American and Peruvian isolates is probably explained by the onion trade route between these countries and the similar growing conditions.

Sequence analysis of Potato virus M isolates from Czech Republic

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Potato virus M (PVM), a member of the genus *Carlavirus* is one of the most common viruses of potato. PVM has been reported from the United States and some parts of Europe. Carlaviruses like PVM have not been as extensively characterized as plant viruses of other genera. There are only a limited number of complete genome sequences of PVM available in GenBank.
Detection and elimination of viruses in potato seed stock is very important for potato health and production worldwide. When new virus isolates or strains occur, the standard tests must be updated to provide the most efficacious detection possible. Potatoes infected with PVM are often infected with another carlavirus, mainly with Potato virus S. The simultanous infections of potatoes with more carlaviruses are dangerous, because it could lead to recombination events between similar sequences of related viruses and give rise to a new virus with new properties. One example of such an event came from the Idaho (USA) where a new strain of PVM was found having significant differences in coat protein sequences as well as in serological activity. Sequence analysis of PVM isolates and comparison of their sequences with other known carlaviruses are therefore important for production of new antibodies based on structural or nonstructural viral proteins.
We designed primers covering the whole PVM genome and amplified overlapping genomic fragments. These fragments were sequenced and the obtained sequences were arranged into a single contig. Genomic sequences of Czech PVM isolates were compared with sequences of other already sequenced PVM isolates and other related carlaviruses available in GenBank. Suitable sequences were chosen for protein expression and antibodies production.
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Use of plant viruses in non-food agriculture

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Transient expression of heterologous proteins in plants represents an attractive strategy for vaccines production combining cost-effectiveness and safety. We investigated the possibility to transiently express *human papillomavirus* type 16 (HPV-16) epitopes-based vaccine in plants using potato virus X-based vector and to induce an immune response against HPV.

The optimized expression of recombinant *Potato virus X* (PVX) coat proteins (XCP) carrying different epitopes from HPV-16 was developed. Epitopes derived from the L2 minor capsid protein and E7 oncoprotein were joined as N-terminal or C- terminal fusions with XCP of a *Potato virus X* based vector and these recombinant proteins were initially expressed in *E. coli* to prove their ability to form virus-like particles (VLPs). Then, the transient expression in plants using *Agrobacterium tumefaciens* mediated inoculation was performed. To increase the level of the produced proteins the transgenic *Nicotiana benthamiana* plants expressing the *Potato virus A* HC-Pro gene were tested. Immunogenicity of these recombinant viruses was tested after immunization of mice. Recombinant viruses were injected subcutaneously or administered by a tattooing device. In animal sera the antibodies against the XCP and the L2 epitope were found after both methods of vaccine delivery.

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Genetic diversity of Pepino mosaic virus in the U.S. and identification of a tomato infecting strain capable of inducing disease on potato

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Growers were once reluctant to remove Pepino mosaic virus (PepMV)-infected tomato plants because its effect on yield was considered mild. Pepino mosaic has now become an endemic disease problem on greenhouse tomatoes in the U. S. Recently, viroids (i.e., Tomato chlorotic dwarf viroid - TCDVd) were identified to naturally infect tomatoes. Mixed infection of PepMV and TCDVd appeared to result in more dramatic disease symptoms on tomato. The objectives of the present study were to evaluate the host range via mechanical inoculation, to conduct molecular characterization of disease causing agents on various alternative host plants, and to determine whether the disease was caused by a synergistic effect of PepMV and TCDVd or involved some other unidentified components. Four field isolates were selected for host range study with 38 plant species in 9 families. In addition to the detail examination of disease symptoms exhibited on the indicator plants, appropriate detection methods (ELISA or RT-PCR) were employed to determine the PepMV and/or viroid infection. Infectivity of PepMV and/or viroid on the alternative host plants was confirmed through back-inoculation onto tomato 'Money Maker'. Nucleotide sequences obtained through direct sequencing of PCR amplicons for PepMV or Pospiviroid were analyzed against GenBank database. The results showed that in addition to the prevalent EU-like genotype, the CH2 genotype has now expanded to other states in the U.S. A tomato-infecting EU-like strain was capable of inducing disease symptoms on potato upon mechanical inoculation. Although the original pepino strain of PepMV was capable of infecting potato, to our knowledge this is the first time that a tomato strain of PepMV was demonstrated to infect potato. These results indicated that tomato growers in the U.S. are facing difficulties in effectively managing their viral disease problems due to the emergent of new PepMV genotype (CH2) and the introduction of viroids.

Evolution of resistance-breaking in *tomato spotted wilt virus:* response to selection by *sw-5* mediated resistant tomato.

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The rapid evolution of resistance-breaking (RB) in RNA plant viruses circumvents human efforts to develop disease-resistant crops. Increasing selection for RB through repeated use of crops expressing the same resistance gene has important implications for their long-term utility. Understanding fitness as well as virulence of a virus under intense selection will aid in development of models to predict the consequences of using plants with dominant single gene resistance. To explore the evolution of virus fitness and virulence in RB isolates, we selected *tomato spotted wilt tospovirus* (TSWV) and cultivated tomato (*Solanum lycopersicum)* as a model system. We are testing the hypothesis that selection for RB viral phenotypes through sequential passage in hosts with the *sw-5* dominant single gene for resistance will increase virus fitness in subsequent resistant hosts and decrease virus fitness in susceptible hosts. We are also predicting that RB isolates serially transferred through susceptible hosts will be less fit when returned to a resistant host. By increasing the number of transfers in one host type, we expect to see a more significant decrease in virus fitness when returned to the alternate host type. Measurements of the variation in virulence from one host transfer to the next will be used to predict the consequences of RB epidemics in sequential cropping systems vs. systems with host alternation. Preliminary results suggest a change in viral phenotypes under selection by resistant hosts. Future directions will explore changes at the molecular level to determine the strength of selection by the host plant and make predictions about the evolution of RB in TSWV.

Molecular characterization of endogenous plant pararetroviruses in wild *Dahlia* spp from natural habitats

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We recently described an endogenous plant pararetrovirus (DMV-D10) belonging to the genus *Caulimovirus* in dahlia (genus *Dahlia*). The central mountain ranges of Mexico are home to the greatest diversity for this genus. First described in 1791, the genus has 35 recognized wild species in addition to the cultivated forms, known as either *D. pinnata* or *D. variabilis*. Majority of these wild dahlia species were found in the Mexican mountain ranges. Recent survey on incidence of the three caulimoviruses [*Dahlia mosaic virus* (DMV), D10-US, and Dahlia common mosaic virus (DCMV)] in wild dahlia species showed the presence of DMV-D10 sequences and no evidence of DMV or DCMV. To better understand the genetic diversity of DMV-D10 from wild species, the genome structure and organization of DMV-D10 from wild dahlia species, *Dahlia coccinea* (D10-DC), *Dahlia sherffii* (D10-DS) and *Dahlia tenuicaulis* (D10-DT) were studied and compared to that of D10-US from cultivated species. The complete ca. 7 kb dsDNA genome of D10-DC, D10-DS, and D10-DT had the structure and organization typical of a *Caulimovirus* species and shared 89.3 to 96.6% amino acid sequence identity among various ORFs when compared to those of D10-US from *D. variabilis.* The absence of an aphid transmission factor homolog and the truncated coat protein open reading frame that was fused with the reverse transcriptase gene were also common among these DMV-D10 isolates from wild and cultivated *Dahlia* species. Molecular characterization of plant pararetroviruses in wild plant species in their natural habitats could provide important clues about the possible emergence, co-existence and co-evolution of pararetroviruses and their host plants.

Molecular epidemiology of African cassava mosaic viruses in Yangambi, Northeastern Democratic Republic of Congo

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A molecular epidemiology study on *Begomoviruses* involved in the Cassava Mosaic Disease (CMD) was conducted around Yangambi (DRCongo). The incidence and disease severity was systematically evaluated and compared to results from targeted PCR and derived sequences from both cassava, leguminous and whiteflies collected in the visited fields.

The results showed that CMD viruses are widely spread throughout the investigated area, possibly along circulation axis. Up to 17 ACMV and 10 EACMV DNA A sequences along with more than 140 sequences of AC2/AC4 genes were obtained. Dual infections of cassava samples are common (66 %). The EACMV Uganda severe was predominant, in relationship with highly infested fields. Data analysis shows a distribution of the viruses in relationship to the type of environment in which cassava is grown. The viruses isolates are related to the major ecosystems : moderate isolates are closer to the evergreen humid forest in which agriculture activity is extensive and the severe isolates are limited in the secondary forest, in which the cassava crop is intensive. EACMV isolates are randomly spread as well in primary forest as in the mix secondary forest or savannah.

However, dual infections were also frequent in whiteflies (59 %). The various biotopes and the long lasting presence of cassava makes this region of special interest for studying the spread and development of CMD. Therefore, knowing the constant evolutionary process of begomoviruses, our study focused on the potential contribution of forest plants species to the emergence of different viruses: ACMV and EACMV were detected in two leguminous *Fabaceae* (*Centrosema pubescens* and *Pueraria javanica*) showing the role of whiteflies in spreading the disease. The wide presence of EACMV-UgV, the high incidence and severity of the CMD raises the question whether they should be considered at the forefront of the CMD pandemic by now.

Three commonly co-occurring perennial grass species have less herbivore and pathogen attack in their introduced range than in their native range

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The role that herbivores and pathogens (natural enemies) have in invasions by introduced species is currently under debate. The enemy release hypothesis states that introduced species lose natural enemies upon introduction, and are "released" from population control by their enemies. We surveyed several populations of each of three perennial bunchgrass species introduced to the United States from Europe in their native and introduced ranges. We surveyed natural enemy attack on populations of *Anthoxanthum odoratum*, *Holcus lanatus*, and *Schedonorus arundinaceus* in Oregon, Washington, North Carolina, New York, and Wisconsin. In Europe we surveyed populations in Switzerland, Austria, Germany, and the Netherlands. We found significantly higher percent leaf area damaged and more types of enemy attack on all three species in their native range than in their introduced range. The only exception was the number of types of natural enemy attack on *A. odoratum*, which did not differ significantly between the two ranges. Individual plant aboveground biomass was higher for all three species in the introduced range than in the native range. Our results are consistent with the predictions of the enemy release hypothesis.

Wednesday, June 23, 2010

Vector Biology/Virus Transmission

The seemingly simple non-circulative transmission of a plant virus is hiding an extremely sophisticated interplay between virus, plant and vector

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Nearly all plant viruses use vectors to spread between hosts, most common vectors being insects, especially aphids. A highly common strategy for virus–vector interaction is non-circulative transmission, where the virus is taken up by a vector in an infected plant, adsorbed somewhere on the cuticle lining of the inner part of the feeding apparatus, and subsequently released into a new host plant. While the viral components involved in this phenomenon are well established in several viral species, the counterpart binding sites in the insect vector remain vastly elusive[1]. Similarly, the direct involvement of plant factors in the vector-transmission of viruses has rarely been investigated[2].

1- We have recently demonstrated the existence of a protein receptor for *Cauliflower mosaic virus* (CaMV), in the stylets of aphid vectors. An *in vitro* system allowed visualization of the interaction between dissected stylets and the CaMV ligand protein. The receptor molecules are concentrated in a tiny area at the extreme distal tip of the maxillary stylets, lining the bed of the common food/salivary duct. Recently, high resolution scanning and transmission electron microscopy have allowed the direct visualisation of the anatomical structure containing these receptors molecules. This anatomical structure in aphid stylets has been previously overlooked and its physiological role remains to be elucidated.

2-Regarding transmission, host cells are usually conceptualised as simple bags where the virus accumulates and is eventually taken up by a vector feeding on cell contents. Several lines of evidence radically changing this simplistic view will be presented on the example of CaMV. Our results demonstrate that the virus is carefully preparing its encounter with the vector, through specific and astonishingly complex interactions with the plant cell. Several converging preliminary results are indicating that the virus is “sensing” the presence of its vector, and immediately changing its accessibility within infected cells, thereby increasing its chances of acquisition by the aphid vector.

An aphid gut binding peptide impedes entry of Pea enation mosaic virus into the aphid hemocoel

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Aphids transmit more than 275 plant viruses that result in considerable economic loss within the agricultural sector. Methods to block virus transmission by aphids could lead to novel and broad-spectrum approaches for management of plant viruses. Viruses in the Luteoviridae are obligately transmitted by aphids in a persistent manner that requires virion accumulation in the aphid hemocoel. To enter the hemocoel, the virion must bind and traverse the aphid gut epithelium. The molecular mechanisms involved in this process are poorly understood. By screening a phage display library, we identified a twelve residue gut binding peptide (GBP3.1) that binds to the midgut and hindgut of the pea aphid *Acyrthosiphon pisum* (Harris). Binding was confirmed by labeling the aphid gut with a GBP3.1-green fluorescent protein fusion. GBP3.1 reduced uptake of Pea enation mosaic virus from the pea aphid gut into the hemocoel. GBP3.1 also bound to the gut epithelia of the green peach aphid and the soybean aphid. These results suggest a novel strategy for inhibiting plant virus transmission by at least three major aphid species.

