Advances in biology, ecology and control of Xiphinema index, the nematode vector of GFLV

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6th International Symposium on Phylloxera

Rootstock perspectives

August 28-30, 2013
ISVV - Institute of Sciences for Vines and Wines
BORDEAUX, FRANCE

6th Symposium on Phylloxera
Bordeaux France 2013

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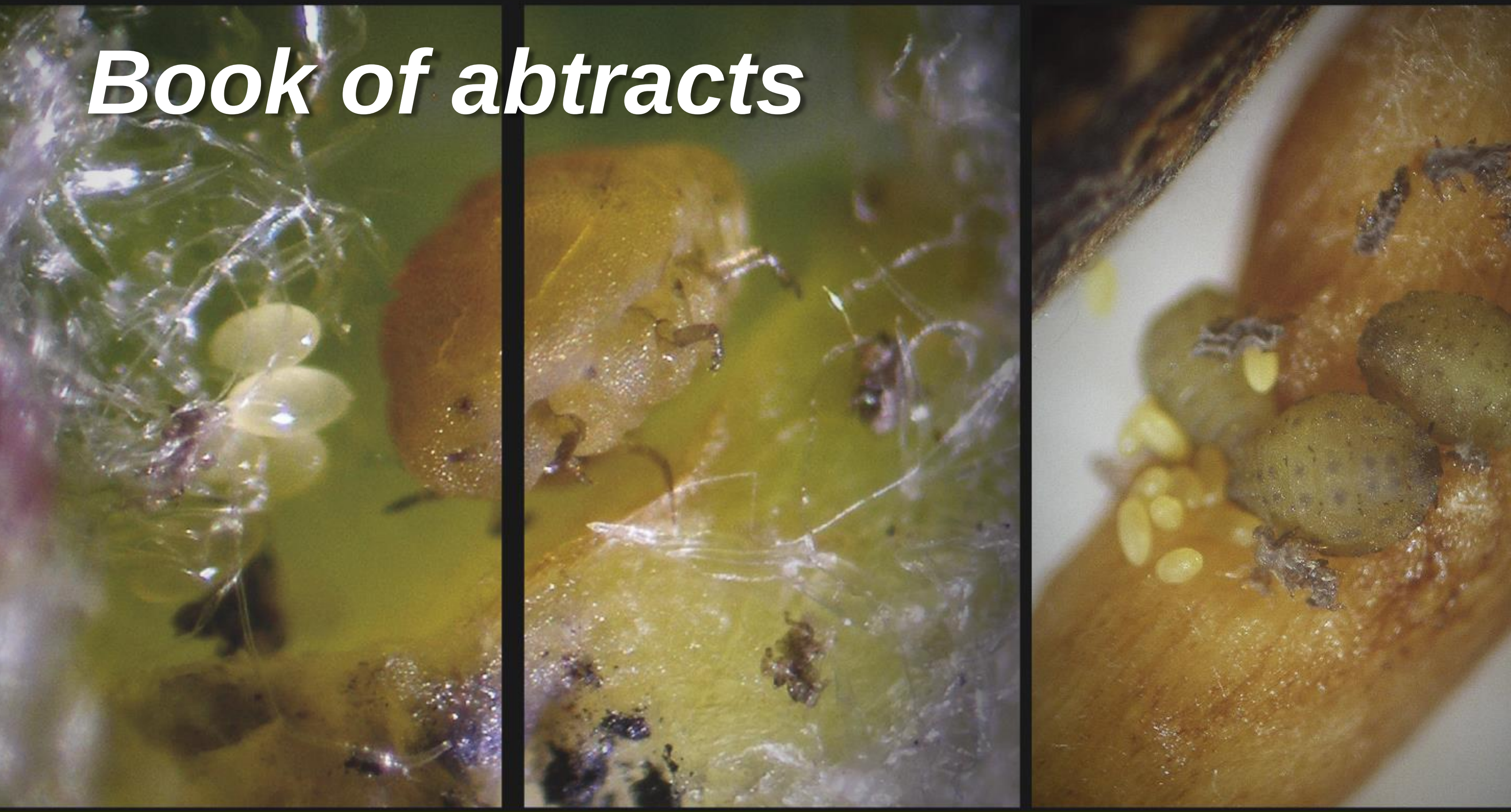

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6th International Symposium on Phylloxera

Rootstock perspectives

Co-convenors
N. OLLAT
D. PAPURA

Bordeaux-France, August 28-30, 2013
ISVV- Institute of Sciences for Vines and Wines

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6th Symposium on Phylloxera
Bordeaux France 2013

Bordeaux, 2013, the 1st of August

Dear colleagues and friends

One hundred and fifty years after the first decays observed in South East France vineyards and the first description of the insect by an entomologist from Oxford, J.O. Westwood, Phylloxera (*Daktulosphaira vitifoliaie* Fitch) remains the *sine qua non* reason for grafting grapevine in the world. Introduced in Europe from North American in the early 1850th, where, it became a devastating pest of the highly susceptible European grapevine species *V. vinifera*. This aphid almost destroyed the French and European vineyards within the following 50 years and spread to almost all viticulture regions of the world (North America, Europe, Australia, South Africa, South America, Asia and the Middle East, etc). Meanwhile, the mobilization of scientists to find a solution and save the French viticulture of the 19th century- and consequently the traditional viticulture based on the use of *Vitis vinifera* species – was unique. Entomologists from France, UK, United States, botanists, breeders have been working together or concomitantly to solve this problem by grafting the sensitive *Vitis vinifera* species onto resistant (or tolerant) American species. Considering the new challenges for grape growing throughout the world, this period should remain a model of scientific approach and international collaboration which allowed the saving of European viticulture industry.

Today, phylloxera is still a major threat for viticulture imposing the grafting of *V. vinifera* (80 to 85% of the vineyards worldwide are grown on rootstocks) and quarantine regulations in areas (e.g. Australia, Chile, Armenia and China) where varieties are predominantly grown own-rooted. Many rootstock breeding programs developed in different countries led to the release of dozens of rootstocks, most of them highly tolerant to Phylloxera and adapted to various environmental conditions. Because grafting is such an efficient biological way to control Phylloxera, studies on the pest itself have not been so active. This resulted to a lack of scientific knowledge which may be considered as a bottleneck for the efficiency of the actual breeding programs and a weakness to maintain the sustainability of resistance. Only few scientists throughout the world working group on Phylloxera are still active on the issue of the biology and genetics of Phylloxera. However a new interest is rising with the ongoing Phylloxera genome sequencing project which aims to improve our understanding of some specific biological traits or particular aspects in the biology of this invasive pest. In addition to cope with Phylloxera, the rootstocks have to be adapted to various environmental conditions. In the frame of climate change, especially because of the threat of increased drought, research projects which aim to understand and improve drought resistance of grapevine rootstocks are becoming more and more numerous.

The VIth edition of the International Phylloxera Symposium aims to be, after 150 years, the showcase of this convergent scientific experience in Phylloxera and grapevine rootstocks. Presenting the most recent studies on these issues, as well as giving a tribute to our historical predecessors, has been the wish of the conveners of this meeting. We would like to thank every member of the Scientific Committee and of the Organization board for their contribution to the preparation of this Symposium. We acknowledge also deeply our supporting institutions and the funding organizations.

Bordeaux is unique in the world of grape growing and Premium wine making. Bordeaux has also been very active in the past Phylloxera research with Léo Laliman, Alexis Millardet, and more recently with Roger Pouget and co-workers. Bordeaux is now hosting one the largest and famous research institute for vine and wine research (ISVV) in the world. We are so pleased to welcome you in this place. We hope you will get interesting scientific exchanges and unforgettable memories of this stay in Bordeaux. We did our best for that purpose.

Have a pleasant symposium!

Nathalie OLLAT

Daciana PAPURA

Acknowledgments: we are grateful to the below mentioned organizations which supported the organization of the symposium

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6th Symposium on Phylloxera

Bordeaux France 2013

Final Program

Day	Time	Authors			Page
28/08	*8h00-9h00*	*Registration*			
28/08	**9h00-9h15**	Serge Delrot, scientific director of ISVV	Symposium launchment		
28/08	9h15-10h00	Yves Carton	An exemplary partnership of American entomologist (C. V. Riley) with French entomologists in control of the Grape Phylloxera in France (1868-1895).	Introductionary lecture	13
28/08	10h00-10h30	Astrid Forneck	Phylloxera, scientific and viticultural challenges	Opening lecture	14
28/08	*10h30-10h50*	*Pause*			
		Session 1	***Biology, Ecology, genomics of grape phylloxera***		
28/08	10h50 11h20	F. Delmotte, A. Forneck, K.S. Powell, C. Rispe and Denis Tagu	The genome project of grape phylloxera	Session 1	15
28/08	11h20-11h40	C. Rispe, D. Papura, F. Legeai, J. Jaquiéry, A. Bretaudeau, D. Tagu and F. Delmotte	A study of phylloxera transcriptomes, comparing root and leaf feeding morphs	Session1	16
28/08	11h40-12h00	L. Kocsis and I. Bari	Preference and performance of leaf-galling grape phylloxera on Vitis sp. cultivars	Session1	17
28/08	poster	K. Wieczorek, S. Hensler and A. Forneck	Displaying sink-source flux in phylloxerated grapevines by microscopic techniques	Session 1	18
28/08	*12h00-13h15*	*Lunch*			

28/08	13h15-14h00	Poster session	5 mn to every poster authors		
		Session 4	***Strategies to control soil-born pests : detection, quarantine, alternative control options***		
28/08	14h00-14h30	Kevin S. Powell	Root-feeding grape phylloxera: Approaches for improved detection and reduced quarantine risk	Session 4	19
28/08	14h30-14h50	S. Tramontini and J.C. van Lenteren	The European Food Safety Authority (EFSA)'s pest risk assessment on Daktulospharia vitifoliae for the EU territory	Session 4	20
28/08	14h50-15h10	A.Nankivell, C. Collins, R. Correll, A. Downs, D. Giblot-Ducray, A. McKay, and K. Opel-Keller	Determining the sampling protocol to ensure quality material for DNA testing for detecting phylloxera in a vineyard	Session 4	21
28/08	15h10-15h30	D. Benheim, K.S. Powell, I. Potter, E. Robertson, and S. Rochfort	Metabolomics as a tool for early detection of grape phylloxera infestations	Session 4	22
28/08	15h30-15h50	K.S. Powell, R.J. Bruce and G.A. Korosi	Assessing the risk of phylloxera survival during white grape processing	Session 4	23
28/08	15h50-16h10	L. Zhang, X. Zang, Y. Du and H. Zhai	Bio-control plant selection of grape phylloxera	Session 4	24
28/08	16h10 – 16h30	M.van Helden, C. Laveau and G. Darrieutort	Monitoring *Xiphinema* virus vectors to adapt the fallow period in vineyards of Gironde	Session 4	25
28/08	poster	L. Kocsis and R. Andor	Efficacy of pesticides on grape phylloxera populations in vitro and in situ	Session 4	26
	17h00-18h30	*Cocktail and meeting of the working groups : phylloxera / water stress,*			
	19h30-20h30	*Visit of Bordeaux historical downtown*			
29/08		***Session 3***	***Grapevine resistance and rootstock breeding for phylloxera and nematode resistance***		

29/08	8h30-9h00	Andrew Walker	Breeding grape rootstocks for resistance to phylloxera and nematodes – it's not always easy	Session3	27
29/08	9h00-9h20	K.S. Powell, G.A. Korosi, P. Clingeleffer, R. Walker, M.R. Thomas, H. Smith and B. Smith	Genetic markers for phylloxera resistance: Preliminary observations using two endemic phylloxera genetic strains	Session3	28
29/08	9h20-9h40	A. Forneck, M. Griesser, N.C. Lawo, N. Stralis Pavese and D. P. Kreil	Changes in the expression of genes in root galls of grapevines infected by phylloxera (*Daktulosphaira vitifoliae* Fitch)	Session 3	29
29/08	9h40-10h00	K.S. Powell and G.A. Korosi	'Taking the strain' selecting the right rootstock to protect against endemic phylloxera strains	Session 3	30
29/08	*10h00-10h20*	*Pause*			
		Session 2	***Nematode and fan leaf virus control***		
29/08	10h20 10h50	Daniel Esmenjaud, G. Demangeat, M. Van Helden and N. Ollat	Advances in Biology, Ecology and Control of Xiphinema index, the Nematode Vector of GFLV	Session 2	31
29/08	10h50-11h10	P. Schellenberger, P. Andret-Link, C. Keichinger, B. Lorber, C. Sauter, P. Bron, S. Trapani, M. Bergdoll, A. Marmonier, E. Vigne, V. Komar, D. Esmenjaud, O. Lemaire, M. Fuchs, C. Ritzenthaler and G. Demangeat	Structural and functional characterization of Grapevine fanleaf virus capsid determinants involved in the transmission by Xiphinema index	Session 2	32
29/08	11h10-11h30	M. Claverie, L. Audeguin, P. Bloy, D. Esmenjaud and N. Ollat	Nemadex Alain Bouquet, a rootstock that delays Grapevine fanleaf virus (GFLV) infections	Session 2	33
29/08		***Session 5***	***Abiotic stress tolerance by rootstocks : what are the***		

			underlying mechanisms ?		
29/08	11h30-12h00	Felicidad de Herralde, X. Aranda, C. Biel, and R. Savé	Roots and rootstocks ecophysiology: mechanisms to cope with a changing environment	Session 5	33
29/08	*12h00-13h00*	*Lunch*			
29/08	13h00-13h20	M. Morales, G. Toro, A. Riquelme, G. Selles, R. Ferreyra and M. Pinto	Effect of different rootstocks on photosynthetic and nutritional response of grapevines cv. Sultanina under flooding stress	Session 5	35
29/08	13h20-13h40	J. Lecourt, SJ. Cookson, V. Lauvergeat, N. Ollat and P. Vivin	The mineral composition of grafted grapevines depends on rootstock genotype and nitrogen supply	Session 5	36
29/08	13h40-14h00	F. Meggio, B. Prinsi, A. S. Negri, G.S. Di Lorenzo, G.Lucchini, A. Pitacco, O. Failla, A. Scienza, M. Cocucci and L. Espen	Responses of the new grapevine rootstock M4 to drought: a biochemical and physiological characterization	Session 5	37
29/08	14h00-14h20	A. Peccoux, L. Rossdeutsch, B. Golard, H. R. Schultz, S. Delrot and N. Ollat	Drought-sensitive and drought-tolerant grape rootstocks differ in root xylem development and hydraulic conductivity	Session 5	38
29/08	14h20-14h40	M. Hadaynedjad, A. Ebadi, A. Musavi, R. Fatahi, L. G. Santesteban and M.A.Nejatian	The effect of drought stress on photosynthetic traits and certain gene expression of some Iranian grapevine candidate rootstocks	Session 5	39
29/08	*14h40-15h00*	*Pause*			
29/08	15h00-15h20	C. Pagliarani, C. Forcato, M. Ferrero, D. Alberto, G. Valle and A. Schubert	Expression differences induced by drought on conserved and novel miRNAs in grapevine	Session 5	40
29/08	15h20-15h40	F. Barrieu, A. Peccoux, L. Rossdeutsch, E. Marguerit, S.	Molecular mechanisms of grapevine rootstock adaptation to drought	Session 5	41

		Cookson, M. di Filippo, V. Lauvergeat , P.F. Bert and N. Ollat			
29/08	15h40-16h00	B. Prinsi, M. Cocucci and L. Espen	Root proteome responses to drought in two grapevine rootstocks with different susceptibility	Session 5	42
29/08	poster	A.S. Negri, M. Cocucci and L. Espen	Drought differentially affects the root and shoot metabolome in two grapevine rootstocks having different tolerance to water deficiency	Session 5	43
29/08	poster	M. Vitali, C. Pagliarani, C. Lovisolo and A. Schubert	Ecophysiological responses to stress of the M4 rootstock	Session 5	44
29/08	poster	E. Claverie, J. Lecourt and P. Vivin	Rootstock genotype and nitrate supply modify grapevine tissues metabolic content	Session 5	45
29/08	*16h00-23h00*	*Departure for Haut Bailly estate visit and gala diner in Chateau Luchey Halde*			
30/08		***Session 6***	***Genetic determinism and rootstock breeding for abiotic stress tolerance***		
30/08	9h00-9h30	Ernst H. Rühl and J. Schmid	Rootstock breeding - between site adaptation and abiotic stress tolerance	Session 6	46
30/08	9h30-9h50	L. Kocsis, B. F. Lajterné, Gy. Németh and G.M. Kocsisné	Grape rootstock breeding program of the Georgikon Faculty	Session 6	47
30/08	9h50-10h10	F. Emanuelli, D. Grossi, G.B. Simone di Lorenzo, S. Lorenzi, L. Brancadoro, O. Failla, M.S. Grando and A. Scienza	Drought tolerance in grapevine rootstocks: an association genetics approach	Session 6	48
30/08	10h10-10h30	E. Marguerit, A. Calonnec, C. Van Leeuwen, S. Delrot and N.	Analysis of the control of shoot growth by the rootstock under drought conditions by an integrated	Session 6	49

		Ollat	approach of quantitative genetics and ecophysiology		
30/08	poster	S.Grundler, J.Schmid, O.Bitz and E.Rühl	*Vitis berlandieri* – A potential partner for rootstock breeding	Session 6	50
30/08	poster	A.Scienza, M. Lucchin, A. Schubert, M. Zamboni, M. Gardiman and L. Espen	SERRES: a Italian project to develop and to validate markers for the selection of new grape rootstocks resistant to abiotic stresses	Session 6	51
30/08	*10h30-11h00*	*Pause*			
30/08	11h00 - 12h30	Convenors + representative of working groups + ISHS phylloxera group chairwoman + next symposium convenor	Symposium conclusions : reporting of the working groups Next symposium Farewell walk		
30/08	*12h30-13h30*	*Lunch*			
30/08	*13h30-17h30*	*Departure for the nursery visit*			

Abstracts

An exemplary partnership of American entomologist (C. V. Riley) with French entomologists in control of the Grape *Phylloxera* in France (1868-1895)

Y. Carton
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C.V. Riley (1843-1895) was born and reared in England; he made his career as an agricultural entomologist in the United States. Nevertheless, his tenure at a boarding school in Dieppe, France (1855-1857) established Francophile patterns that persisted throughout his life. Most important, Riley's early French contact prepared him, as Missouri State Entomologist (1868-1877) and Chief U.S. Entomologist (1878-1894), to join with J. E. Planchon, (Montpellier University Professor, 1823-1888),J. Lichtenstein, V. Signoret (entomologists), and other French scientists in the historic campaign against the grape *Phylloxera* in France. Riley's American *Phylloxera* specimens, field studies ansd experiments helped establish key aspects of the *Phylloxera's* life history. With American and french data, he confirmed with his French colleagues, that the American and European *Phylloxera* were identical and that the species originated in America; he also discovered the previously unknown American root form.