Grapevine leafroll-associated viruses – mealybug transmission biology and ecology

Rodrigo P.P. Almeida and Kent. M. Daane

Mealybug-borne viruses cause several plant diseases of economic importance. Grapevine leafroll is an emerging disease caused by a complex of closteroviruses, primarily in the genus *Ampelovirus*, which can be mealybug-borne. We are studying several aspects of this disease system in California vineyards, focusing on transmission biology and ecology, and pathogen ecology. Transmission experiments with one of the primary mealybug vectors, *Planococcus ficus* (Hemiptera: Pseudococcidae), showed that *Grapevine leafroll-associated virus 3* is transmitted in a semi-persistent manner. In addition, transmission assays using different mealybug (*Pseudococcus* spp.) and virus species, coupled with phylogenetic analyses, indicated no virus-vector specificity in this complex; although the number of mealybug-ampelovirus combinations used was limited. We are also studying the role of seasonality and plant tissue on both mealybug and virus populations, aiming to incorporate these parameters on disease spread models, and preliminary data will be discussed. Lastly, because this is a disease complex and the leafroll virus(es) driving the current epidemic in California has not been identified, we are surveying vineyards in affected regions using a hierarchical approach, starting at the leafroll species level to strains of one apparently dominant species.

Examining the impact of elevated CO_2 on wheat, *Cereal yellow dwarf virus* and its vector

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The Intergovernmental Panel on Climate Change released their fourth assessment report in 2007 which concluded global warming is occurring presently and that changes in the global climate system will continue into the foreseeable future. These changes are expected to have a major impact on agricultural systems, particularly as both CO_2 and temperature are expected to increase and more frequent severe weather events, such as drought, are expected to occur. However, there is a lack of empirical data on the effects of elevated CO_2 and temperature on agricultural pest and pathogen populations. Consequently, predictions on the future of our major monoculture crops such as wheat, have not been fully explored.

The Department of Primary Industries Victoria, the University of Melbourne and the Department of Climate Change have established a Free-Air CO_2 Enrichment (FACE) research facility at Horsham, Victoria, to study the effects of elevated CO_2 on wheat production in Australia. This facility is being used to study the effects of projected CO_2 concentrations (550ppm) under field conditions on wheat, the *Cereal yellow dwarf virus* (CYDV) RPV isolate, as well as the population dynamics of its aphid vector *Rhopalosiphum padi* (Homoptera, Aphididae). Results from studies on wheat plants conducted at the FACE facility show changes in C:N ratio, increase in plant height, biomass, number of tillers, and surface area in response to elevated CO_2 with root biomass and root length unaffected. However, variable field conditions have proved difficult for studying the impact of elevated CO_2 on CYDV and its vector, therefore in addition to the FACE facilities, controlled environment growth chambers are being used to study the physiology and feeding behaviour of *R. padi* and its ability to acquire and transmit CYDV under various climatic conditions and CO_2 concentrations. Preliminary experiment data from the FACE facility and growth chambers will be described and results presented. Potential ecological and epidemiological consequences will be discussed.

Virus-vector interactions mediating the specific retention and whitefly transmission of criniviruses

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Vector transmission is a crucial step in the development of virus disease epidemics, and is mediated by critical processes in virus acquisition, retention and inoculation. For members of the emerging and economically important genus *Crinivirus*, transmission is achieved in a semi-persistent manner by specific whitefly vectors. Viruses exhibiting this mode of transmission are acquired and inoculated within minutes to hours; they are retained in the vector from hours to days but do not circulate through the vector, and are not retained beyond vector molting. In addition, retention of virions in the insect's foregut appears to be involved in the vector transmission of some of these viruses. Among criniviruses, transmission of *Lettuce infectious yellows* (LIYV) by the whitefly *Bemisia tabaci* biotype A is best studied. However, little is known about its biology within the vector. To address this deficiency, we have developed an immunofluorescent localization assay aimed at investigating this poorly understood aspect of transmission. Our studies revealed that fluorescence was seen in the foregut of viruliferous vectors, and virus transmission was frequently observed when the signal was present in >39% of the whiteflies. In contrast, fluorescence was rarely seen in the foregut of non-vectors, and no virus transmission was observed following virus acquisition. A second assay, which could complement the immunofluorescent localization studies, involves testing the transmission and retention binding of engineered mutants recovered from plants infected with LIYV constructs delivered via an agroinoculation procedure. We demonstrated the feasibility of this approach by testing the transmission of wild type LIYV and a transmission defective mutant identified with a truncated minor coat protein. This is only the first step; many issues remained unexplored. What is/are the viral protein(s) that mediate(s) foregut retention? Are transmission defective mutants retained in the foregut of vector whiteflies? These and other developments will be discussed.

Sequence polymorphism of a glassy-winged sharpshooter phytoreovirus reveals a bottleneck in the Californian population

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The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis* Germar) is an invasive insect introduced to California circa 1989. Native to the southeastern U.S. and northeastern Mexico, GWSS is of economic concern as a vector of the Pierce's disease bacterium *Xylella fastidiosa*. Recently, a novel phytoreovirus species (*Homalodisca vitripennis* reovirus, HoVRV) infecting GWSS was characterized. As viral genomes evolve at a rate many log units greater than their hosts, it was postulated that HoVRV polymorphism may serve as a marker to discriminate GWSS populations. Complete genome sequences of five Californian and four southeastern HoVRV isolates were evaluated for polymorphism. Pairwise diversity was ~10-fold less for HoVRV in California (~0.1%) compared to the southeastern U. S. (~1%). Phylogenetic analysis of each dsRNA segment indicated that the Californian isolates grouped as a monophyletic lineage. In contrast, relative placement of southeastern U. S. isolates varied among dsRNA segments. To sample diversity at single locations, dsRNA Segment 11 was sequenced for nine additional isolates each from Riverside, CA and Johnston Co., NC. Whereas 9 of 10 Riverside isolates were identical (the tenth varied at one position), diversity among Johnston Co. isolates approached that of the southeastern U. S. population. Coalescent analyses estimated median age of the Californian population at 11.6 to 26.3 years, depending upon the demographic model employed. Estimates of median clock rate for the Californian population were 1.662×10^{-5} to 5.502×10^{-5} nt substitutions/site/year. Collectively, the results indicate that HoVRV diversity in the native range of GWSS was high relative to a newly established population, and that the Californian population of HoVRV was subjected to a bottleneck coinciding with introduction of GWSS. By proxy, the results suggest that GWSS establishment in California resulted from a limited introduction and that HoVRV genotype may serve to identify source of the Californian GWSS population.

Differential transmission rates of PVY^{O} and PVY^{NTN} from two inoculum sources by three aphid vectors

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Several aphid species vector *Potato virus Y* (PVY), the most economically important virus affecting potato [*Solanum tuberosum* (L.)] production in the United States (US), in a non-persistent manner. We studied the transmission of PVY^{O} and PVY^{NTN}, two PVY strains present in the Pacific Northwest (PNW) of the US, to potato by three aphid vectors: the most efficient potato-colonizing aphid vectors of PVY, the green peach aphid *Myzus persicae* and the potato aphid *Macrosiphum euphorbiae*, and the most abundant cereal aphid that migrates in large numbers through Idaho potato fields during the middle of the growing season, the bird cherry-oat aphid *Rhopalosiphum padi*. Two inoculum sources were used for these experiments: hairy nightshade, *Solanum sarrachoides*, and potato. Hairy nightshade is a prevalent annual solanaceous weed in the PNW and a preferred host for *M. persicae* and *M. euphorbiae*. Percentage transmission of PVY^{NTN} by *M. persicae* and *M. euphorbiae* was twice as high (46 and 34%, respectively) from hairy nightshade to potato than from potato to potato (20 and 14%). Although no significantly different, percentage transmission of PVY^{O} by *M. persicae* and *M. euphorbiae* was also higher (20 and 20) from hairy nightshade to potato than from potato to potato (14 and 16%). Percentage transmission of either PVY strain by *R. padi* was lower than the one of the two other aphid vectors and not affected by the inoculum source. These results show that hairy nightshade may be an equal or better virus reservoir than potato and thus, becoming important in the epidemiology of PVY.

Vector fitness on infected plants affects virus epidemiology

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The frequency of different viral isolates in the field is in part due to their transmission efficiency by its vectors. Other factor affecting virus epidemiology could be the effect of viral infected plants on the performance of their vectors. *Citrus tristeza virus* (CTV), one of the most devastating viral diseases worldwide, is transmitted by several aphid species in a semi-persistent manner. *Aphis gossypii* is the main vector of CTV in the Mediterranean area. The low frequency of specific CTV isolates in the field could be due to lower quality of infected trees as hosts for *A. gossypii*, therefore sustaining smaller aphid colonies and producing lower number of dispersing winged aphids. In this work we compare the fitness of *A. gossypii* (juvenile survival, development and reproduction of aphids) developing on two *Citrus* hosts (Mexican lime and sweet orange) infected with four CTV-isolates differing biologically and genetically. Additionally, the transmission efficiency of these four isolates by *A. gossypii* was studied on both citrus species. Our results show that CTV-infection of the host plant affects fitness of *A. gossypii* developing on them. The effect of the different CTV-isolates on *A. gossypii* fitness depends on the citrus host plant and the isolate and it is not related to virus virulence or to the geographical origin of the isolate. Transmission rates of the isolates tested differed between the two host plants studied. On sweet orange, the isolate resulting in lower fitness for *A. gossypii* was transmitted at the highest rate. The comparison of these results with the data on epidemiology of CTV indicates that the low frequency of some CTV-isolates in Spanish citrus orchards could be explained by its detrimental effect on *A. gossypii* rather than by their rate of transmission by aphids. Overall, our results suggest that vector fitness on virus infected plants plays an important role in viral epidemiology.

Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts.

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Vector-borne pathogens can alter the phenotypes of their hosts and vectors in ways that influence the frequency and nature of interactions between them, with significant implications for the transmission and spread of disease. For insect-borne pathogens, host odors are particularly likely targets for manipulation, because both plant- and animal-feeding insects use volatile compounds derived from their hosts as foraging cues. Here, we document the effects of a ubiquitous and economically important plant pathogen, *Cucumber mosaic virus* (CMV), on the quality and attractiveness of one of its host plants (*Cucurbita pepo* cv. Dixie) for the aphid vectors *Myzus persicae* and *Aphis gossypii*. Our results indicate that CMV greatly reduces host-plant quality—aphids performed poorly on infected plants and rapidly emigrated from them—but increases the attractiveness of infected plants to aphids by inducing elevated emissions of a plant volatile blend otherwise similar to that emitted by healthy plants. Thus, CMV appears to attract vectors deceptively to infected plants from which they then disperse rapidly, a pattern highly conducive to the non-persistent transmission mechanism employed by CMV and very different from the pattern previously reported for persistently transmitted viruses that require sustained aphid feeding for transmission. In addition to providing a documented example of a pathogen inducing a deceptive signal of host-plant quality to vectors, our results suggest that the transmission mechanism is a major factor shaping pathogen-induced changes in host-plant phenotypes. Furthermore, our findings yield a general hypothesis that, when vector-borne plant or animal pathogens reduce host quality for vectors, pathogen-induced changes in host phenotypes that enhance vector attraction frequently will involve the exaggeration of existing host-location cues.