Riley was able to explain, on Darwinian grounds, that the consistent failure of European vines in eastern North America was due to their lack of resistance to *Phylloxera*. Riley's support of Planchon and Lichtenstein—who held that *Phylloxera* was the cause of the dying vines, and not an effect as maintained by Signoret and other defenders of "pure" French vineyards—helped the "Americanists" prevail in their diagnosis of the problem. His summary of resistant American vines helped to convince French vintners that the eventual solution lay in grafting French vines on American rootstock.

When Riley represented the U.S. Department of Agriculture at the International Exhibition in Paris in 1889, the French government presented him with the Legion of Honor, its highest award, for his assistance in *Phylloxera* affairs. Official recognition, along with collegial relations with French scientists, and general acceptance and gratitude among the French people were merited. Riley's partnership with the French in the *Phylloxera* challenge not only allowed him to repay his debt of gratitude to France but also to demonstrate how the fledgling field of economic entomology could meet a major insect crisis in the international arena.

(C. SORENSEN, J. SMITH AND E. SMITH and Y. CARTON, 2008. Charles Valentine RILEY, France and *Phylloxera*. *American Entomologist,* 54, 3: 134-149; Y CARTON, C. SORENSEN, J. SMITH AND E. SMITH. 2007. Une coopération exemplaire entre entomologistes français et américains pendant la crise du *Phylloxera* en France (1868-1895). *An. Soc. Entomol. Fr.*, 43(1), 11-33

Introductionary lecture

Phylloxera – Challenger of Viticulture and Science

A. Forneck
BOKU *University of Natural Resources and Life Sciences*, Vienna, Austria

Phylloxera (Daktulosphaira vitifoliae Fitch) have changed the face of Europe's viticultural landscape since their arrival in the 19th century and the following destruction of almost the whole viticultural basis. Although research efforts and publications with focus on the insect and its interaction with the host plant have been immense, a lot still lies in the dark. Because of the brutality of consequences for the local wine industry, once Phylloxera invade an area, the problem still is of vital essence. This lecture will try to give a synopsis of challenges Phylloxera still put to science and viticulturists when it comes to understanding as the basis to find reliable means of preventing, detecting and fighting infestation. Also in the face of these challenges, the lecture comes to the conclusion that expanding our knowledge is the best way to find strategies in dealing with the enduring threat.

The genome project of grape phylloxera

F. Delmotte[1], A. Forneck[2], K.S. Powell[3], C. Rispe[4] and D. Tagu[4]
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In the framework of the International Aphid Genomics Consortium (IAGC) and the i5K initative, we propose the sequencing of the grape phylloxera (*Daktulosphaira vitifoliae* Fitch) genome through an integrated and collaborative approach. Phylloxera, because of its basal phylogenetic relationship to aphids, provides an interesting model for comparative genomics studies between aphids (in the broad sense). The phylloxera sequence will also fill the gap between aphids and related taxa such as cicadas and leafhoppers, psyllids and whiteflies.

A genome project is only the very first step for following uses in genetics, evolutionary biology and functional analyses. Knowledge of the genome of phylloxera will considerably improve our understanding of many of the specific biological features of this invasive pest. This includes identifying genes for complex traits including those with relevance to the genetic basis of host-plant interaction, to leaf gall and root gall formation, to nutrition on grape, and to the developmental causes of extreme phenotypic plasticity. Finally, knowledge of the phylloxera genome is also relevant to human and economic well being by contributing to reduced environmental cost and quarantine risk in viticulture.

The genome project itself requires several steps including DNA extraction (in large quantity and quality) from, where possible, low heterozygous lineages, DNA sequencing using new high throughput technologies, assembly and annotation. In this presentation, we will provide a general overview and milestones of the project, as well as an update of the first steps that are conjointly performed in our respective groups. Currently, DNA sequencing and pre-assembly of a *D. vitifoliae* Australian clone have been obtained; its sequence is compared with gene sequences obtained by RNA-Seq from a French clone to estimate sequence divergence between the two strains. In the meantime, first genome sequence data will be obtained from the French clone to improve this comparison. This project is also a collaboration with the 1Kite initiative for the transcriptome acquisition and with the BGI (Beijing Genomics Institute) (China) for DNA sequencing. .

Acknowledgements: INRA Grant AIP Bioressource (France) and DEPI Victoria, Strategic Innovation Funding (Australia).

A study of phylloxera transcriptomes, comparing root and leaf feeding morphs

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Grape phylloxera (*Daktulosphaira vitifoliae*) has been a historical major pest of viticulture, and is still a species of concern in many areas of the world. In addition, its phylogenetic position as a basal species to true aphids (Aphidoidea) makes it an interesting biological model in the perspective of understanding the evolution of this plant-feeding insect group as a whole. Phylloxera has an unusually complex life-cycle with a potential alternation of sexual and asexual reproduction, and also between leaf- and root-feeding morphs. In the last case, the nutritional and environmental differences among these morphs are expected to impact the physiology of the insect, resulting in specific patterns of gene expression. To study this point, and also with the aim of generating the first large scale collection of transcript sequences for this species, we generated high throughput transcriptomes (RNA-Seq, with llumina sequencing) with two replicates for both root- and leaf-feeding individuals (collecting mixed stages) of a clone collected from the field in Bordeaux, France.

Sequences from both morphs and from all replicates were assembled with Trinity, and resulted in 105,697 contigs. Then expression for each condition and replicate was counted, and compared statistically (using DESEQ). A list of genes with statistically significant expression differences among morphs was generated. The annotation of contigs, and the comparison of genes specific to gall or root feeding has been undertaken, and allows to identify functional groups characteristic of each morph.

Finally general patterns of the transcriptome have been described through a comparison with the pea aphid genome (*Acyrthosiphon pisum*), such as the number of different pea aphid genome best hits (10,413), and compositional patterns; a markedly higher AT content characterizes coding sequences of phylloxera, compared to the pea aphid (which has already an AT-rich genome).

Preference and performance of leaf-galling grape phylloxera on *Vitis* sp. cultivars

L. Kocsis[1]and I. Bari

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In the last decade, there have been increasing numbers of reports on the proliferation of leaf galling forms of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) on *Vitis vinifera* L. cultivars (Szalai et al., 2005, Botton and Walker, 2009, Győrffyné-Molnár et al., 2009). In addition, Kocsis et al. (2010) reported on the economic damage caused by the leaf-galling form of grape phylloxera in a *V. vinifera* vineyard. Because of these reports, we initiated studies of the impact of leaf galling on different *V. vinifera* cultivars. Kimberling and Price (1996) developed a method to evaluate preference and performance of grape phylloxera under field conditions. Omer et al. (1999) evaluated a root bioassy to assess preferential feeding by root feeding forms. Given our experience with previous evaluation methods, we established a potted vine experiment under glasshouse conditions, using 3 rootstocks and 7 *V. vinifera* cultivars. We found that the leaf galling form of phylloxera had a significant preference among cultivars based on their feeding behavior, and that scion/rootstock combinations impacted feeding results. Phylloxera preferred cultivars with glabrous, succulent leaves, like ‘Portugieser’ or ‘Cserszegi fuszeres’, and did not prefer cultivars with tougher leaves with some degree of pubescence like ‘Merlot’. A quick in vitro method to evaluate leaf susceptibility and preference was designed that allowed phylloxera eggs to hatch and leaf tissues to be galled. This leaf galling assay needs to be further tested. We have also observed un-pruned own-rooted *V. vinifera* ‘Kékfrankos’ and ‘Leányka’ vineyards in which phylloxera populations were greatly reduced and no perceptible damage occurred on 20 year-old-vines.

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Displaying sink-source flux in phylloxerated grapevines by microscopic techniques

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Grape Phylloxera (*Daktulosphaira vitifoliae* Fitch, Homoptera: Phylloxeridae) is a global pest on grapevine (*Vitis* spp. L.). The insect induces galls (nodosities) within the meristematic zone of root tips, where it stays attached and induces changes in the uptake and transportation of water, minerals and assimilates. Here, we postulate that nodosities induced by phylloxera form strong sink to increase phloem flow and symplastically transfer sucrose to the insect. To analyze this, we applied histological approaches to visualize the sugar symplast from leaves towards and within the root gall. We applied fluorescent dye carboxyfluorescein diacetate (CFDA) on leaves as a marker of sucrose phloem transport as well as symplastic phloem unloading to visualize the transport path towards phylloxerated roots. Our results show that CFDA was strongly symplastically transported towards and into the nodosities associated with the L2 phylloxera stage at 4-5 dai. To further analyse this transport at the single-cell level, we prepared sections of CFDA-loaded nodosities and examined them under both an inverted fluorescence microscope and the confocal laser-scanning microscope. Obtained results confirm the whole mount observations and showed strong fluorescent signal in phloem elements from which it spreads within endodermis towards the incision point and penetration site.

Our results contribute to understand the mechanisms involved in the transfer of assimilates from leaves into nodosity and to gain further knowledge on the compatible phylloxera-root interaction.

Root-feeding grape phylloxera: Approaches for improved detection and reduced quarantine risk

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Unlike many other insect pests, grape phylloxera is relatively difficult to detect in the vineyard due to a combination of small size, unpredictable spatial distribution and delayed expression of symptoms in the host plant. In the last decade a range of ‘novel’ approaches have been tested to improve the efficacy of early detection for root-feeding grape phylloxera. Approaches have included ‘primary’ detection which focus on the insect or its DNA, and ‘secondary’ detection which focus on either the host plant or the soil environment. Primary detection can be done through the effective use of simple insect trapping techniques or the use of a phylloxera-specific molecular probe. Secondary detection can be achieved through the use of remote sensing techniques and metabolomics. However, the efficacy of each detection approach is dependent on biotic, abiotic and edaphic factors which influence spatiotemporal distribution of phylloxera. The risks of phylloxera dispersal from an infested site can be minimized by the use of scientifically validated pre- and post-harvest quarantine protocols and selection of the most appropriate rootstocks. An integrated approach to detection and quarantine is described which would improve the efficacy of detection and reduce the risks of human-assisted dispersal to a minimum.