Comparative genome analysis of an asymptomatic *citrus tristeza virus* isolate with its symptomatic aphid transmitted sub-isolates

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Citrus tristeza virus (CTV), is a closterovirus transmitted mainly by aphids in a semi-persistent manner. The main vector for the transmission is *Toxoptera citricida*, the brown citrus aphid (BCA). The molecular interactions between CTV and its aphid vectors have not been determined. In addition the gene/s responsible for symptoms also is unidentified. Studies were done to better understand the relation of 10 different CTV genes (p33, p6, p65, p61, p27, p25, p18, p13, p20 and p23) with symptoms produced and the changes in CTV genotypes after the BCA transmission. A CTV isolate from Corsica, CTV-B192, and its single aphid transmitted (AT) sub-isolates were studied since the AT sub-isolates produced different symptoms from the asymptomatic source isolate. Using genotype specific primers, the parent CTV-B192 was identified as mixture of T30 and VT genotypes whereas the T30 genotype was absent in 1st or 2nd level AT sub-isolates. Moreover, two AT sub-isolates contained an additional genotype with VT in mixed infections and symptoms of severe vein clearing, vein corking in Mexican lime and stem pitting in sweet orange and grapefruit were produced. The VT genotype was determined to be the minor genotype in the source isolate but was identified as the major and most transmissible genotype in the AT sub-isolates. Relative quantification of the genotypes showed that the VT genotype was higher than T30 genotype in the AT sub-isolates but was lower in the source isolate. In addition some intermediate genotypes also were detected in the AT sub-isolates and were found to be recombinants. The CTV genotypes in the source isolate and graft transmitted isolates of AT sub-isolates remain unchanged overtime. These results indicate that aphid transmission may be an important factor in CTV recombination and evolution

Alterations of capsid protein amino acid positions internal to the virion disrupt nonpersistent virus transmission by aphids

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In the atomic model of Cucumber mosaic virus (CMV), six amino acid residues form stabilizing salt bridges between subunits of the asymmetric unit. To evaluate the effects of these positions on virion stability and aphid vector transmission, six charged amino acid residues were individually mutated to alanine. Five of the six engineered viruses, mutants D100A, K127A, D176A, D179A, and K182A, were viable and able to systemically infect Nicotiana tabacum and to locally infect Chenopodium quinoa; mutant K101A could not be recovered. In order to assess the physical stability of mutants, virions were purified from plants and tested in a urea disruption assay. All five mutant viruses were stable during purification in the presence of 1.5 M sodium and chloroform and exhibited wild type levels of virion stability in the presence of urea. Aphid vector transmissibility of three of the five mutants, D100A, K127A, and D176A, was nearly eliminated. After a series of mechanical passages, additional second site mutations were selected in four of the five mutant viruses, including in one compensatory mutation that restored wild type levels of aphid transmission. It is hypothesized that non-surface associated amino acids involved in acid-base pairing affect the dynamic properties of virions that are in turn required for aphid vector transmission.

New insights on the transmission mechanisms of plant viruses by their aphid vectors

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Aphid saliva has a crucial role in the mechanisms of aphid stylet penetration in plant tissues and the inoculation of plant viruses. Watery saliva contributes to the inoculation of the circulative *Pea enation mosaic virus* (PeMV) during the very early phase of intracellular stylet punctures (so-called subphase II-1). During such phase, typical nonpersistent viruses (*Cucumber mosaic virus* and *Potato virus Y*) are also inoculated readily by their aphid vectors.
However, the mechanism of inoculation of semipersistent aphid-transmitted viruses is not well understood. We conducted experiments with *Cauliflower mosaic virus* (CaMV, *Caulimovirus*), a semipersistent virus known to be retained in a specialized anatomical structure of the common duct and inoculated during successive intracellular stylet punctures (pd) in the epidermal and mesophyll cells. By using electrical penetration graphs (EPGs) we compared the behavioural events associated to the inoculation of CaMV with those of the nonpersistent *Turnip mosaic virus*, using the same vector -*Brevycorine brassicae* L.- and test plant –turnip-. Unlike for nonpersistent viruses, CaMV was not inoculated during the II-1 sub-phase (associated to intracellular salivation) and required the II-2 subphase to be readily inoculated to receptor plants. This result suggests that a mechanism other than salivation might be involved in the inoculation of CaMV.
Circulative viruses are inoculated by accessory salivary gland secretions during sieve element punctures, although they can be secreted much earlier as shown for PeMV. In a series of EPG-controlled experiments using *Cucurbit aphid-borne yellows virus* (CABYV, *Polerovirus*), we investigated the role of aphid saliva during stylet penetration in superficial plant tissues. Viruliferous aphids and leaf disk samples obtained from the penetration site were used for the detection of CABYV by quantitative RT-PCR (qRT-PCR). Virus particles were detected from leaf disks even before the first intracellular stylet puncture was produced, indicating that watery saliva is secreted by aphids at the very beginning of stylet intercellular penetration activities.

Identification of *Myzus persicae* proteins that interact with PVY HC-Pro *in vitro*

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Many reports discuss the possible existence of aphid receptors involved in the transmission of non-persistent viruses by aphids. However, little information is available about the nature of these putative receptors and their exact location inside the aphid body. In this work we identified three aphid cuticular proteins that interacted *in vitro* with *E. coli* expressed PVY-HC-Pro by screening a cDNA library of 15360 aphid clones (Ramsey *et al*. 2007). The screen was done by spotting individual clones onto Genetix Q-bot high-density colony filters, inducing expression of protein, incubating the filters with purified HC-Pro containing an N-terminal histidine tag then identifying the bound HC with an anti-Histidine antiserum. The three clones were sequenced and analysis revealed that one of them shared 92% sequence identity with a characterized cuticle protein containing a RR-1 chitin-binding domain reported to interact with the HC-Pro of ZYMV potyvirus (Dombrovsky *et al*., 2007). The others were identified as PR-2 cuticle and RR-3 exoskeleton proteins.

The genes were amplified, re-cloned into the PQE vector, expressed in bacteria and purified then used in protein overlay assays to confirm the interaction with *E.coli* expressed HC-Pro protein or protein purified from plants infected with a PVX virus-based vector carrying HC-Pro (Sasaya *et al*., 2000). Weak interactions were obtained in these experiments; however, this may reflect the use of relatively small quantities of purified PVY HC-Pro used (10-20µg/ml). To overcome this limitation, the his-tagged HC-Pro of another potyvirus, TEV (Blanc *et al*., 1999) was purified from infected tobacco plants and used in the same assay. Preliminary data suggests that TEV HC-Pro (at 100-200µg/ml) interacted readily with the identified cuticle proteins. This finding supports other reports that the potyvirus aphid receptors are of a cuticle nature (e.g. Dombrovsky *et al*., 2007). Further experiments are in progress to confirm the interaction and to investigate the location of these proteins inside the aphid body.

Biomarkers distinguishing virus transmission competent and refractive insect populations identified by coupling genetics with quantitative intact proteomics

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Yellow dwarf viruses cause the most economically important virus diseases of cereal crops world-wide and are vectored by aphids. Previously, we generated *Schizaphis graminum* (greenbug) F2 populations by crossing transmission competent and refractive genotypes. F2 genotypes differed in their transmission phenotype and barriers: the gut or the salivary gland. Competent and refractive F2 genotypes were compared using difference gel electrophoresis (DIGE) and LC-tandem mass spectrometry. Thirty-five proteins involved in energy metabolism, membrane trafficking and the cytoskeleton were identified as differentially expressed between competent and refractive aphids; among them a receptor with homology to the human Epstein Barr virus receptor, PITP, GAPDH3, tubulin and proteins from the aphid's obligate and maternally-inherited bacterial endosymbiont *Buchnera*. The genetic system also helped to pinpoint where the proteins are expressed. For example, to identify proteins that promote virus translocation through the gut, refractive aphids with a pure salivary gland barrier (refractive type-2) were grouped with competent aphids because both permit virus movement through the gut. These were compared using ANOVA to refractive type-1 aphids, which have a strong gut barrier. In this class, cuticle proteins were identified. Portions of the aphid gut are known to be lined with cuticle, therefore our analysis suggests a novel involvement for cuticle proteins in virus movement across the gut epithelium. Ten proteins had pI isoforms specific to competent or refractive aphid F2 genotypes. We tested the robustness of these proteins as biomarkers to distinguish between transmission-competent or refractive aphid populations using DIGE with field-isolated aphid biotypes of uncharacterized vector competency. Based on the biomarker profiles, we predicted 3 biotypes to be competent and 7 to be refractory. We then conducted virus transmission assays. Expression of at least 9 biomarkers found in the competent F2 genotypes distinguished between competent and refractory aphids of different biotypes with 100% accuracy.

Determination of aphid transmission efficiencies for N, NTN and Wilga strains of *Potato virus Y*

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Potato virus Y (PVY, genus *Potyvirus*, family *Potyviridae*) causes high economic losses worldwide, especially in the production of seed potatoes (*Solanum tuberosum).* PVY control systems rely on measuring virus pressure and vector pressure in the field. Calculation of the vector pressure is based on the relative efficiency factors (REFs) of aphid species, which express the transmission efficiency of aphid species in relation to the efficiency of *Myzus persicae*, the most efficient vector of PVY.

In the Netherlands, in the 1980s, aphid's REFs were determined using aphids caught alive in the field. Thus, experiments were conducted using limited numbers of aphids and only during the potato growing season. We have now developed a system which allows us to test virus transmission whole year round, using aphid clones reared in insect chambers.

Using the new system, we determined the aphids' relative transmission efficiency factors (REFs) for six isolates of the PVY strains PVY^{N}, PVY^{NTN} and $PVY^{N\text{-}Wi}$. Biotype Mp2 of *M. persicae* showed comparable average transmission efficiencies for all isolates, and was used as an internal control to determine the REFs of 18 other aphid species. The newly determined REFs for PVY^{N} were comparable to previously reported values. New REFs for the PVY^{NTN} strains were overall comparable to the REFs for PVY^{N}, except for *Aphis frangulae* and *Schizaphis graminum*. For $PVY^{N\text{-}Wi}$ six aphid species showed higher REFs (*Acyrthosiphon pisum, A. fabae, Aphis nasturtii, Aphis* spp., *P. humuli* and *R. padi*). Only *A. frangulae* shows a lower REF for $PVY^{N\text{-}Wi}$. In addition three aphid species (*Aulacorthum solani*, *Myzus ascalonicus* and *S. graminum*) for which no REF was determined earlier were found to be capable to transmit PVY and their REFs were determined.

ANTAGONISTIC EFFECTS OF PVY-INFECTED POTATO PLANT ON APHIDS

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Host plant selection by aphids can be positively or negatively affected when plants are infected by phytoviruses. Modifications can occur on orientation and feeding behaviour of the aphid colonizer, but also on its demographic parameters.
A potato plant infected by the PVY non-persistent virus is reported to modulate settling behaviour and growth parameters of *M. persicae* and *M. euphorbiae*.
Using the Electro penetrographic technique (EPG), we demonstrated that PVY-infected plants influence the feeding behaviour of these two species. A positive effect was observed on *M. persicae* whereas a negative effect was observed on *M. euphorbiae*.

Variation in transmission of *Tomato spotted wilt tospovirus* among isolates and populations of a vector, *Thrips tabaci*

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Tomato spotted wilt tospovirus (TSWV) is transmitted in nature exclusively by thrips, and ranks among the most economically important insect-vectored plant viruses worldwide. Although *T. tabaci* is one of 10 reported thrips vectors of TSWV, significant variation in transmission efficiency has been reported among populations of *T. tabaci* in Greece and in the USA. To better understand the role of the virus and the thrips in this observed variation, ten populations of *T. tabaci* were tested for their ability to transmit seven TSWV isolates. Results showed that vector competence of *T. tabaci* varied among isolate populations in a manner that was TSWV isolate specific. Moreover, transmissibility of isolates by *T. tabaci* varied in a manner that was *T. tabaci* population specific. These results indicate that geographic variation in the competence of *T. tabaci* as a vector of TSWV is strongly influenced by variation in both the vector and the virus.

Do secondary bacterial endosymbionts of *Sitobion avenae* clones affect vector specificity or transmission efficiency for barley yellow dwarf virus?

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Aphids harbour different bacterial endosymbionts. The primary bacterium *Buchnera aphidicola* is critical for their survival and reproduction and is responsible for the production of a protein, called symbionin. Symbionin from vector or non-vector aphids binds different luteoviruses in vitro, but the binding capacity is not correlated with the transmission ability. The role of symbionin in the transmission process remains in question; anyhow, it is not involved in vector specificity.
Aphids also harbour secondary endosymbionts related to the same family of *B. aphidicola*; they contribute to different life history traits in aphids, such as host plant adaptability. But their role in vector specificity, transmission ability or efficiency has not been investigated in any aphid species.
We tested the vector-specificity, transmission ability and efficiency of *Sitobion avenae* clones, harbouring different bacterial secondary endosymbionts with regard to different BYDV isolates. Our results unravel the role of secondary bacterial endosymbionts in the ability and efficiency of *S. avenae* as vector of BYDV.

What's new in polerovirus transmission?