The European Food Safety Authority (EFSA)'s pest risk assessment on *Daktulospharia vitifoliae* for the EU territory

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Following a request from the European Commission, the EFSA Panel on Plant Health (PLH Panel) has been asked to deliver a scientific opinion on the risk posed by *Daktulospharia vitifoliae* for the European Union territory and to identify and evaluate risk management options for this pest. In particular, the Panel was asked to provide an opinion on the effectiveness of the current EU requirements against this organism, which are laid down in Council Directive 2000/29/EC, in reducing the risk of introduction of this pest into, and its spread within, the EU territory. The PLH Panel created a specific working group with the objective to answer the request by the end of May 2014. The activity will be conducted in accordance with methodologies developed by the same Panel and in agreement with the International Plant Protection Convention.

This mandate is the first received by the Panel on an important pest of *Vitis* sp. As such, the identification of information gaps and expertise which the group might need will be the first step in the process of risk identification. A major task of the working group will be to try to answer the question if there are aggressive strains of *Daktulospharia vitifoliae* outside the EU territory which might form a risk for EU grapevine.

Determining the sampling Protocol to ensure quality material for DNA testing for detecting Phylloxera in a vineyard

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Detecting Phylloxera has traditionally been based on observation of vines with low vigour followed up with a systematic grid pattern of ground truthing by digging up roots and a visual inspection with a magnifying glass to confirm the presence of Phylloxera or Phylloxera damage. This method dates back to the 19th Century.

The aim of this study is to further investigate the potential of a quantitative Polymerase Chain Reaction (qPCR) assay developed previously (Herbert *et al*., 2008), in combination with the soil DNA extraction developed by Ophel-Keller *et al.* (2008) to quantify Phylloxera in soil samples. qPCR was used to detect Phylloxera in soil samples collected from known infected vines and stored for 0, 1, 3 or 12 days at 10, 20 or 35°C. Other soil samples were collected from three vineyards at different depths and positions relative to infected vine trunks and tested for Phylloxera by qPCR.

Phylloxera detection results in the various samples will be presented. Storage of soil samples before molecular analysis and optimal sampling depth and position will be discussed.

Eventually, this study will assist designing a cost effective, reliable and easy to use surveying method that is easily delivered by regulators and vineyard owners alike. The method, in combination with qPCR, should allow early detection of Phylloxera in soil samples, well before low vigour is observed with the potential to improve management systems. Other soil borne pests and diseases could also be assessed on the same samples providing specific assays are available.

Metabolomics as a tool for early detection of grape phylloxera infestations

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Grapevine phylloxera (*Daktulosphaira vitifoliae* Fitch) is a destructive, root feeding insect that is difficult to detect and easily spread by the use of shared farm equipment. Early detection of this pest is essential to ensure the sustainability and profitability of the viticulture industry. Late detections of the insect cause significant economic damage to affected vineyards, with replanting onto phylloxera resistant rootstocks costing AUS $20,000 - $25,000 per hectare.

Metabolomics offers a new and exciting approach for the early diagnosis of phylloxera infestation. Studies of leaf material obtained from field studies in the Yarra Valley, Victoria, Australia indicated that there were metabolic differences between non-infested and infested vines. Analysis suggested that certain flavonols, as well as other, as yet unidentified, metabolites could be useful biomarkers of phylloxera infestation.

Validation of these putative biomarkers is crucial for the provision of useful diagnostic tools. Challenges in validation involve the accurate discrimination between biomarkers or metabolic profile changes caused by phylloxera infestation when compared to other environmental stressors. In order to address this challenge, glasshouse-based trials have been conducted to test the metabolic response of vines to nutrient, water and phylloxera induced stress. It is therefore critical that biomarkers of infestation can be detected under green house conditions where the vines are relatively immature and have had less exposure to phylloxera compared to field conditions.

This presentation will describe both LC-MS and NMR-based analyses of grapevine leaf material from both glasshouse trials and a field study and subsequent statistical analysis of the LC-MS and NMR data which showed discrimination between infested and non-infested grapevines.

Assessing the risk of phylloxera survival during white grape processing

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In the absence of a food source grape phylloxera can survive up to three weeks representing a significant quarantine risk. Dispersive stages of grape phylloxera, particularily first instars, can be inadvertently transferred on several potential 'vectors' including post-harvest grape products such as juice. Fermentation of red grape juice for at least 72 hours or filtering will kill or prevent phylloxera first instar transfer. White juice is processed differently to red juice, has different physiochemical properties and undergoes a range of treatments prior to filtering. For example, tartaric acid may be added to ensure pH is within a range of 3.0-3.4. At various stages in the white wine-making process sulphur dioxide is generally added, as either potassium or sodium metabisulphite. At maturity Baumė is usually between 10º and 12.5º depending on variety. Cool temperatures are generally used for white grapes with fermentation occurring at 10-16ºC or lower, clarification occurring below 15ºC and cold stabilisation occurring at -4 to +2ºC. The impact of ph, Baumé, white juice, sulphur dioxide and cold temperature on first instar phylloxera mortality was tested and results are described.

Bio-control plant selection of grape phylloxera

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Grape phylloxera (*Daktulosphaira vitifolia Fitch)* is one of the most terrible pest among the world. While phylloxera rampantly reemerged in China in 2005, this seriously threat to root cuttings propagation viti-viniculture industry in China. The aim of this research is to select one or more efficacious bio-control plant of phylloxera. Seventeen different kinds of Chinese medical plants (*Blumea balsamifera* (L.) DC., *Lablab purpureus* (Linn.) Sweet, *Nicotiana tabacum*, *Aloe vera* var. chinensis (Haw.) Berg, *Andrographis paniculata* (Burm. f.) Nees, *Melia toosendan* Sieb. et Zucc., *Datura stramonium* Linn., *Quisqualis indica* L., *Zingiber officinale* Rosc., *Juglans regia*, *Hyoscyamus niger*, *Astragalus membranaceus* (Fisch.) Bunge, *Scutellaria hypericifolia* Levl., *Zanthoxylum bungeanum* Maxim., *Nepeta cataria* L., *Tripterygium wilfordii* Hook. f., *Solanum nigrum* L.) were used in the tests. Both mortality and LT_{50} of the eggs and adults were obtained during the tests. All the select tests were in the lab, and the best one was chosen to carry out the pot trial. The results showed that *Nicotiana tabacum* and *Blumea balsamifera* were better than others in both eggs and adults control experiments. Highest mortality was present in *Nicotiana tabacum*, in the first 24 h mortality reached 100% in the lab and the LT_{50} is 1.5 h. Mortalities were higher in adults than eggs. The results perhaps showed some functional compositions exist in the selected plants. Other works can be done as a developmental direction of phylloxera bio-pesticide.

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Monitoring *Xiphinema* virus vectors to adapt the fallow period in vineyards of Gironde

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Over a 5-year period, a total of 108 uprooted virus diseased vineyard plots were sampled for the presence of nematode vectors *Xiphinema index* and *X. diversicaudatum*, responsible for transmission of *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV) in viticulture, respectively. A total of 711 ditches (approx. 10 per ha) were dug and sampled for nematode extraction. Frequency of *X. index* (310 samples; 43%) was higher than that of *X. diversicaudatum* (81 samples; 11%). Nematode numbers were very variable among plots and among samples of the same plot. We never found high numbers of both species in the same plot. No link was found with any management factor, except for high levels of living roots in the absence of devitalisation that were often associated with high nematode numbers. We propose to use the maximum number of nematodes found in a sample in a plot to adapt the fallow period using 4 classes: 0 (‘absent’, no nematode found: no need for fallow); 1 (‘few’, <= 5 nematodes per sample: 2.5 years); 2 (‘medium, >5 to 20: 3.5 years) and 3 (‘high’, >20: > 4 years). Applying this rule for the two species would result in 22% of plots that can be replanted almost immediately, 28% in class 1, 21% in class 2, and only 29% in class 3 requiring 4 or more years of fallow period. Monitoring nematodes to adapt fallow periods can be useful both to reduce unnecessary long fallow periods in the absence of nematodes and to avoid costly mistakes when replanting in the presence of high nematode numbers.

Efficacy of pesticides on grape phylloxera populations *in vitro* and *in situ*

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Until recently, the use of pesticides to control the root form of grape phylloxera has failed since it was first tried at the end of the 19th century (Buchanan and Godden, 1989, Granett et al. 2001). A range of pesticides (carbamates, organophosphates, organochlorines, pyrethroids, neonicotionids) with different insecticidal mechanisms have now been developed to control insects with sucking mouth parts (Heinrichs, 1979, Jeschke et al., 2011), and they have been successfully used against leaf galling forms of grape phylloxera in Hungary. The effect of these pesticides against root forms is not well understood, because of difficulties applying them through soil, however a few trials with thiamethoxam and imidacloprid showed promising results (Nazer et al., 2006, Herbert et al., 2008). In our study the efficacy of three insecticides was studied on insect survivorship and fecundity using a root bioassay; the efficacy of the most promising compound was studied under field conditions. Spirotetramat did not allow nymphs to develop in the root bioassay while thiamethoxam and abamektin did. The adult stage was reached by the 19th and 21st days after 1-6 day old eggs were placed on excised roots that were treated with abamektin and thiamethoxam, respectively. Spirotetramat was tested under field conditions using own-rooted *Vitis vinifera* cv. Chasselas rouge and blanc. The number of colonies was reduced with foliar spray application of spirotetramat when sampled vine roots were compared to the root samples before treatment. Treated plants displayed more vigor, but more years of testing are needed to verify that vineyard health was improved.

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Breeding grape rootstocks for resistance to phylloxera and nematodes – it's not always easy.

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Grape rootstocks were first developed to address the phylloxera crisis during the late 1800s, and many of these rootstocks continue to be used. However, changes in the climate, water availability, pest pressure, and pest control practices require the development of new and better adapted rootstocks. Many of the traits we need to address these issues are from *Vitis* species that have not been widely used in the past, either because they rooted poorly or had marginal phylloxera resistance.

The UC Davis grape rootstock breeding program has been studying phylloxera resistance for many years. Recent studies have examined the development of phylloxera strains capable of aggressive nodosity-based feeding on resistant rootstocks and the occurrence of foliar feeding strains, once rare in California. In order to better understand the origin of these strains, more than 500 leaf gall phylloxera samples were collected from 19 States along a meandering 5,000km transect across phylloxera's native range. Analysis of SSR data from 26 markers found that phylloxera populations primarily grouped by host.

Efforts to utilize *Muscadinia rotundifolia*'s exceptional pest resistance continue with an emphasis on resistance to nematodes, phylloxera and fanleaf degeneration. Large hybrid populations between various rootstocks and *Vitis* species x *Muscadinia rotundifolia* have been created. Many of these have strong resistance and some root moderately well, however no fertile progeny have been found. We are testing the breadth and durability of phylloxera resistance of a few fertile *V. vinifer*a x *M. rotundifolia* hybrids in hopes of using these to introgress *M. rotundifolia'*s pest and disease resistance into commercial rootstocks. We are also using *V. arizonica* and a number of related species from the southwestern United States that possess strong resistance to *Xiphinema index*, Pierce's disease, drought and salinity. These breeding efforts include developing strongly linked markers from SSR-based genetic maps to expedite traditional breeding and the physical mapping of resistance genes. Five rootstocks have been released from the program with resistance to aggressive root-knot nematode strains; X. index; these nematodes in a combined inoculum and at high soil temperature; and resistance to lesion, citrus and, in one case, ring nematode.

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Genetic markers for phylloxera resistance: Preliminary observations using two endemic phylloxera genetic strains

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In grapevine rootstock breeding programs, the utilization of molecular markers linked to key traits, such as phylloxera resistance, are essential for employing marker assisted selection aimed at reducing costs and time associated with extensive *in vitro* and *in planta* screening. A recent study identified a dominant resistance locus to phylloxera on linkage group 13 that is near two molecular markers (Gf13_1 and Gf13_9) in a *Vitis cinerea* hybrid[1]. To examine the genetics of phylloxera resistance further, a preliminary glasshouse-based study was conducted using a *Vitis cinerea* x *Vitis vinifera* F1 mapping population, which was screened with two phylloxera clonal lines (G4 and G30). Replicated trial resistance ratings were compared by quantifying relative phylloxera survival and nodosity formation after 12 weeks post infestation. Preliminary results using the Gf13_1 and Gf13_9 DNA markers indicated that these predict phylloxera resistance in the *V. cinerea* x *V. vinifera* F1 mapping population. Moreover, our results also suggest an additional locus that may have a complementary involvement in phylloxera resistance to the G4 and G30 lines. Future studies are aimed at confirming these preliminary observations and identifying the genes that confer phylloxera resistance.

1. Zhang J, Hausmann L, Eibach R, Welter LJ, Töpfer R, Zyprian EM. (2009) A framework map from grapevine V3125 (*Vitis vinifera* 'Schiava grossa' x 'Riesling') x rootstock cultivar 'Börner' (*Vitis riparia* x *Vitis cinerea*) to localize genetic determinants of phylloxera root resistance. Theor Appl Genet 119:1039-51.

Changes in the expression of genes in root galls of grapevines infected by Phylloxera (*Daktulosphaira vitifoliae* Fitch)

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As a worldwide pest grape phylloxera (*Daktulosphaira vitifoliae* Fitch) threatens viticulture. The compatible phylloxera – grapevine (*Vitis* spp.) interaction has a dramatic impact on the host plant response and in reverse will select for adapted phylloxera biotypes to interact. The understanding of these interactions will improve our knowledge of the resistance of rootstocks, evolution of phylloxera biotypes, insect-induced plant responses including the carbohydrate allocation of the host plant and enhances the knowledge base on future plant protection strategies against phylloxera. The aim of the project was to analyze the transcriptome of the phylloxera induced root gall (nodosity) to elucidate significant plant-based responses mainly affecting the carbohydrate metabolism and sink activity of the gall.

The experimental approaches covered gene expression analyses combined with functional tests. As a model, the partially resistant *Vitis* genotype Teleki 5C was tested with a defined phylloxera single founder lineage. Uninfected root tips were compared with nodosities at different development stages and changes between these stages were studied by different methods: gene expression (microarray, qPCR analysis), starch content (using an enzymatic assay) and sugar content. Our results indicate that several pathways are significantly altered in nodosities. The sink activity is greatly enhanced through sucrose synthase activity, activity of expansin genes and the insect feeding activity by effectively tapping the symplast.

The results will impact the scientific field by providing a reference transcriptome of nodosities allowing comparable studies on the host-pest interaction in *Vitis* roots and also provides a modified gene chip to do so.

'Taking the strain' selecting the right rootstock to protect against endemic phylloxera strains

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In Australia to-date 83 genetically different grapevine phylloxera strains have been characterised which vary in their geospatial distribution on both ungrafted and grafted *Vitis vinifera* under field *conditions*. Damage levels on *V. vinifera* also differ. An assessment of phylloxera development and root response to phylloxera feeding on ten *Vitis* rootstocks has been made under both laboratory and glasshouse conditions using six selected genetically diverse strains of grape phylloxera. These strains were screened using both excised roots and whole plant bioassays, which allowed quantification of phylloxera development and damage to mature lignified roots, and to a lesser extent under field conditions. Based on these assessments rootstocks were classed as resistant, tolerant or susceptible. Genetically different phylloxera strains differed in population survival and development on both *V. vinifera* and grafted rootstock hybrids and in addition root damage levels differed between phylloxera genetic strains and rootstocks. A summary of 'genotype-specific' rootstock resistance ratings, based on laboratory and glasshouse screening conducted to-date, for phylloxera resistance are presented. The results highlight the need for further studies focusing on the genetics of both the host plant and the pest insect.

Advances in biology, ecology and control of *Xiphinema index*, the nematode vector of GFLV

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The ectoparasitic dagger nematode *Xiphinema index* specifically transmits *Grapevine fanleaf virus* (GFLV) which is responsible for a progressive degeneration of grapevines occurring in most vineyards worldwide. Since the year 2000, new data on the biology and ecology of the vector nematode and on the virus-nematode and plant-nematode interactions have contributed to draw new alternatives to chemicals for nematode control.

Molecular tools have allowed reliable nematode identification and new insights have been acquired into its genetic diversity at both the world and local scales and into its mode of reproduction. The long virus retention by the nematode in the bare soil combined with the location of the nematode in deep soil layers have highlighted the interest of both the development of nematode-resistant rootstocks as priority control alternative and the fundamental studies on molecular mechanisms of this specific virus retention.

Some Muscadine accessions are currently the most efficient resistance sources. An optimization of their use through the characterization of *Vitis x Muscadinia* F1 and BC1 material for spectrum, histological mechanisms, genetics and durability is in progress. In the perspective of an integrated control, the resistance strategy could be completed by fallow crops showing an antagonistic (direct or indirect) effect on nematode numbers between two successive grapevine plantings.

Structural and functional characterization of *Grapevine fanleaf virus* capsid determinants involved in the transmission by *Xiphinema index*

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Grapevine fanleaf virus (GFLV) and *Arabis mosaic virus* (ArMV), the main agents responsible for the fanleaf degeneration, are specifically transmitted by two ectoparasitic nematodes species, *Xiphinema index* and *X. diversicaudatum* respectively. Previous results showed that the transmission specificity maps to the capsid protein (CP). The objective of our study was to characterize the determinant of GFLV CP involved in this transmission specificity. To address this issue, an approach combining reverse genetics, X-ray crystallography and electron cryomicroscopy was performed. The structural study of the GFLV CP shows that the virus has an icosahedral architecture composed of 60 subunits organized according to a pseudo $T = 3$ symmetry. Comparisons of the atomic structures of a wild-type and a weakly transmitted GFLV variant, revealed a positively charged cavity at the surface of the virus particle. This pocket consists of three loops within the B domain of the CP. The function in GFLV transmission of two of these highly exposed loops was demonstrated by a series of site directed mutagenesis. Altogether, our results give a first structural insight into the molecular mechanism needed for the specific binding of a plant virus to its retention sites within the feeding apparatus of the nematode vector.

Nemadex Alain Bouquet, a rootstock that delays *Grapevine fanleaf virus* (GFLV) infections

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In tribute to Alain Bouquet

Since the 70's, through clonal selection, French vine growers have access to plant material which is free of the most problematic grape virus diseases. However, because of infection after plantation, GFLV is still a big issue in the French vineyards. In some producing areas, vine growers cannot afford a ten-year fallow between two plantations for economical reasons. Using Nemadex Alain Bouquet (Nemadex AB) could shorten that duration.