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Poleroviruses are strictly transmitted by aphids in a circulative and non propagative manner. Virions are acquired by aphids when ingesting sap from infected plants. Virus particles cross the gut epithelium to be transported into the hemolymph and then to the accessory salivary glands cells, before being released, together with saliva, into the plant during a subsequent feed. Although viral determinants involved in the transmission process have been identified, data are lacking on (i) the role of post-translational modifications of the structural proteins in virus transmission, (ii) on the involvement of plant proteins in the transmission process, (iii) on the deregulation of aphid genes during virus acquisition and inoculation and (iv) on the nature of polerovirus receptors in aphids. We have developed different approaches to decipher some of these transmission steps. We have observed that acquisition of virions in aphid intestinal cells did not induce a strong gene deregulation, suggesting that the virus hijacks a well-conserved endocytosis mechanism. We have identified several phloem proteins able to bind purified virions *in vitro* and have shown that these proteins can stimulate virus transmission by aphids when added to the aphid diet together with purified virus. Identification of virus partners in phloem cells are pursued by screening Arabidopsis cDNA libraries using the yeast double hybrid system. We have also demonstrated that poleroviruses particles are not phosphorylated, nor glycosylated and that glycosylation does not play a role in the transmission process as suggested before. Finally, experiments are in progress to identify virus partners in aphid vector by developing yeast screening of aphid cDNA libraries.

Transmission of several isolates of *Tomato spotted witl virus* (TSWV) by *Frankliniella occidentalis*.

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Tomato spotted wilt virus (TSWV) causes important economic losses in several crops worldwide. It is transmitted in a persistent manner by several thrips species (Thysanoptera: Thripidae), being *Frankliniella occidentalis* (Pergande) its main vector. In Spain, the problem of TSWV seemed to be solved by the use of field tomato and pepper cultivars carrying the resistance genes *Sw-5* and *Tsw*, respectively, obtained by plant breeding. However, resistance breaking TSWV isolates appeared in the field after a few years of using resistant cultivars. In order to evaluate the dispersal ability of these new resistance-breaking isolates, we compared the transmission efficiency of several resistance-breaking and non resistance-breaking TSWV isolates by *F. occidentalis*. Our results showed that virus titer in source plants, estimated by real time RT-PCR, did not affect transmission rates. No significant differences of transmission rates between different TSWV isolates were found suggesting that the resistance-breaking and non resistance-breaking TSWV isolates have similar ability of dispersal by the thrips *F. occidentalis*.

Transmission of two isolates of *Broad bean wilt virus* 1 (BBWV-1) by several aphid species

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Broad bean wilt virus 1 (BBWV-1) is transmitted by several aphid species in a non persistent manner. Transmission efficiency by vectors is a key factor for understanding virus epidemiology. In this work, we compared the transmission rates of two BBWV-1 isolates (PV-132 from USA and Ben from Spain) by nine aphid species: *Acyrthosiphon pisum* (Harris), *Aphis gossypii* Glover, *Aphis fabae* Scopoli, *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *Megoura viciae* Buckton, *Myzus persicae* (Sulzer), *Nasonovia ribisnigri* (Mosley) and *Rhopalosiphum padi* L. Transmission experiments were performed with broad bean (*Vicia faba* L.) as virus source and receptor plants, using five aphids per each receptor plant. Virus titer in source plants, estimated by real time quantitative RT-PCR, had a positive effect on transmission rate. The Spanish isolate Ben was transmitted at a higher rate than the American isolate PV-132 by six aphid species and at lower rate by two aphid species, although these differences were not statistically significant. *Macrosiphum euphorbiae* showed the highest transmission rate (91.6% and 93.7% for isolate Ben and PV-132, respectively), followed by *Acyrtosiphum pisum*, (68.75% and 59.37%), whereas *Aphis fabae* (25% for both isolates) and *Nasonovia ribisnigri* (50% and 18.75%) had the lowest transmission rates.

Responses of *Myzus persicae* to headspace vilatiles of of *Nicotiana benthamiana* infected with artificial mutants of *Potato leaf roll virus*

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The effect of virus infections on volatile organic compounds (VOC) released from plants, and on the responses of aphids to these compounds, were examined using the *Nicotiana benthamiana-Potato leafroll virus* (PLRV)-*Myzus persicae* pathosystem. Point mutations were induced within the PLRV viral gene, open reading frame 4 (ORF4). The mutations were designed to avoid disrupting gene ORF3/4 (which overlaps ORF4), thereby isolating the effects of ORF4 variation. Six of the resulting mutant strains were agroinoculated into *N. benthamiana*. Virus-free agroinoculated and non-inoculated plants were employed as controls. PLRV titer was assessed with coat protein TAS-ELISA or RT-PCR; viral RNA from the plants was sequenced to validate successful transmission. Approximately 3 weeks after inoculation, VOC trapped from plant headspace were compared using gas chromatography/mass spectrometry. Total VOC production and the specific VOC blend differed significantly among PLRV mutants, one mutation eliciting particularly greater VOC release than the others, notably through greater release of nicotine. The emigration of apterous *M. persicae* in response to plants infected by the PLRV mutants was also studied. The bioassay employed measures aphid emigration rates while on screening placed above leaflets of the test plants, thus preventing aphids from assessing tactile and gustatory cues, and is conducted in darkness to eliminate visual cues. The mutation eliciting the greatest VOC production was more arrestant than other PLRV mutations or the wildtype. The result indicates that differences in the genetic sequence of gene ORF4 in PLRV can influence VOC release from infected potato plants and associated changes in aphid behavior.

Bird cheery-oat aphid behavior in response to barley yellow dwarf virus disease infection of wheat

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The bird-cherry oat aphid (BCOA), *Rhopalosiphum padi* (L.), is an important vector of *Barley yellow dwarf virus* (BYDV) on wheat. Recent research has shown that BCOA prefers virus-infected compared to non-infected plants and that plant volatile cues mediate such responses. The objective of this study was to examine for the first time the influence of BYDV disease progression on BCOA behavioral responses. Assays were conducted with two wheat genotypes, virus-susceptible Lambert and a BYDV-resistant, Lambert-derived transgenic (103.1J) that expresses the BYDV coat protein. All treatments were planted simultaneously and plants were either sham- or BYDV-inoculated at 2, 3, 4 or 5 weeks after planting. Sham-inoculated plants were challenged with nonviruliferous aphids as a control for effects induced by aphid feeding alone. Choice-test bioassays were conducted 7 weeks after planting to examine preference of nonviruliferous, apterous BCOA among treatments. Leaves still attached to plants were placed in contact with a platform from which aphids could move onto the leaves. A single platform included 4 treatments, BYDV- or sham-inoculated Lambert or 103.1J, and 4 platforms (one for each of the disease progression stages) were tested simultaneously. Thirty aphids were placed in the center of each platform and numbers settling onto plants were recorded every 15 min for 2 hours. The bioassay allows detection of short-term effects likely due to olfactory, visual, tactile and gustatory host cues. Headspace volatiles were collected to examine relationships between disease progression, virus-induced volatiles and BCOA preference. ELISA tests were performed to determine virus titer. Results will be presented and potential ecological and epidemiological implications discussed.

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Virus Disease Management/Detection/Diagnosis

Clean potato seed programs in Kenya

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Potato, whose production is growing the fastest of any crop in the developing world, is the second staple in Kenya and serves a unique dual role of both cash crop and important food security crop in the highlands of Eastern and Southern Africa. Average yields in this region only amount to about 7-10 t/ha, although the yield potential of predominant varieties is over 25 t/ha. The availability of clean seed is the predominant factor in limiting yields in the region, and over 99% of farmers source their seed from their own ware crops, from neighbors or small ware from markets. Virus surveys in Kenya indicated that more than 90% of "seed" from markets were virus infected and the existing capacity to produce clean seed is very low in most countries in the region. *Potato virus Y*, *Potato leafroll virus* and bacterial wilt are thought to be the major seed-borne diseases limiting this yield potential. Through engagement with the private sector, introduction of new rapid multiplication techniques (aeroponics) and the development of a new limited generation seed system, significantly increased amounts of clean seed, of improved late blight resistant varieties, have been produced and distributed to small private seed multipliers and individual small holder farmers. A very significant effect of seed age on yields obtained "on-farm" was observed and growers trained to recognize virus symptoms, and to practice positive selection of their own seed, also obtained significant yield increases.

Cryotherapy of shoot tips as an efficient means for virus and phytoplasma elimination and healthy plant production

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Cryotherapy represents an application of plant cryopreservation techniques to eradication of viruses and phytoplasma at a high frequency (Wang et al. 2009, *Annals of Applied Biology* 154, 351-363). In cryopreservation, biological samples are stored at ultra-low temperature, usually in liquid nitrogen (-196°C), which is considered as an ideal means for long-term storage of plant germplasm. However, in cryotherapy shoot tips are treated briefly in liquid nitrogen using adjusted cryopreservation protocols and healthy plants are regenerated from the surviving pathogen-free meristematic tissue. The least differentiated meristematic cells are most tolerant of the cryo-treatment and also the most likely to be virus-free, but their mechanical excision manually and subsequent regeneration may be impossible by traditional means of meristem culture. Therefore, cryotherapy shows typically a higher efficiency in virus eradication and can be enhanced further by thermotherapy of the shoots prior excision of the shoot tips. The method facilitates treatment of large numbers of samples and is independent of shoot tip size. So far, cryotherapy has been applied to eliminate a total of nine viruses from banana, *Citrus* spp., grapevine, *Prunus* spp., raspberry, potato and sweetpotato. Furthermore, sweet potato little leaf phytoplasma and Huanglongbing bacterium causing 'citrus greening' have been eliminated from sweetpotato and *Citrus*, respectively, using cryotherapy. Cryopreservation protocols are available for a wide variety of plant species and can be used or adjusted for the purpose of cryotherapy.

Ecology and management of whitefly-transmitted vegetable viruses in Florida

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Whitefly-transmitted *Squash vein yellowing virus* (SqVYV) was identified in squash and watermelon in Florida in 2005 and shown to cause a severe decline of watermelon vines as crops approach harvest. Florida is most economically impacted by SqVYV although the virus has more recently been detected in Indiana and South Carolina. The origin and evolutionary history of SqVYV remains a mystery. Sequence diversity of SqVYV isolates collected at different times, from different locations and from different plant species is being analyzed for insights into the origin of the virus. More recently, *Cucurbit leaf crumple virus* (CuLCrV) and *Cucurbit yellow stunting disorder virus* (CYSDV), also whitefly-transmitted, have been found in Florida watermelon. Numerous surveys have been conducted in the region to identify alternate hosts for these three viruses. Cucurbit weeds including Balsam-apple (*Momordica charantia*), creeping cucumber (*Melothria pendula*) and smellmelon (*Cucumis melo* var. *dudaim*) provide reservoirs for SqVYV, CuLCrV and CYSDV. Green bean (*Phaseolus vulgaris*) also can provide a reservoir for CuLCrV. The effectiveness of insecticides and silver plastic mulch to manage whiteflies and mitigate SqVYV has been demonstrated. In addition, potential sources of SqVYV resistance have been identified in wild watermelon germplasm and single plant selections are underway to develop resistant lines for use in breeding programs. Lastly, a comprehensive map of 82,928 acres of vegetable fields in the three counties comprising the majority of the southwest Florida vegetable production area has been developed to identify “hot spots” and reservoir crops for viruses and whiteflies, and will be useful in evaluation of management strategies on virus incidence in commercial fields.

Combination of natural and engineered resistance to rhizomania in sugar beet

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Rhizomania is one of the most devastating sugar beet diseases. It is caused by *Beet necrotic yellow vein virus* (BNYVV) belonging to the genus Benyvirus. The virus is transmitted by the soil borne vector *Polymyxa betae.* It is generally considered that use of sugar beet varieties with resistance to BNYVV is the only way to maintain a profitable yield on rhizomania-infested fields. Rhizomania resistance in most sugar beet varieties is conferred by the gene *Rz1.* In some regions there are BNYVV isolates that can overcome *Rz1.* In the future it can be highly important with varieties that are based on different sources of resistance and engineered resistance can be one alternative.

We explored the transgenic expression of viral dsRNA for engineering resistance to rhizomania. The transgenic plants expressed an inverted repeat of a 0.4 kb fragment derived from the replicase gene of BNYVV.

Evaluation of the resistance level in sugar beets with natural and/or engineered resistance was performed by growing plants in soils with different strains of BNYVV. The experiments were done in greenhouse and field, the concentration of BNYVV in samples of root sap was measured. The transgenic resistance without or in combination with *Rz1* compared very favourably to natural sources of resistance and thus offers an attractive alternative for breeding rhizomania resistant sugar beet varieties.