Nemadex AB, registered in the Official French Catalogue in 2011, is a cross between the F1 hybrid VMH 8771 (*Vitis vinifera* 'Cabernet Sauvignon x Alicante Henri Bouschet' and *Muscadinia rotundifolia* 'NC 184-4') and 140 Ruggeri.

Under controlled conditions, Nemadex AB slows down the multiplication of the nematode vector *Xiphinema index*. As a consequence, we observed in two long-term field experiments that this rootstock delayed significantly over time the infection by GFLV, in comparison to SO4 or 110 R.

Its intrinsic characteristics are: high resistance to Phylloxera, low production of cuttings, good aptitude for herbaceous and woody grafting. Conferred vigour to the scion varieties is low, similar to Riparia Gloire de Montpellier. Yields are low to medium. Nemadex AB is not adapted to calcareous conditions and is susceptible to drought.

Additional field experiments are now in progress i) in infested soils (10 locations including both new plantations and replacements of individual vines) and ii) in non-infested soils from various areas of production of wine and table grapes (19 locations). This experimental network was set up to define the agronomical conditions for using this rootstock in the French vineyards.

The first results from this national network have brought enough information to advise nurserists and producers. Nemadex AB has to be limited to infested vineyards where vines have been devitalized before being pulled out, and planted after at least 12 months of fallow.

Keynote lecture, session 5

Roots and rootstocks ecophysiology: mechanisms to cope with a changing environment

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Rootstocks are imperative for grapegrowing in those regions where phylloxera is present. Nevertheless, rootstocks have many more functions. They are the root anchorage and structural support of vines in the ground, exploring soil searching for water and nutrients, which are absorbed and transported to the canopy. Moreover, they act as a barrier against other soil pathogens and supply mechanisms to cope with abiotic stresses such as drought, salinity, anoxia, pH, etc. Besides, rootstocks play other important physiological roles such as hormonal production, water status regulation, or reserve accumulation. Lately it has been described that not only rootstocks can influence on scion performance in terms of growth, yield, quality or regulation, but also scions have an influence on rootstock performance.

In the context of the global change, in most parts of the world, drought is going to be one of the most important abiotic stresses due to climate change, mainly under Mediterranean conditions that grapevines must cope with in the near future, particularly where irrigation water can be unavailable or very restricted. Knowing the genetic bases and the ecophysiological mechanisms related to the avoidance and the tolerance to water stresses are key to develop sustainable viticulture in the future. Recent advances in root growth and distribution, hydraulic properties, genetic basis and the interaction between rootstock and scion are reviewed and discussed.

Acknowledgments: This work has been partially funded by the Spanish Ministry of Economy and Competitiveness project AGL2011-30408-C04-02 “Rootstock contribution to water and carbon balances: structural and dynamic components and relation with grape and wine yield and quality”.

Effect of different rootstocks on photosynthetic and nutritional response of grapevines cv. Sultanina under flooding stress

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The effect of flooding on young grapevine plants grafted on two rootstocks: Harmony and Freedom was evaluated. Grafted and non-grafted plants were grown for 6 weeks in pots under flooding and normal condition. Flooding condition was obtained by keeping the water 3 cm above the soil. Oxygen diffusion rates measurements was 0,2 µg cm^{-2} min^{-1} (hypoxia) in flooding treatment, while in the control was 4,6 µg cm^{-2} min^{-1}.

At the beginning of flooding, plants grafted on Harmony showed a reduction in net CO_2 assimilation (*A*). This reduction reached a minimum after at 8 days of flooding. By contrast, the plant grafted on Freedom did not show any reduction on *A* until 17 days of flooding. This indicates that this last rootstock confer to the scion a tolerance to hypoxia. On the other hand, the reduction in *A* was well correlated with dry matter production in both rootstocks. In fact, Harmony grafted plants presented the highest reduction (54%) in dry matter accumulation, while Freedom grafted plants presented 36% of reduction. As the reduction in *A* was not correlated with stomatal conductance, we discuss the effects of hypoxia on processes as chlorophyll degradation, changes in photochemical parameter and reduction on mineral nutrition.

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The mineral composition of grafted grapevines depends on rootstock genotype and nitrogen supply

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Rootstocks are widely used in viticulture and are known to confer different degrees of vigour to the scion. Despite this knowledge, the determinism of the scion vigour conferred by the rootstock is poorly understood. We hypothesize that conferred vigour is linked to differences in the capacity of rootstocks to absorb and allocate minerals to the scion. Minerals accumulation in the plant is controlled by several physiological processes that occur in the root and the shoot of the plant. Alteration of one of these processes can potentially alter the uptake of one or several elements. Among all the minerals supplied to the scion by the rootstock, Nitrogen is a major actor of rootstock conferred vigour as N is well known for its role as both a nutrient and signal that regulates root:shoot ratio. Recent studies highlighted strong relationships between N and other minerals uptake processes, leading us to wonder if a modification of the concentration of N in the soil can affect other elements accumulation and growth of the grafted plant. To test this hypothesis, an experiment was set up in pots. A unique scion, Cabernet Sauvignon, was grafted upon two rootstock genotypes: Riparia Gloire de Montpellier that confers a low vigour and 1103 Paulsen conferring a higher vigour to the scion. Plants were watered with 3 nutritive solutions differing in their nitrate concentration. Roots, stems and leaves concentration in 13 macro and micro-elements were measured 60 days after the beginning of N treatments. It appears that rootstock genotype affects plant minerals allocation between the shoot and the root and modifies not only N but also other minerals profile of the scion in response to N supply.

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Responses of the new grapevine rootstock M4 to drought: a biochemical and physiological characterization

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In this study a new selected genotype, named M4, was compared with the commercial rootstock 101.14, analyzing the responses evoked by water stress (WS) in both the shoot and the root organs.The effects of progressive WS were studied in controlled environmental conditions on plants grown in pots.Sixty-six plants for each genotype were randomized to obtain two pools that were used as i) control, plants that were maintained at the 80% of the soil field capacity; ii) water stress, plants in which water supply was progressively reduced until the 30% of the field capacity. The effects of WS was studied for an experiment period of 10 days.Shoot growth, plant water status and leaf photosynthetic parameters were measured. Moreover, the levels of sugars, amino acids and total proteins as well as the contents of the more abundant ions were determined. M4 genotype resulted to have a greater capacity to tolerate WS, maintaining photosynthetic activity also under severe stress conditions. In this genotype, the root system appeared to play a central role to sustain biochemical and physiological responses evoked by WS, as suggested by the greater capacity to adequate inorganic and organic osmolytes in this organ. The experiments conducted in grafted plants, in which Cabernet was the used as scion, confirmed the good performances of M4.This study produced a first characterization of the new putative rootstock M4, in which the greater tolerance to WS, resulted strictly related to root integrity/functionality, so confirming that these aspects have to be considered in the further selection programs.

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Drought-sensitive and drought-tolerant grape rootstocks differ in root xylem development and hydraulic conductivity

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In order to control soil-borne diseases, shoot vigor and drought tolerance, *Vitis vinifera* L. is grafted onto American rootstocks. For grafted plants, the rootstock genotype is responsible for water acquisition and transport to the scion, and plays the main role in the sensing of soil water content. It is well known that abscisic acid acts as a chemical signal, transmitted from the root to the shoot, which controls stomata aperture. New discoveries show that a hydraulic signal is also involved in drought shoot response. This mechanism is dependent on hydraulic conductivity mediated mainly by xylem anatomical structure.

Four different soil moistures were applied for two weeks in one-year-old potted plants of Cabernet Sauvignon (CS) grafted into a drought-sensitive, Riparia Gloire de Montpellier (RGM), and a drought-tolerant, 110-Richter (110R) rootstock. Adventitious root conductivity was on average 3-fold lower for 110R than for RGM and was not influenced by soil water deficit. Anatomical observations of root tips, collected at 25 cm distance from the trunk, showed significantly more and smaller xylem vessels in 110R than in RGM with the same total conduit area. Despite no significant difference in conductivity, we observed a shift in xylem conduit diameter class in moderate stress, especially for 110R. We used the Hagen-Poiseuille law to calculate the theoretical hydraulic conductivity by diameter class and showed that a larger proportion of conductivity is mediated by small vessels under stress for 110R.

We conclude that the drought tolerance conferred by 110R rootstock seems to have two mechanisms associated to hydraulic conductivity: a general lower conductivity, which limits water depletion (maybe controlled by aquaporins activity), and a reduction of vessels size to limit and prevent cavitations induced by drought.

The effect of drought stress on photosynthetic traits and certain gene expression of some Iranian grapevine candidate rootstocks

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Grapevine rootstocks are able to increase cultivar adaptation to different climates, soil types and a variety of adverse conditions. The main objective of this study was, studying the photosynthetic traits and gene expression in candidate drought tolerant rootstock cultivars. Three selected Iranian candidate rootstocks (Yaquti, Askari and Rotabi) as well as Sultana (control) were exposed to moderate (-1 MPa), severe (-1.5MPa) and very severe (>-1.5MPa) drought stress. Results showed that drought stress and cultivar had significant effect on photosynthesis rate, internal concentration of CO_2, chlorophyll A as well as gene expression of Rubisco activase. Drought stress reduced photosynthesis rate, internal concentration of CO_2 and chlorophyll A compared to control. Cultivars showed two distinct behaviors under stress levels. Yaquti and Rotabi as tolerant cultivars showed higher photosynthesis rate than Askari and Sultana as semi tolerant and sensitive cultivars. The concentration of CO_2 was more stable in Yaquti and Rotabi under stress levels than Askari and Sultana cultivars. Based on results, the relative expression of Rubisco activase gene increased rapidly in tolerant cultivars (Rotabi and Yaquti) after exposing to moderate stress, but these reaction occurred later in semi tolerant and sensitive cultivars (Askari and Sultana) after severe stress with significant increase in Askari. Increase of Rubisco activase resulted in more photosynthesis rate under stress conditions and reduced negative effects of low stomata conductivity. Time and quality of responses to different levels of drought stress could discriminate tolerant cultivars (Yaquti and Rotabi) from semi tolerant (Askari) and sensitive (Sultana) cultivars, especially in gene expression level.