Development of broad, stable and durable resistance to monopartite and bipartite begomoviruses in Brazilian tomato lines

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Begomoviruses cause a major disease affecting tomato crops in tropical and subtropical regions worldwide. It is caused by distinct single-stranded-DNA-containing virus species of the genus *Begomovirus* (Family *Geminiviridae*) that are transmitted by whiteflies. An extremely diverse tomato-infecting bipartite *Begomovirus* species complex has emerged after the spread of the whitefly *Bemisia tabaci* biotype B in Brazil. This complex comprises several recognized species and some additional provisional ones. The tomato yellow leaf curl disease (TYLCD) is also caused by a complex of at least eight highly aggressive monopartite begomovirus species, which have not been detected in Brazil. Control of begomoviruses is difficult and is mainly based on the intensive use of insecticides to reduce *B. tabaci* populations, with limited success. Therefore, genetic-host resistance is the most desirable alternative. Recently, we identified an effective recessive resistance against different species of begomoviruses in the *Solanum lycopersicum* Brazilian line TX-468. The genetic basis of resistance was characterized and inheritance studies indicated that this wide-spectrum *Begomovirus* resistance in 'TX 468-RG' is largely explained by a single recessive locus (named *tcm-1)* which could facilitate the incorporation of this trait in elite tomato lines. Plants of TX-468 inoculated with different species of monopartite and bipartite begomoviruses exhibit no disease symptoms and limited virus accumulation in infected tissues. Field resistance was also effective even under high inoculum pressure. The effect of use of this resistance on virus spread by *B. tabaci* on TX-468 was investigated. Primary and secondary spread was significantly reduced by the use of this resistance line. Therefore, these results supported that the use of *tcm-1*-containing lines might result in a significant control of monopartite and bipartite begomovirus spread. On going genomic and proteomic analysis may reveal virus and host determinants involved in this resistance.

Modeling vector flights to increase effectiveness of foliar protectant programs

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In recent years, Potato virus Y (PVY) has re-emerged as a serious disease problem in potato production areas in both the United States and eastern Canada. Asymptomatic cultivars which express mild or no symptoms when infected with PVY combined with an increase in recombinant strains of this virus prevent accurate field identification and rouging of infected plants. Further, limited information currently exists to document the unique dispersal biology of the soybean aphid (*Aphis glycines*), a vector species of PVY recently introduced into the US and Canada in the early 2000's. Aphid vector flights were monitored over several sites in 2009 to document the relationship of vector movement and PVY disease progress in susceptible potato. PVY disease progress was more closely associated with the temporal patterns of selected aphid vectors, including the soybean aphid. Late season inoculation of plants by migrating soybean aphids often results in infection with limited foliar symptoms in mature plants, yet tuber infection can be significant. At present, there is a lack of effective strategies to reduce the incidence of PVY infected plants and tubers, and there is a need to improve cost-effective, reduced-risk tactics of limiting PVY levels in seed lots.

A mutation in the NIb cistron of *Potato virus Y* confers virulence towards the *Pvr4* resistance of pepper and a high competitiveness cost in susceptible cultivar

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The *Pvr4* resistance of pepper against potyviruses is highly durable in field conditions but *Potato virus Y* (PVY) virulent variants can be selected in laboratory conditions. To understand this discrepancy, we studied the molecular mechanisms which generated these variants and their consequences on viral fitness. We found that the region coding for the NIb protein (RNA-dependent RNA polymerase) of PVY was the avirulence factor corresponding to *Pvr4* and that a single non-synonymous nucleotide substitution in that region was sufficient for virulence. This substitution imposed a high competitiveness cost to the virus against an avirulent PVY variant in plants devoid of *Pvr4.* In addition, during serial passages in susceptible pepper plants, the only observed possibility of the virulent mutant to increase its fitness was through the reversion of the virulence mutation, strengthening the high durability potential of the *Pvr4* resistance. This is in accordance with the high evolutionary constraint exerted on the NIb protein of PVY and other potyviruses and with a model predicting the durability of virus resistances as a function of the evolutionary constraint applied on corresponding avirulence factors.

Modulation of virus-host plant interplay in the tomato yellow leaf curl disease by using insect resistance in the tomato host

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An essential step for viruses to infect plants is to reach the host plant and to target host plant cells efficiently. Therefore, virus-host plant interplay can hypothetically be modulated by means of an insect resistance in the host. This was studied in the tomato (*Solanum lycopersicum*) breeding line ABL 14-8, nearly isogenic to cv. Moneymaker but exhibiting a resistance to the whitefly *Bemisia tabaci* introgressed from the wild relative *S. pimpinellifolium*. The insect resistance in ABL 14-8 plants was based on the presence of foliar type IV glandular trichomes and the production of acylsucroses. *B. tabaci* is the vector of the tomato yellow leaf curl disease (TYLCD) which seriously limits tomato production worldwide. TYLCD is caused by a complex of single-stranded-DNA-containing virus species of the genus *Begomovirus* (family *Geminiviridae*), including tomato yellow leaf curl virus (TYLCV), among others. Currently, control of TYLCD damage is mostly based on intensive insecticide use to control *B. tabaci* populations, with limited success. In addition to antibiosis, antixenosis to *B. tabaci* was observed in ABL 14-8 plants that resulted in a restricted virus spread. In fact, we demonstrated that *B. tabaci* exhibited a significantly lower preference to ABL 14-8 plants compared to cv. Moneymaker and that presence of glandular trichomes difficulted feeding on ABL 14-8 plants, based on electrical penetration graph studies. Consequently, a significantly reduced primary spread of TYLCD was observed in ABL 14-8 plants. Moreover, even if ABL 14-8 plants can be infected, they were demonstrated to be far less efficient sources for secondary virus spread. Therefore, resistance to the insect might be used to modulate virus-host interplay to control TYLCD.

Mild and aggressive Pepino mosaic virus isolates: tomato transcriptomic responses and the potential of cross-protection as a control strategy

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Pepino mosaic virus (PepMV) is a highly infectious Potexvirus and a major disease of greenhouse tomato (Solanum lycopersicum) crops worldwide. Damage and economical losses caused by PepMV vary greatly, and can be attributed to differential symptomatology caused by different PepMV isolates. A custom-designed Affymetrix tomato GeneChip array with probe sets to interrogate over 22,000 tomato transcripts was used to study transcriptional changes in response to a mild and an aggressive PepMV isolate from the CH2 genotype that share over 99% nucleotide sequence identity, and to a mild isolate from the EU genotype with 79% nucleotide sequence identity to the CH2 isolates. Our results show that PepMV temporarily suppresses host primary metabolism and induces a broad range of defense responses, including the RNA silencing pathway. The defense response was stronger upon inoculation with the aggressive isolate. Interestingly, flavonoid and lycopene biosynthesis pathways were affected, which might be correlated with the typical PepMV-induced fruit marbling. Subsequently, the potential of the two mild isolates to protect a tomato crop against the aggressive CH2 challenge isolate was assessed in greenhouse trials. After challenge inoculation, enhanced symptom display was recorded in plants that were pre-inoculated with the mild EU isolate. A quantitative genotype-specific TaqMan assay revealed that the accumulation of the challenge isolate only temporarily slowed down in these plants. By contrast, efficient cross-protection was obtained using the mild CH2 isolate. In this case the aggressive CH2 challenge isolate was barely detectable in the pre-inoculated plants, suggesting that the interaction between PepMV isolates largely depends on RNA sequence homology. Altogether these results suggest that isolate aggressiveness correlates with the intensity of defense responses, and that post-transcriptional gene silencing plays an important role in the interaction of PepMV with its host.

Controlling the banana bunchy top disease pandemic in sub-Saharan Africa

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Banana bunchy top virus (BBTV; genus *Babuvirus*) - a 'new encounter pathogen' of banana and plantain (*Musa* spp.) first recognized in 1958 in Democratic Republic of Congo (DRC) - is maintaining the invasive phase and has spread into 11 countries in sub-Saharan Africa (SSA). Our recent investigations on causes of unabated BBTV spread suggested farmers' traditional practices of exchanging planting materials within and between countries as the main factor for inter-continental spread of the virus. Introduced infected material in turn have been serving as 'founder source' for subsequent spread by the banana aphid (*Pentalonia nigronervosa*), the vector of BBTV widely prevalent in SSA. All the banana and plantain clones were found susceptible to virus, however, landraces were found to have tolerance to disease affects; in contrast, disease affects and scale of economic impact were most severe in introduced desert banana (Cavendish Williams). The nucleotide sequences of DNA-S and DNA-R segments of SSA BBTV isolates were highly similar and they formed a unique linage, and aligned with the 'South Pacific' group that includes BBTV isolates from Australia, South Asia and South Pacific. Increased cultivation of introduced desert bananas; severe shortage of virus-free planting material; use of infected suckers as founder planting material in newly cleared forests; and poor awareness about the disease among the growers, extension and quarantine agencies are the major factors favouring the frequency and distribution of BBTV outbreaks during the last two decades. Lack of resistance to BBTV in *Musa* germplasm and difficulties in implementing the phytosanitary measures pose formidable challenges to contain the virus spread in SSA. This situation underscores a need for research to identify novel tactics for sustainable management of the virus and vector in countries where the disease has already been established and prevent virus spread into unaffected regions in SSA.

Improved virus diagnostics to support seed certification in Australia

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Virus level estimates in seed potatoes in Australia are based on visual inspections. While latent or symptomless field infections are almost impossible to detect by this method the existing Grow-on ELISA test is considered too time consuming and expensive by industry. The aim of this study was to determine the efficiency of direct tuber testing for potato viruses using RT-PCR when compared to the Grow-on ELISA method.

Potato tubers were collected from field grown virus-infected plants and used to compare direct detection of viruses in dormant tubers compared to grow-on plants by both ELISA and PCR. ELISA detected PLRV in 7/12 and PCR 9/12 tubers that were directly tested while only 6/12 and 8/12 positives were obtained from the grow-on plants tested for by ELISA and PCR respectively. For PVY, 8/33 and 28/33 tubers direct tested were positive by ELISA and PCR respectively and 26/33 and 28/33 grow-on plants were positive. ELISA detected PVS in 26/39 and PCR 34/39 tubers tested directly and 28/39 and 29/39 grow-on plantlets respectively. Direct tuber testing for TSWV detected 15/27 and 22/27 positives for ELISA and PCR. Significantly ELISA detected TSWV in only 1/27 and 3/27 grow-on plantlets for ELISA and PCR respectively. This result indicates poor translocation of TSWV from the infected tuber into the potato sprout.

A comparison of the distribution of PVY strains within a potato tuber indicates that the PVY^{NTN} strain is more evenly spread thorough the tuber and is easier to detect by PCR than the PVY^{o} strains. Variation of PVY distribution within a tuber has also been recorded between potato cultivars.

The implications of these findings for potato seed certification will be discussed.

Epidemiology of *Plum pox virus* (PPV) in nursery blocks and evaluation of the effect of horticultural mineral oil treatments.

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The susceptibility to *Plum pox virus* (PPV) natural infection of the main Prunus rootstocks used in the Spanish stone fruit industry was evaluated during two consecutive vegetative periods (2006-2008) in two experimental plots with different PPV-D inoculum pressure. Samples from both experimental plots were analyzed by ELISA 5B-IVIA and Spot real-time RT-PCR. Significant differences in the susceptibility were found between the assayed rootstocks under a high inoculums pressure. Adesoto 101 and Mariana GF8-1 were the most susceptible rootstocks. Cadaman and Garnem rootstocks presented the lowest number of PPV infected plants, only detected by Spot-real time RT-PCR. Practical differences among the assayed rootstocks were not found under low PPV inoculum pressure.

Aphid species were monitored by Moericke yellow water traps and sticky plant methods in both experimental plots in May 2006 and 2007. *Aphis spiraecola* Pagenstecher was the most abundant aphid species monitored. The average of *A. spiraecola* carrying PPV PCR-amplifiable targets resulted in 30.4% of the tested individuals in the plot with high PPV inoculum pressure whereas 8.0% in the plot with low inoculum pressure.

The effect of horticultural mineral oil treatments (Sunspray Ultrafine at 1%) on natural PPV spread was evaluated in the same plots. The treatments did not avoid PPV infection but significant differences in PPV incidence were obtained between Mariana GF8-1 treated and non-treated plots after one year challenge in natural conditions under high PPV-inoculum pressure.

The use of less susceptible or resistant rootstocks to natural infection, and/or mineral oil treatments could constitute two possible strategies to reduce PPV incidence in nursery blocks of Prunus.

Biological control of vectors affects virus dispersal

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Biological control limits the population growth of arthropod vectors and consequently reduces the frequency of virus dispersal in a crop. However, reduction of vector abundance is not the only effect of biological control. Natural enemies might induce antipredator behaviour that affects development, feeding and dispersal of vectors, and therefore virus spread. Several studies explore the effect of biological control on aphid dispersal and virus transmission by aphid vectors. Results are controversial, since positive to negative effects of natural enemies on virus spread are found. One of the factors explaining these differences is the feeding 'style' of predators, which induce different level of disturbance on aphid colonies, and consequently different degrees of aphid dispersal. Other factor explaining different outcomes is the mode of transmission of the virus.

We compared the behaviour and dispersal of the aphid *Myzus persicae* in presence of two biological control agents: the coccinellid *Adalia bipunctata* (adults and larvae), and the syrphid *Sphaerophoria rueppellii* (larvae). The effect of these predators on dispersal of *Broad bean wilt virus* 1, a Fabavirus non persistently transmitted by aphids, is also studied, both through the effect of natural enemies on acquisition and on transmission of BBWV-1 by *M. persicae*.

Aphids showed higher dispersal rate to uninfested plants in presence than in absence of natural enemies. Antipredator behaviour of aphids in front of coccinellids differed from antipredator behaviour in presence of syrphids. Consequences of these behaviours on transmission of BBWV-1 by *M. persicae* and on virus epidemiology are discussed.

A cucumber mosaic virus mutant that induces resistance to its aphid vector in tobacco

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Cucumber mosaic virus (CMV), the type species of the *Cucumovirus* genus, has a tripartite positive sense RNA genome and the largest host-range of any known plant virus. It is transmitted by several species of aphid in a non-persistent manner, making successful control of CMV transmission difficult to achieve. During transmission, virions are bound to the insect mouthparts by sequences of the CMV coat protein and no additional viral factors are required for binding. However, we have found that the CMV 2b protein plays an indirect but important role in transmission by protecting the vector against the induction of anti-insect defenses. The 2b protein is a counter-defense factor that suppresses the initiation of RNA-silencing and inhibits salicylic acid-induced resistance to the virus. It is also a major symptom determinant that can impair microRNA-mediated host gene expression. While investigating the potential of CMVΔ2b (a CMV mutant in which the gene for the 2b protein is deleted) as a cross-protection agent, we noted that aphid (*Myzus persicae*) infestation was inhibited on CMVΔ2b-infected plants of tobacco (*Nicotiana tabacum*). We found statistically significant decrease in aphid survival and an increases in aphid mortality on CMVΔ2b-infected plants compared to mock-inoculated plants or plants infected with wild-type CMV. The data indicates that in CMVΔ2b-infected plants a viral gene product other than 2b (or the stress of viral infection) induces resistance to aphids. However, in plants infected with wild-type CMV suspect that this effect is neutralized by the 2b protein. Thus, the results suggest that an RNA-silencing suppressor may also target anti-aphid defenses as well as anti-viral responses in plants. Gene expression analysis of jasmonic acid-regulated genes (involved in anti-insect defence responses) in tobacco support this hypothesis. Furthermore, aphid transmission of CMVΔ2b is also drastically reduced in comparison to wild-type CMV.

Rhizomania of sugar beet –Similarities and differences between the Iranian and European situation

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Rhizomania is a major soil-borne disease of sugar beet. There is a major concern about the emergence of resistance-breaking isolates of the virus in intensive beet cropping areas, though up to now several hypothesis are still under investigation to explain the phenomenon.
With the view of unraveling the question, extensive surveys were conducted in severely disease-affected areas in Europe around the Pithiviers area (France), in Belgium as well as in Asia in countries like Turkey or Iran. Root samples collected from both in disease-affected or non disease-affected areas were tested for the presence of *Beet necrotic yellow vein virus*, causal agent of the disease or of other soil-borne viruses like *Beet soil-borne virus*, *Beet virus Q* and *Beet black scorch virus*. Samples were tested by RT-PCR for the detection of BNYVV. A specific fragment was amplified from the gene P25 located on RNA-3 and from the gene P26 located on RNA-5.
Results from the analysis of more than 400 samples reveal a wide range of variations within the BNYVV RNA-3 as well as contrasting situations according to the area surveyed, with evidences for mix BNYVV type infections. Methodological questions regarding the virus distribution in the root, presence and distribution of the virus in the soil will be addressed.

Survival of *Pepino mosaic virus* in aqueous environment reveals the need for efficient detection system suitable not only for plant but also for environmental samples

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Pepino mosaic virus (PepMV) is threatening tomato industry worldwide. It is easily mechanically transmissible therefore it is suspected that PepMV can be stably present in the environment, opening the door to potential additional routes of infection, such as soil or water. We have experimentally confirmed that PepMV remained infective in water at room temperature at least for three weeks. Moreover, preliminary experimental results indicate that PepMV can infect tomato plants, grown in greenhouse and watered with PepMV infected water via root system.

In order to detect, even very low PepMV concentrations that might be present in contaminated water samples, we developed several sensitive one-step RT-qPCR assays, which also enable the discrimination of currently circulating PepMV genotypes. The following genotype combinations, European tomato-Peruvian, Ch2, and US1, were successfully distinguished within the tested isolates. The method enables studies on the demographic distribution of the circulating PepMV genotypes. It was successfully tested on plant, water and seed samples. It can detect as little as one naturally infected seed among 5000 uninfected seeds. At the same time, the disposability of few gene targets for the detection of PepMV RNA, increase the reliability of the virus detection in samples with low expected virus concentration, such as latent infections or irrigation waters.

In addition, we are investigating the potential of monolithic chromatographic supports (CIM Convective Interaction Media) for concentration of PepMV. CIM (Convection Interactive Media) monolithic supports are chromatographic media that, due to their highly porous structure, have proven ability to bind, purify and concentrate large biomolecules, such as viruses and nucleic acids. The combination of both technologies (CIM for concentration and qPCR for detection) will allow detection of extremely low concentrations of PepMV from environmental or irrigation waters. The same methodology proved to be successful in the case of *Tomato mosaic virus*, *Cucumber mosaic virus* and Rotaviruses.

Viruses in weeds in *Dioscorea* yam fields in Nigeria

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Seventy-four leaf samples were collected during surveys of *Dioscorea* yam fields in six States of the Guinea Savanna agro-ecological zone of Nigeria in the wet and dry seasons of 2009 and 2010. The leaf samples showing symptoms (mosaic, mottle, necrosis and chlorotic) and those without visible symptoms were analyzed by serological tests (ELISA) for the identification of different viruses. A total of 26 out of 54 (48.1%) and 19 out of 20 (95.0%) leaf samples collected during wet and dry seasons, respectively, were infected. Seven viruses were detected; *Yam mosaic virus* (YMV), *Cucumber mosaic Virus* (CMV), *Cowpea mild mottle virus* (CPMMV), *Pepper venial mottle virus* (PVMV), *Bean common mosaic virus* (BCMV), *Telfaria mosaic virus* (TeMV) and *Cowpea yellow mosaic virus* (CYMV). The weed species and the viruses detected in them during the wet season were *Hibiscus esculentus* (YMV, CMV and CPMMV), *Amaranthus spinosus* (CMV), *Physalis angulata* (YMV and CMV), *Procumbane* Linn (CMV), *Phyllanthus amarus* (YMV and CPMMV), *Ludwigia abyssinica* (YMV), *Amaranthus spinosus* (YMV), *Galinsoga culiata* (YMV), *Eclipta prostrate* Linn (YMV), *Justicia flara* (YMV and CMV), *Euphorbia heterophylla* Linn (YMV and CMV), *Phyllanthus amarus* (CMV), *Melan podium divaricatum* (YMV and CMV) and *Saccivlepsis africana* (YMV). In the dry season, the weeds and the viruses that were detected in them were *Melanpodium divaricatum* (CPMMV), *Crotalaria rutusa* (YMV and CMV), *Aspelia bussei (CPMMV), Aneilema acquinotide* (CPMMV), *Pueraria phaseloides* (YMV), *Platostoma africana* (YMV), *Conyza summtrensis* (YMV, BCMV, PVMV and TeMv), *Chroniolea oduratiu* (YMV and CYMV), *Phyllanthus amarus* (YMV), *Mitracarpus villosus* (CMV) and *Sclerocarpus africanus* (YMV, BCMV, PeMV and TeMV). Weeds growing around yam may serve as alternate hosts of viruses infecting yam.

Simultaneous Detection of Two Important Bean RNA Viruses By Multiplex Reverse Transcription Polymerase Chain Reaction

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Two important seed borne bean viruses have been described; *Bean Common mosaic virus* (BCMV) and *Bean Common mosaic necrosis virus* (BCMNV). Multiplex RT-PCR, the amplification of multiple RNA targets in single reaction described for simultaneous detection of more than one targets. Amplification of multiplex RT-PCR could greatly reduce the cost of routine detections and therefore cause great help to the seed certification program of beans. With use of internal control, the risk of false negative results reduced. In this study a multiplex RT-PCR was developed for simultaneous detection of BCMV and BCMNV. Specific primers were designed based on the some available sequences of BCMV and BCMNV from the Gene Bank and we used RT-PCR for amplification of respective viral band. Infected BCMV samples amplified 586bp and BCMNV 1081bp. In addition to, one primer pair drive from 18s ribosomal RNA was used as internal control. The fragment of internal control was constantly amplified from all healthy and infected plant cultivars. We described the primer pairs and the multiplex RT-PCR conditions for detection of BCMV and BCMNV. The specificity of three pairs of primer was proved by the size of bands and sequenced of the product bands.

Distribution of PVY strains in susceptible and moderately resistant North American cultivars.

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Different strains of Potato virus Y (PVY) are present in the United States. Twenty four isolates from different geographical regions representing three strain types (PVY^{O}, PVY^{NTN}, and PVY^{N-Wi} [syn. $PVY^{N:O}$]) were used in bioassay studies. To determine the amount of spread to the daughter tubers, eleven cultivars showing differing levels of PVY susceptibility were mechanically inoculated using isolates from each strain. Tubers saved from the primary infected PVY positive plants were evaluated for PTNRD and non-symptomatic tubers were grown-out and ELISA tested for PVY. The total numbers of infected tubers were used to determine the extent of PVY spread to daughter tubers. Results show that a few of the cultivars had resistance to one strain (mainly PVY^{O}) or they had different levels of resistance to isolates within the same strain. Some of the treatment combinations in resistant cultivars had multiple negative ELISA results, but produced tubers that were ELISA positive in grow-outs indicating low virus titre in the mother plant. Other combinations showed infection in the mother plant, but no virus-positive daughter tubers. These results are being used to characterize PVY strains in the U.S. and determine PVY strain susceptibility in cultivars grown widely in the U.S.

Detection and molecular epidemiology of *Oat sterile dwarf virus*

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Severe outbreaks of oat sterile dwarf disease occur periodically in the central parts of Sweden. Affected oat plants show abnormal formation of side shoots, stunted shoots, few and small kernels, dark green colour and sometimes enations, or vein swellings, on shoot bases. The disease is caused by *Oat sterile dwarf virus* (OSDV; genus *Fijivirus*; family *Reoviridae*), which is transmitted in a persistent manner by the planthopper *Javesella pellucida* (family Delphacidae). OSDV has a double-shelled spherical particle containing double-stranded (ds) RNA. Fijiviruses replicate in planthoppers, and all except Nilaparvata lugens reovirus (NLRV) also replicate in the phloem of susceptible monocotyledonous plants.

Isolation of double-stranded RNA from symptomatic plants revealed 10 bands corresponding to the genome components of OSDV. Sequence analysis of a region of segment 8 (709 bp) from four Swedish isolates collected in different years (1987, 1994, 2002 and 2007) showed a very close relationship and a sequence identity of 99%. This suggests that the disease is caused by a single strain of OSDV. Molecular tools were developed for detection of OSDV. Virus was detected in extracts of individual planthoppers with dot-blot hybridization using a non-radioactive probe or with RT-PCR. OSDV in oat samples was detected by incubating plant extract in PCR tubes followed by RT-PCR. The detection methods were shown to be a very valuable tool for determining the level of viruliferous planthoppers, which is necessary for predicting disease outbreaks in the following year's crops, and the identification of infected plants. For the development of ELISA, the complete gene for the outer capsid protein of OSDV was amplified by RT-PCR and cloned. A GST-tagged viral protein was expressed in *Escherichia coli* and purified. Antibodies were produced against the capsid protein and could specifically detect OSDV in oat extracts using both western blot analysis and ELISA.