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Expression differences induced by drought on conserved and novel miRNAs in grapevine

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Plant microRNAs (miRNAs) play a pivotal role in regulating gene expression not only during developmental and physiological processes, but also in response to biotic and abiotic stresses, such as drought.

In this study, we investigated the effects of water stress on miRNA abundance, focusing on expression changes occurring in leaf and root tissues of two grapevine genotypes, *Vitis vinifera* cv. Cabernet Sauvignon and the M4 rootstock (*Vitis vinifera* X *Vitis berlandieri*).

Potted grapevines were subjected to a water stress treatment lasting 12 days in summer 2012. During the treatment, physiological parameters (leaf water potential and leaf gas exchanges) were daily monitored on both treated (WS) and irrigated (IRR) plants, in order to assess the moment of maximum stress optimal for sampling ($g_s < 0.05$ mol H_2O m^{-2} s^{-1} and $\Psi_{leaf} \sim -1.4$ MPa).

Total and low molecular weight RNA (LMW RNA) were extracted in triplicate from WS and IRR leaves and roots of the two varieties. RNA samples were thus used both to prepare cDNA libraries, which were sequenced by SOLiD platform, and to carry out expression experiments by RT-qPCR assay.

Sequencing results identified 105 miRNA sequences: 77 conserved miRNAs and 28 putative novel miRNAs. The conserved miRNAs known to be involved in water stress response, such as miR159, miR393, miR156, were also analyzed by RT-qPCR and expression changes of their target transcripts were studied in the same samples, in order to gain evidence of miRNA effect on their abundance.

Among novel miRNAs, some showed significant expression differences upon WS in at least one of the two considered genotypes, whereas others seemed exclusively activated in either CS or M4 independently of the treatment. Further analyses are currently underway on target transcripts to complete the characterization of the novel miRNAs resulting from sequencing and already analyzed by RT-qPCR.

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Molecular mechanisms of grapevine rootstock adaptation to drought

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A multidisciplinary approach (ecophysiology/genomic/genetic) that uses the most recent facilities and technologies was undertaken for a comprehensive analysis of grapevine response and adaptation to water-limiting conditions. Using a phenotyping platform that allows the progressive application of well-defined levels of water deficit, drought experiments have been conducted with four rootstock/scion combinations between the two *Vitis vinifera* varieties Syrah and Grenache and the two rootstocks Riparia Gloire de Montpellier and 110 Richter, exhibiting contrasted sensitivity and response to water deficit. Fine ecophysiological measurements were performed and biological samples from root were collected and subjected to global transcriptome analyses using Nimblegen grape whole-genome expression arrays. In parallel, phenotyping experiments were conducted in control and water deficit conditions with an interspecific Cabernet Sauvignon x Riparia Gloire de Montpellier progeny used as rootstock and Cabernet Sauvignon as a single scion to identify zones in the genome which control the different responses to water deficit. We are currently in the process of analyzing these important data sets to understand the phenotypic plasticity of grapevine in response to water deficit and to identify candidate genes involves in the root response to water deficit. The resultst of this study will contribute to the identification of molecular markers for drought tolerance that might be used for rootstock selection and improvement.

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Oral presentation, session 5

Drought differently affects the root and shoot metabolome in two grapevine rootstocks having different tolerance to water deficiency

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In the field of Mediterranean viticulture, the need of facing the new unfavorable condition related to the climate change made emerge the growing request of new genotypes able to cope with situation of low water availability. In order to fulfill this aim, it is mandatory to deepen the knowledge on the mechanisms allowing the tolerance to drought and to pinpoint in a perennial plant such as *Vitis*, what are the weights of leaves and roots and how they cross-talk. In this study, we investigated the main changes occurring at the metabolomic level in the leaves and roots of in-pots-grown plants in response to a progressive water stress reaching the 30% of the field capacity. The experiments were conducted on two rootstock genotypes, 101.14 and M4, characterized by a different tolerance capacity, with the M4 genotype being the more resistant. The metabolomic analyses were conducted by means of the Gas Chromatography – Mass Spectrometry technique (GC-MS) on the trimethylsylyl-derivatized metabolites of the polar fraction. Through the following batch-quantification, taking into account the deconvoluted spectra to match against the Golm Metabolome Database, it was possible to identify around 120 metabolites in both organs. The generated dataset was analyzed through multivariate statistical techniques, such as PCA and PLS-DA, which showed that for the tolerant genotype M4, especially in the roots, the response to water stress was more marked and involved many compounds known to play a role in the osmotic component of the tolerance response to the stress (i.e. proline, beta-alanine, myo-inositol, mannitol, sucrose). At the same time, the higher sensibility of the GC-MS when working in the Selected Ion Monitoring mode (SIM) allowed quantifying the levels of ABA on the samples which being the main signal of the stress through the plant. I also allowed evaluating, in addition to the peculiarity of the M4 tolerance to drought, the relevance of root and leaves in the evolution of these mechanisms of response.

Acknowledgments: Work was supported by SERRES-AGER project, grant n° 2010-2105.

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Ecophysiological responses to stress of the M4 rootstock

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Adaptation to global climate change is increasingly required for quality viticulture, and rootstocks may represent a tool to manage grape responses to environmental stresses. Most grape rootstocks have been selected long before the occurrence of global climate warming and do not fulfill adequately these aims. Grapevine is a highly flexible species as concerns responses to abiotic stress, and *V. vinifera* genotypes follow different patterns of water conductance control in response to drought. It is this conceivable that such differences may be also found within the rootstock genetic pool. The M series of grapevine rootstocks was obtained at the University of Milan within a breeding program started in the 1980s. These rootstocks support scion berry quality in conditions of both water availability and water stress. Here we report an ecophysiological characterization of pot-grown plants of the M4 rootstock (41B x Resseguier n. 1: vinifera x berlandieri) exposed to drought stress. When Cabernet sauvignon (CS) was grafted on itself or on 101-14, it showed very similar stomatal conductance (gs) / leaf water potential relationships, suggesting that stomatal conductance was mostly controlled by the scion genotype. When CS was grafted on M4, however, stomata closed at a faster rate than in the other graft combinations thus avoiding the attainment of very low leaf water potential. The influence of M4 on stomatal closure was further confirmed when auto-grafted CS and M4 were compared: While the first closed stomata progressively upon water stress, the latter had a low and constant gs. At severe drought stress levels (<-1MPa), the combinations carrying M4 as rootstocks had the lowest gs. The behavior of M4 can be explained by a stronger root-derived hydraulic or non-hydraulic signal, or by intrinsic lower root conductivity of this genotype. To elucidate this aspect, measurements of hydraulic conductivity are currently underway. This characteristic can represent an interesting viticultural feature as this rootstock may avoid excess water loss upon drought.

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Rootstock genotype and nitrate supply modify grapevine tissues metabolic content

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Grapevine is widely cultivated as a grafted plant, composed of a rootstock that is responsible for nutrient and water supply and a scion that performs photosynthesis. The scion and the rootstock have to communicate to maintain growth in response to environmental variations such as N supply. Despite their importance in plant growth, physiological mechanisms and signals exchanged between the rootstock and the scion are poorly understood. Owing to its role as a nutrient and signal, nitrate might be a major actor of this coordination. Nitrate plays a central role in plant: in the shoot, photosynthesis is strongly dependent on nitrogen, and C and N metabolisms are closely interconnected. Plant nitrogen status such as amino acids, controls nitrate assimilation. Metabolites are principal factors of plant development, regulate genes expression and contribute to plant homeostasis.

We make the assumption that metabolites could characterize a rootstock/scion combination and allow to a better understanding of differential growth in response to nitrogen variation.

In order to test our hypothesis, we set up a pot experiment. Cabernet Sauvignon (CS) was grafted upon two rootstock genotypes, 1103P conferring a high vigour and RGM a low vigour to the scion. Plants were irrigated with 3 nutrient solutions differing in their nitrate concentrations: 0.8mM, 1.6mM and 2.45mM.

To investigate biochemical content of grafted grapevines, several metabolites such as amino acids, proteins, inorganic phosphate, nitrate or malate were measured in leaves roots, trunks, stems and leaves.

Our results highlight some metabolic key factors that could explain shoot: root biomass accumulation and contribute to design a conceptual grapevine model.