Restricted spread by *Bemisia tabaci* of *Tomato yellow leaf curl virus* in the begomovirus-resistant Brazilian line TX-468

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Tomato Yellow Leaf Curl Disease (TYLCD) is a major disease affecting tomato crops in tropical and subtropical regions of the world. It is caused by several distinct single-stranded-DNA-containing virus species of the genus *Begomovirus* (Family *Geminiviridae*) that are transmitted by the whitefly *Bemisia tabaci*. Among them, *Tomato yellow leaf curl virus* (TYLCV) is one of the most widespread and economically important. Control of TYLCD is difficult and is mainly based on the intensive use of insecticides to reduce *B. tabaci* populations, with limited success. Therefore, genetic-host resistance is the most desirable alternative and almost effective control is achieved by the partially dominant *Ty-1* resistance gene. Recently, we identified an alternative, effective recessive resistance against different species of the TYLCD complex in the *Solanum lycopersicum* Brazilian line TX-468. Plants of TX-468 artificially inoculated with TYLCV do not exhibit disease symptoms but limited virus accumulation can occur. Our results suggested that the resistance mechanism operating in TX-468 impairs the long-distance movement of TYLCV. We investigated the effect of use of this resistance on virus spread by *B. tabaci* on TX-468 and cv. Moneymaker (susceptible control). Medium scale primary and secondary TYLCV spread was simulated under greenhouse conditions. For primary spread, viruliferous whiteflies were released on plants of each tomato genotype (nonchoice tests) or on plants in a 1:1 mixture of both genotypes (choice tests) in small insect-proof net houses containing 22 plants and virus infection was monitored. Significantly fewer TX-468 plants became infected in both tests. Moreover, secondary spread of TYLCV from an infected resistant or susceptible source plant to susceptible plants indicated a significantly lower risk of resistant plants for TYLCD spread. Therefore, these results supported that the use of this resistance might result in an effective control of TYLCD spread even to genotypes highly susceptible to the virus.

Occurrence, incidence and distribution of viruses infecting yam (*Dioscorea* spp.) in Nigeria

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A survey was conducted during the months of July and August 2009 to determine the occurrence, incidence and distribution of the viruses infecting yam (*Dioscorea* spp.) in six major yam-producing states in the guinea savanna agroecologial zone of Nigeria. Yam leaf samples were collected from 54 fields and were indexed for *Yam mosaic virus* (YMV; *Potyvirus*), *Cucumber mosaic virus* (CMV; *Cucumovirus*) by enzyme-linked immunosorbent assay (ELISA) and *Dioscorea alata badnavirus* (DaBV) by polymerase chain reaction (PCR). Nine percent (117/1350), four percent (47/1350) and less than one percent (9/1350) of the leaf samples tested were infected with YMV, CMV and badnaviruses, respectively. The various symptoms of viral disease associated with yam included chlorotic mosaic, mottling, shoe-string, vein clearing, necrosis and stunted growth. YMV was the most prevalent virus detected (85.5% of total leaves sampled). This is the first comprehensive report of YMV, CMV and Badnavirus infecting yam in the guinea savanna zone of Nigeria.

A *Cucumber mosaic virus* 2b-mutant was not able to establish a systemic infection in bell pepper (*Capsicum annuum* L.).

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The *Cucumber mosaic* virus-Fny 2b mutant (CMV-FnyΔ2b) was evaluated for its ability to infect bell pepper plants in comparative tests with its parent virus, CMV-Fny. 'Calwonder' pepper plants inoculated with CMV-FnyΔ2b did not develop local or systemic symptoms, whereas CMV-Fny-infected plants developed systemic chlorosis by 7 days post-inoculation (dpi) followed by mosaic symptoms. Virus accumulation, determined by ELISA, revealed that CMV-FnyΔ2b accumulated in inoculated 'Calwonder' leaves but not in non-inoculated leaves (i.e., no systemic infection). The amount of CMV-FnyΔ2b detected in inoculated leaves was significantly less than that of CMV-Fny. Immuno-tissue blot tests did not detect CMV-FnyΔ2b in the stem of infected plants, whereas CMV-Fny accumulated in all tissues throughout the stem. Mesophyll protoplasts isolated from 'Calwonder' leaves were inoculated with CMV-FnyΔ2b RNA comparatively with CMV-Fny RNA. CMV-FnyΔ2b accumulated to lower levels than CMV-Fny in protoplasts collected at 24 hpi in each test performed. Co-infection of 'Calwonder' pepper plants with CMV-Fny and selected Potyviruses resulted in a synergistic disease response. In contrast, co-infection of CMV-FnyΔ2b and selected Potyviruses led to disease symptoms similar to those of each Potyvirus alone with no alleviation in the restricted movement of CMV-FnyΔ2b. These data suggest that the CMV 2b protein is needed for systemic infection of 'Calwonder' pepper plants and for efficient accumulation at the cellular level.

Incidence and prevalence of *Strawberry mild yellow edge virus* (SMYEV) in Argentina

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Strawberry mild yellow edge virus (SMYEV) was first detected in California, USA, in *Fragaria vesca*, by Horne in 1922. It is one of the most widespread virus of the cultivated strawberry. SMYEV is a *Potexvirus* vectored by aphids of the genus *Chaetosiphon.* The virus was first reported in Argentina in 2008, in strawberry (*Fragaria x ananassa*). It was detected in asymptomatic and symptomatic plants (dwarfing, marginal chlorosis, stocky plants, leaf mottle, and distortion) associated with other viruses. A survey was conducted to determine the incidence and prevalence of SMYEV in the main strawberry production regions of Argentina: Santa Fe, Corrientes and Tucumán, in 2009. A total of 1255 samples of the cultivars 'Camarosa', 'Earlibrite', 'Festival', 'Camino Real' and Albion, from 34 fields were analyzed by enzyme-linked immunosorbent assay (DAS-ELISA), with SMYEV antiserum (BIOREBA, Switzerland). Samples were collected from randomized plots (5 to 10 plots, depending of field size). Each plot consisted on a 20-meter planting bed portion, where six plants were taken (two at the beginning, two in the middle and two at the end of the plot). The index of incidence of SMYEV was approximately the same for the three locations: 3.3% in Santa Fé, 4.0% in Corrientes, and 3.7% in Tucumán. The index of prevalence was: 54.5% in Coronda, 50.0% in Bella Vista and 64.7% in Tucumán.

Plum pox potyvirus

Vahida Seremet

I have succeded in making the autochtonus plum pozegacka – cacanka – madzarica – bistarica“ to produce healthy fruit again.
The plum is located in Tuzla, Bosnia and Herzegovina.
I have discovered that the disease is not brought on by the wind, insects or garden scissors, but by something completely different.
The problem is soil pollution.
This reffers to the SO_2 , which combined with water, gives H_2SO_3, followed by H_2SO_4.
Depending on the levels of soil concentration there are areas with different levels of pollution, i.e. the degree of PPV. The affected plant can be healed with change of soil.
In my garden, i also grow red roses (Rosa Centifolia). Unfortunatley, there are some brown spots on its leaves, and the leaves are falling of the stalk and the flower is completely deformed. I tried to heal the soil with one of my products and, surprisingly, i got a completely healthy plant. The flowers are also bigger with lots of petals. Now, the rose is rich with many flowers. This confirms my theory that not only plums are affected by polluted soil.

A tospovirus new to North America: Virus detection and discovery through the use of a macroarray for viruses of solanaceous crops

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Winter planted tomato plants with virus-like symptoms of leaf distortion, mottle-mosaic, and necrotic stem streaking were observed in south Florida in January 2010. Extraction of total plant RNAs and analysis using a macroarray for the detection of viruses of solanaceous crops revealed hybridization to 70-mer oligonucleotide probes designed for the detection of tospoviruses. Probes with hybridization signals contained sequences from the genomes of *Tomato chlorotic spot virus* (TCSV), *Impatiens necrotic spot virus*, and *Tomato spotted wilt virus*, with the majority from TCSV. The macroarray did not contain probes for the related *Groundnut ringspot virus* (GRSV), as this virus had not been reported from solanaceous plants. Sequencing of an M RNA derived, cDNA fragment showed the closest relationship with TCSV (92-95% identity) and GRSV (89-92% identity). By contrast, sequences from the S RNA showed 91% identity with GRSV but only 78% identity with TCSV. A more complete analysis of the viral genome and investigation of its vector relationships in progress will provide a better understanding of the nature of the virus.

Toward aphid-resistant transgenic plants

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While transgenic plants expressing *Bacillus thuringiensis* (Bt)-derived toxins have met with widespread success for management of lepidopteran and coleopteran pests, Bt-derived toxins are not effective for management of the sap-sucking insects within the order Hemiptera. Indeed, in some instances damage caused by hemipteran pest species which include aphids and plant bugs, has compromised the success of the Bt-based technology. Plant viruses which are transmitted by aphids in a persistent, circulative manner enter the aphid hemocoel by a receptor-mediated process. We have shown that the coat protein (CP) of such a virus, *Pea enation mosaic virus* (PEMV: *Luteoviridae*), when fused to an effector protein delivers the fusion protein into the aphid hemocoel. For example, a CP-P-EGFP fusion protein with a proline-rich linker derived from the virus (-P-) was delivered into the aphid hemocoel. Uptake of this fusion protein showed that the virion structure is not required for uptake of CP from the aphid gut. PEMV CP fused to the spider-derived insecticidal ω-atracotoxin Hv1a was tested for aphicidal activity by using membrane feeding assays with *E. coli*-expressed fusion proteins, and by transient expression of fusion proteins in *Nicotiana benthamiana*. The results of these experiments show promise for use of this approach for production of aphid-resistant transgenic plants.

Potyviruses of legume weeds and *Passiflora* spp. from Western Australia: biological properties and phylogenetic placement of coat protein sequences

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In 2005-2009, potyvirus isolates were obtained from naturalized wild *Passiflora foetida* (stinking passion flower), cultivated *P. edulis* (passionfruit), cultivated *P. caerulea* (blue passion flower), and naturalized wild legume species. The legume isolates came from plants of *Macroptilium atropurpureum* (siratro), *Clitoria ternatea* (butterfly pea), *Vigna trilobata* (wild mung bean), and *Rhynchosia minima* (Rhycho). The isolate collection localities were Broome, Carnarvon, Geraldton, Kununurra and Perth in geographically and climatically different parts of Western Australia. All samples were tested initially by ELISA using generic potyvirus antibodies and samples reacting positively were studied further.

When the coat protein genes of the 25 potyvirus isolates obtained were sequenced and their nucleotide sequences subjected to phylogenetic analysis, the isolates from legume species were *Bean common mosaic virus* (BCMV) (3), Passiflora virus Y (PaVY) (9), Passiflora mosaic virus (PaMV) (1), and one that did not fit well into any established potyvirus grouping. With the isolates from *Passiflora* species, *Passionfruit woodiness virus* (PWV) (7), PaVY (5), and one unknown potyvirus were detected. The two unknown potyviruses detected were from *C. ternatea* and *P. foetida* and were sufficiently distinct that they apparently represent unknown viruses. When isolates of PaVY (10), PWV (7), BCMV (3), PaMV (1), and the unknown potyvirus from *C. ternatea* (1) were inoculated to standard indicator plants, all infected *Nicotiana benthamiana,* which is native to Western Australia, systemically. Some isolates of PaVY and BCMV also caused local infection in *Chenopodium* spp. but other indicator plants remained uninfected. Subsequent inoculations from infected *N. benthamiana* to plants of *P. edulis*, *P. caerulea* and *M. atropurpureum* satisfied Koch's postulate by reproducing the symptoms in the original isolation hosts.

Incidence and control of cucurbit viruses in NWFP Pakistan

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Symptoms like mosaic, mottling, chlorosis and deformation were quite common in the cucurbit crops grown in different districts of NWFP, Pakistan. Surveys were conducted during 2007-2009 in seven districts including Peshawar, Nowshera, Mardan and Swabi in the plain areas and Swat and Dir districts in hilly areas of NWFP. Six different viruses were found commonly infecting the cucurbit crops with CMV as the most dominant virus beside CGMMV, ZYMV, WMV, PRSV and TLCNDV. Multiple infections were quite common, where a single plant was infected with two or more than two viruses. Among the different viruses highest infection was recorded for CMV (64 %) at district charsada and least for PRSV (20 %) at district Peshawar. In Northern hilly areas highest incidence was recorded for CMV (45 %) and least for WMV (24 %) both at district Swat. Among the different crops highest infection was caused by CMV (44 %) in Luffa and least by CGMMV (15 %) in Squash. Different strategies were employed to control the vector transmitted viruses and also thermotherapy and chemotherapy were tried to check the efficacy of these treatments in controlling the seed transmission of CGMMV. Seed treatment at 80 C alone or in combination with chemotherapy gave maximum protection. Attenuated strain of CGMMV was also effective in controlling the severe strain of CGMMV in lageneria crop.