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Rootstock breeding - between site adaptation and abiotic stress tolerance

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The introduction of phylloxera into Europe in the middle of the 19th century resulted in a lasting change in viticulture. It not only led to an economic disaster for individual growers and whole regions, it also marked the end of own-rooted vines. With the overcoming of the phylloxera disaster by using tolerant or resistant rootstocks, instead of own-rooted plants, we now have two quite different genotypes combined in one plant, one that forms the shoot and grapes, conducts photosynthesis and transpiration and another one that grows roots and provides the vine with water and nutrients. This combination of two genotypes also provides chances to overcome abiotic stress caused by soil factors and gain a better site adaptation independent of the scion, something which is not possible in ungrafted vines. Certainly, the first abiotic stress to overcome was lime-induced iron chlorosis, actually caused by using American rootstocks. As most North American soils are rather acidic, most wild *Vitis* species are adapted to this kind of soil and the first rootstock candidates suffered from chlorosis on the predominant calcareous soils of Europe. The search for some more tolerant genotypes in species like *V. riparia* resulted in rootstocks like Gloire de Montpellier or Riparia #1 Geisenheim, which tolerate some lime, but not high amounts. Crosses of American species with *V. vinifera* cultivars showed medium to good lime tolerance, but low tolerance to phylloxera. The introduction of *Vitis berlandieri* into rootstock breeding led to a better lime tolerance combined with adequate phylloxera resistance. Berlandieri x Rupestris and Berlandieri x Riparia crosses conquered the world: less than ten rootstocks are grown on more than ¾ of vineyards worldwide. This proves their versatility, but also raises the question of whether ten rootstocks can be the optimum solution for all the sites they have been planted on. A larger range of rootstocks could certainly provide more well-adapted solutions for a number of soils and sites. The problem with adaptation studies is that a good adaptation of a rootstock at a particular site cannot be attributed to one single factor, like abiotic stress, but is caused by several factors. Consequently, site adaptation studies have been until today, and will most likely remain so for some time, very much empirical studies, and results may only be transferable to a certain degree to other, similar looking locations.

Among abiotic stress factors future challenges are still lime-induced iron chlorosis but also drought and, in irrigated arid regions, salinity. Chlorosis is still a problem at a number of sites and the use of a larger range of *V. berlandieri* germplasm might provide the material needed to overcome it. Drought tolerance can be attributed to a number of mechanisms, e.g. a deep root system, a better water absorptive capacity or a more economic water use efficiency. A mechanism that works at one site might fail at another. A deep root system is certainly an advantage in a Mediterranean climate on deep soils, where winter rain can be stored in the soil profile and ensures water supply during summer, but a deep root system may be a disadvantage in an irrigation situation, where surface soil contains more water than subsoil and subsolum. Consequently, breeding for drought tolerance has to address different drought tolerance mechanisms as well as site characteristics and vineyard management.

Grape rootstock breeding program of the Georgikon Faculty

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Grapevine rootstock breeding commenced in the 1970s at the Georgikon Faculty of Pannon University by utilizing genetic resources from a collection of more than 110 rootstock genotypes. The main aim was to develop highly lime tolerant rootstocks, because about half of Hungary's grape growing regions suffer from lime-induced chlorosis. The lime content in some of these soils even precludes the use of the Teleki rootstocks. Ease of propagation was also selected for – Georgikon 28 is one of the main results of these efforts. This rootstock has an extremely high lime tolerance, is easy to propagate, but because of its *V. vinifera* genetic background its phylloxera resistance needs to be strengthened. As the program progressed drought tolerance was also added to the suite of breeding goals. Seedling populations were made in the mid-1990s after parents had been characterized and selected; their evaluation continues. The drought tolerance of Georgikon 28 x Börner seedlings will be discussed in detail. Field performance of this seedling populations was evaluated in a rootstock block and in a vineyard planting using standard rootstock cultivars as controls in 2011 and in 2012. Both years were extremely dry during the growing season. Harvested cane production was used as an easy and efficient indicator of productivity to select promising candidates among the seedlings. The performance of grafted plants was judged by the fruit quality (number of clusters, berry size, yield per stock, berry weight, sugar content, acid content) and cane production. The most promising genotypes produced higher yield, with better quality in 2012 than scions grafted on 1103 Paulsen, Fercal or Georgikon 28 and their cane production were satisfactory as well.

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Drought tolerance in grapevine rootstocks: an association genetics approach

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Water deficit is widely recognized as one of the major constraint in the Mediterranean and semi-arid regions where a large part of the world_premium wines are produced. Since the introduction of new varieties in the wine industry is not so straightforward, breeding grapevine rootstocks for tolerance to drought is becoming a key strategy for the future. A Genome Wide Association Study (GWAS) and a Candidate Gene (CG) approach were used to investigate the genetic basis of drought response mechanisms in an ad hoc core-collection consisting of 96 different genotypes of Vitis spp. and hybrids, selected to ensure the maximum genetic variability of a larger population of commercial, germplasm and new bred rootstocks. The physiological and growth responses to water deficit of more than 500 one-year old potted cuttings were evaluated over 30 days in semi-controlled conditions. For each genotype, three well-watered control plants were mantained at 90% of the Field Capacity (FC) determined by gravimetric method and three plants were subjected to water stress. After 7 days, water deficit was gradually established to reach first a moderate stable water deficit (50% FC for 7 days) and then a more severe and stable water deficit (30% FC for 7 days). Finally, stressed plants were fully irrigated to evaluate the level of plant recovery. Stomatal conductance was correlated with plant growth evaluation (leaves and shoots biometric measures) to define different response classes and thermal infrared imaging was implemented for the first time as a valuable remote sensing tool for high-throughput phenotyping in a GWAS experiment.

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Analysis of the control of shoot growth by the rootstock under drought conditions by an integrated approach of quantitative genetics and ecophysiology

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Water is the main limiting factor for yield in viticulture. Vine water status also strongly impacts grape quality. The objective of this work is to analyze the genetic determinism of shoot growth induced by the rootstock under drought conditions.

A mapping pedigree consisting of 138 F1 individuals, derived from the inter-specific cross of *V. vinifera* Cabernet Sauvignon × *V. riparia* Gloire de Montpellier, was used as root-stock. Cabernet Sauvignon was the scion grafted on each genotype of this population. The experiment was carried out in pot, in a greenhouse. Water deficit intensity was evaluated daily by weighing each pot individually with a 150 scale platform. Irrigation was applied daily in order to keep all the pots at the same water content. After 10 days without any stress, a progressive water limitation was applied for 10 days, then followed by a stable water deficit stress for 15 days. Pruning weight, root and aerial dry weight were recorded during three years.

Growth curves in relation to growing degrees days (summation of daily temperatures minus a 10°C base temperature since the beginning of the experiment) in each pot were established and mathematically fitted. A large variability was observed within the studied population. A parameter of growth curve equations and the area under the curves for control and drought conditions were used as plasticity traits of growth regulation. QTL analysis was then performed for all the traits already cited. Stable QTLs over the three years were detected on 9 linkage groups. These results demonstrate that growth regulation of the scion by the rootstock is determined genetically. Common QTLs were identified for a same trait in different water status conditions

This is the first genetic quantitative study taking into account the growth plasticity to assess water deficit tolerance.

Vitis berlandieri – A Potential Partner for Rootstock Breeding

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The invasion of phylloxera during the 19th century nearly led to the collapse of viticulture in Europe. This could only be prevented by the introduction of phylloxera tolerant American wild grapevine species, used henceforth as rootstocks for grapevine grafting. While phylloxera control is the major aim of rootstock breeding, a wide ranged suitability to different abiotic environmental stress factors, particularly with regards to soil adaptation, is of great importance as well. The majority of the European vineyards, typically characterized by higher lime contents, are not compatible to most of the American wild *Vitis* species. The American species *Vitis berlandieri* is very well adapted to limey soils, and thus makes it an interesting partner for rootstock breeding.

In the beginnings of rootstock breeding only a few *V. berlandieri* individuals had been available. However, mostly all of the today used rootstock varieties comprise only a very small fraction of the *V. berlandieri's* genetic information.

To study the variability of *Vitis berlandieri*, seeds were collected in their natural habitat in Texas in the year 2005 and have been germinated and planted in an observation block at Geisenheim. This population of nearly 3.500 individuals should very well suited to represent a more complete picture of the *V. berlandieri's* gene pool.

In our ongoing first project, the ampelographic and genetic characteristics of these *Vitis berlandieri* individuals are being evaluated continuously. Currently, 2.122 seedlings of 21 different mother plants are being monitored by using 12 ampelographic characteristics and 6 microsatellite markers.

First results point out that the offspring of different accessions are showing various distinctive characteristical features, either within one accession, or between different accessions. This indicates a larger genetic and ampelographic variability of *V. berlandieri* than primarily assumed, and it offers a more ample spectrum for future rootstock breeding programs.

SERRES: an Italian project to develop and to validate markers for the selection of new grape rootstocks resistant to abiotic stresses

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The selection of resistant rootstocks represents a crucial factor for the development of future sustainable agricultural models (moderate irrigation and fertilization and recovery of marginal soils) and for assuring optimal maturation profiles of grapes. The selection of genotypes able to cope with stress conditions requires a in depth knowledge of the molecular, biochemical and physiological bases of stress resistance. Aim of the SERRES project is the study of the responses of newly established rootstocks to abiotic stresses in both controlled and open field conditions, taking into account different soil-climatic environments. Genotypes previously selected for resistance to drought, salinity and calcareous soils are characterized at the genetic and physiological levels, agricultural performances and berry quality. In order to identify transcripts, proteins and metabolites involved in the resistance to abiotic stresses and therefore useful as biomarkers, susceptible and resistant rootstocks are compared by the use of high-throughput transcriptomic, proteomic and metabolomic approaches. In addition, expression data are supported by a profitable multivariate statistical integration. Some efforts are devoted to the study of rootstock/scion communication, which implies analysis aimed to the definition of the possible changes in chemical composition (phytoregulators, peptides and small RNAs) of the xylem sap during stress syndromes. The parallel work of selection and definition of new rootstocks with improved performance for drought and saline conditions, and calcareous soils will hopefully be enriched by the identification of biomarkers for abiotic stresses that will reinforce the choices and streamline the process of genotype selection.

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During the Symposium, we will visit one Chateau “Grand Cru Classé” and one nursery. We thank the owners for their welcome.

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During the Phylloxera symposium, you will be able to taste wines which have been kindly offered by the following Chateaux or grower associations. We are deeply grateful for their support.

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