Post-transcriptional gene silencing (PTGS) is a mechanism of plant host-defense against viruses

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Post-transcriptional gene silencing (PTGS) is a mechanism of plant host-defense against viruses. In the course of evolution, the viruses have encoded proteins with the potential to suppress the host RNA silencing mechanism as a counterdefense strategy. Plant viral silencing suppressors can be divided into three families that (*i*) enhance virus accumulation in the inoculated protoplasts, (*ii*) are essential for virus cell-to-cell movement but dispensable on virus accumulation in single cells, and (*iii*) facilitate virus long-distance movement and/or intensify disease symptoms but are not essential for viral replication and cell-to-cell movement. Searches for plant virus silencing suppressors have become an essential part of the functional characterization of viral genomes. Expression of genes in transgenic *gfp Nicotiana Benthamiana* using *Agrobacterium* is a powerful tool for the analysis of gene function in plants. In this work, we have developed this method for screening RNA silencing suppressors candidates encoded by the positive-strand RNA genome of grapevine leafroll-associated virus 3 (GLRaV-3), a widespread agent of grapevine leafroll. We focused in the 3' genes p21, p19.6 and p19.7. The clones were obtained from isolates of Portuguese varieties described previously to have single infections of five phylogenetic groups with a coefficient of differenciation of 88%, based on CP gene.

Development of several laboratory assays for the detection of *Apricot latent virus*

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Grafting experiments showed that *Apricot latent virus* (ApLV), a definitive species of the genus *Foveavirus*, can infect a wide range of *Prunus* species but only peach cultivars and apricot cv. Tyrinthos showed symptoms. Thus, infected but symptomless plum, apricot and cherry cultivars could constitute a major virus reservoir for unwanted ApLV spread through nursery productions. This likelihood should be taken into serious consideration when designing and implementing stone fruit certification schemes so as to restrain, or better, rule out potential contamination of the stocks with this virus. One of the essential elements of successful certification programs for production of healthy propagation material is the availability of sensitive and specific diagnostic methods. Therefore, the optimization and subsequent comparison of several techniques for detection of ApLV was carried out. Based on our partial sequence of the ApLV genome, the new specific sets of primers were designed and used in our study. Six comparative analyses, namely two-step-reverse transcriptional polymerase chain reaction (RT-PCR), one-step-RT-PCR, co-operational RT-PCR, single tube nested RT-PCR, real time PCR and nonisotopic dot blot hybridization using different labeling technologies, are discussed for the purpose of evaluating and determining the most reliable and efficient tests.

Virus diseases of cereal crops in the Czech Republic

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Barley yellow dwarf virus (BYDV), *Wheat dwarf virus* (WDV) and *Wheat streak mosaic virus* (WSMV) are the most important viruses infecting cereal crops in the Czech Republic. One-step RT-PCR followed by an RFLP assay was developed for the typing of BYDV members of Luteovirus genera. A partial coat protein (CP) gene sequencing and RFLP analysis identified the presence of three strains of BYDV: PAV, MAV and PAS. The presence of these strains in winter cereals range 64% PAS, 23% PAV and 13% MAV. Four species of aphid vectors have been observed in cereal fields: *Sitobion avenae, Rhopalosiphum padi*, *Metopolophium dirhodum,* and *Rhopalosiphum maidis.*

Two strains of *Wheat dwarf virus* (WDV), one of which is wheat adapted and one barley adapted, have been confirmed from field samples of wheat and barley. The virus typing was conducted by both PCR-RFLP and sequencing-based methods. The Czech WDV isolates of the barley strain are more variable than the isolates of the wheat strain, separating them into two clades, one representing a pool of divergent isolates. Both RFLP and sequence analysis have shown that the barley strain is restricted to the barley host, while the wheat strain is present in both wheat and barley plants.

Wheat streak mosaic virus (WSMV) is less frequently occurring virus in the Czech Republic. To increase knowledge of genetic diversity of WSMV a multiple alignment of CP nucleotide sequences was performed. The sequence analysis revealed a group of European isolates with an identical 3-nucleotide deletion, resulting in the lack of the Gly2761 codon within the CP region of the polyprotein. All Czech isolates belong to this monophyletic group of isolates (designated as WSMV-ΔE). Two simple assays were developed for specific and accurate detection of WSMV-ΔE isolates: RFLP targeting a ClaI restriction site and specific primers functional in one-step RT-PCR.

Potato leafroll virus (PLRV) resistant potatoes

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There is a growing need for crops resistant to different plant pests. One of the most severe plant pathogens infecting potatoes is *Potato leafroll virus* (PLRV). PLRV is the type member of the genus *Polerovirus* which belongs to the family *Luteoviridae.* The RNA genome of PLRV consists of a ~ 5.9 kb positive-sense single-stranded RNA and encodes eight main open reading frames (ORFs) numbered from 0 to 7. PLRV is considered one of the most damaging potato viruses and is worldwide distributed. One way how to manage PLRV is to use virus-free certified seeds. To obtain suitable sequences of PLRV for design of resistant potatoes complete genomes of three Czech representative PLRV isolates were sequenced. For transgene construction ORF2 gene – encodes motifs typical for RNA-dependent RNA polymerases - was chosen. The expression cassette for ORF2 production in binary vector was designed and the construct was introduced into potato plants of various cultivars. The expression of ORF2 will be detected with our antibodies prepared against recombinant ORF2 antigen and by RT-PCR with PLRV specific primers.
We hope that transgenic potatoes prepared using this construct will demonstrate more stable resistance than it is achieved with normally used coat protein.
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Viral infection of wild orchids in Ukraine

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Preserving plant biodiversity is an important goal. Despite viral diseases of orchids are the subject of extensive research in many countries, spread and characteristics of viruses invading plants of Orchidaceae Juss. Family in natural ecosystems of moderate climatic zone remain practically uninvestigated, particularly in Ukraine. Normally wild orchids of Ukraine belong to rare and/or endangered species. Viral diseases are one of the hypothetical contributors to their extinction with an indeterminate effect. This necessitates control of viruses infecting non-tropical orchids in their natural habitat as well as development of efficient ways of cultivation of such plants in glasshouse conditions. This work aims at detection and identification of virus agents in orchids from glasshouses and natural ecosystems of Ukraine. The following orchid taxa were subjected to research: Comperia, Dactylorhisa, Epipactis, Gymnadeae, Himantoglossum, Limodorum, Listera, Neottia, Ophris and Orchis sampled in Transcarpathian and Crimean regions.

For biological detection of viruses we employed 11 species of bio-indicator plants from 5 families; serological identification was conducted via different types of ELISA and RT-PCR. Analysis of orchid samples from natural ecosystems showed that *Tomato aspermy cucumovirus*, *Cymbidium mosaic potexvirus*, *Tobacco ringspot nepovirus* and *Tomato rattle tobravirus* prevailed in these plants.

Listera ovata (L.) R. Br., Neottia nidus –avis (L.) Rich. and Orchis purpurea Hunds. Demonstrated comparatively higher level of virus infection among wild orchids from natural flora of Ukraine. In Ukraine, these plants belong to rare and endangered (Orchis purpurea Hunds.) orchid species and included into the Red book of Ukraine. Viral diseases of these plants may favour a decline in species biodiversity in orchid habitats or even their complete extinction in Ukraine.

Orchids of Cypripedium L. genus cultivated in glasshouse collection of M.M.Gryshko' National Botanical Garden of NASU also demonstrated high level of virus infection. This may be explained by the decrease of natural plant resistance to viruses when grown in artificial ecosystems with no adequate control of virus diseases in the collection.

Analysis of the temporal and spatial spread of *Plum pox virus* as influenced by USA and Canadian eradication programs

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Plum pox virus (PPV) was recently declared eradicated in Pennsylvania, following the implementation of a statewide eradication program that was initiated in 2000. There were approximately 400 PPV-positive trees detected in 2000, however, the number of PPV-positive trees decreased exponentially in subsequent years until no new PPV-positive trees were detected (in 2007). In Canada, the number of PPV-positive trees was 1,879 in 2000 and has decreased by approximately 200 PPV-positive trees/year since the eradication program was initiated. The success of these eradication programs can be attributed to extensive annual, area-wide surveys and the removal of all susceptible hosts within 500 m of a PPV-positive tree (in Pennsylvania), and the removal of all susceptible *Prunus spp*. in blocks with >1% PPV-infected trees (Ontario). Using 10 years of survey data, a number of spatial analyses were performed at the block and homeowner scale to quantify the spatial and temporal dynamics of the pandemic in Pennsylvania. In 2000, positive blocks were found to be clustered in Pennsylvania for distances between 0.7 and 4.9 km. In later years, the distance to 50% of new PPV-positive blocks/homeowners (D_{50}) increased from 1.3 km in 2000 to 17.2 km in 2005. In 2006, the last year any PPV-positive blocks/homeowners were detected in Pennsylvania, the D_{50} decreased to 6.3 km. An approximate two year periodicity was found to exist arising from previous positive locations, suggesting that the location of a PPV-positive block/homeowner was having an impact on the health status of other *Prunus* blocks at least 2 years after being removed. This was further supported by a nearest neighbor analysis that revealed the time since removal of the nearest positive block/homeowner averaged to be 1.8 years.

Bean Common Mosaic Virus and *Bean Common Mosaic Necrosis Virus* in Mazandaran province of Iran

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French bean (*Phaseolus vulgaris* L.) as pulses crop is extensively cultivated in Iran. During the 2008 season, a severe out break of mosaic diseases occurred in French bean (Chitti cultivar) in farmers fields in Mazandaran province. Symptomatic leaf samples from representative plants tested positive in ELISA tests with *Bean common mosaic virus* and *Bean common mosaic necrosis virus*es. Nl-8, NL-5, NY-15, Fla. ,BCMV-Pstv strains were detected using antisera from DZMZ, Germany. ELISA positive samples were rechecked by RT-PCR using set of primers directed to the coat protein and NIb protein which were designed to detect and characterize the two viral species. The results indicated that both viral species were present in deferent locations in the region . In most cases and with different samples BCMV was detected along with BCMNV, but not visa versa, indicating the prevalence and probably competence impact of these strains of BCMNV in the field.

Precision breeding PVY resistance using a modified potato gene

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Host plant resistance is one of the most successful strategies to control Potato virus Y (PVY) in potato, but cultivars bred to be resistant have gained limited market acceptance in North America due to growers' strong preference for the horticultural characteristics of existing varieties. Engineering resistance while maintaining all other desirable traits has been successful, but widespread acceptance has been hindered by consumer concerns. Intragenic resistance may be an acceptable alternative. The highly conserved eukaryotic translation initiation factor 4E (eIF4E) is a host factor that binds to the 5' cap of mRNA and aids in recruitment to the host ribosomal complex. A number of plant viruses with single-stranded RNA genomes (e.g. PVY) also interact with eIF4E, often through a virus genome-linked protein (VPg), to facilitate translation, replication, and/or cell-to-cell movement of the virus genome. Mutations at the eIF4E locus have evolved in some plants that disrupt the interaction with VPg, resulting in virus resistant individuals. Recessive resistance alleles at the eIF4E locus have been identified in barley, lettuce, melon, pea, and several solanaceous plants, but not potato. Here we show that over-expression of an eIF4E resistance allele from pepper in potato confers resistance to various PVY strains. We then isolate and sequence the previously-uncharacterized potato eIF4E gene and produce a series of novel alleles by introducing mutations resulting in several amino acid changes implicated in disease resistance. These mutant potato alleles were expressed 'intragenically' in potato, and the resulting plants shown to be resistant to different strains of PVY in the greenhouse and the field. The resistance is stable through multiple generations of tuber propagated plants. We show that directed modification of a potato allele can be introduced to recover virus resistant potatoes, a species that is not known to have naturally evolved resistance alleles at the eIF4E locus. This approach of using a modified native gene may be more readily accepted by consumers than non-native genes. In addition, this strategy has the potential to control many plant viruses that are known to interact with eIF4E.

Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy

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The current virus control methods are limited in number, efficacy and environmental suitability and current EU decisions restrict crop improvement strategies employing transgenic plants. To protect plants against existing and emerging virus diseases new methods are urgently needed. A very promising approach is the exploitation of RNA silencing, a natural, endogenous mechanism in plants that is a sequence-specific process leading to viral mRNA degradation.
COST Action FA0806* brings together several EU labs in order to develop suitable, efficient and cost-effective methods to induce anti-viral silencing in crops by the transient application of dsRNA, siRNAs and/or artificial small RNAs (collectively designated as "RNA-based vaccines"). These vaccines are produced either *in vitro* or *in vivo* in large quantities and are applied at laboratory or large scale employing specific delivery machinery.
FA0806 is structured in three Working Groups (WGs), WG1: Development of novel non-transgenic strategies for plant virus control, WG2: Application of novel non-transgenic strategies for plant virus control, and WG3: Socio-economic evaluation of the impact of the novel application methods. In the frame of FA0806, Training Schools and Short Term Scientific Missions provide instruments for scientific exchange and training for early-stage and senior researchers alike. Currently, 50 members from 25 COST countries and four non-COST members, from Argentina, New Zealand, South Africa and Mexico, participate in the Action.