SEVENTH FRAMEWORK EUROPEAN PROGRAMME

SharCo
Sharka Containment

Project summary

The sharka disease is a worldwide serious problem with a severe impact on the productivity and fruit quality of *Prunus* species. It is caused by Plum pox virus (PPV), a virus against which no chemical or biological curative treatment is available. The current short-term solution is the eradication of infected trees and the plantation of virus-free material. SharCo is aimed at providing the EU with tools such as marker-assisted selection to speed up the production of PPV (*Plum pox virus)* resistant plant materials, guidelines, early warning systems and decision-support system in order to anticipate further PPV outbreaks.
For that purpose, the project will, in the field of **epidemiology**, identify driving factors of PPV spread and diversification and develop novel and highthrough-put detection systems warning sharka outbreaks. In the field of **genetics**, it will provide molecular markers for the implementation of marker assisted selection of PPV resistant fruit varieties. In the field of **biology**, SharCo partners will assess innovative biotechnological approaches to broaden resistance to PPV in different fruit tree species. Finally, in order to develop a PPV outbreak **management**, the project will elaborate i) guidelines for endusers and policy makers concerning cultivation and risk management, ii) an early warning system coupled with a decision support system. All knowledge and tools developed by the project will be widely disseminated all over Europe with a special attention paid to PPV endemic countries.

- **The Coordinator**

  The Coordinator of the project is **INRA**, France, represented by **Dr. Véronique Decroocq**..

- **The Executive Committee**
  - Dr. Véronique Decroocq, INRA, project coordinator, France
  - Dr. Miroslav Glasa, Institute of Virology, Bratislava, Slovakia
  - Dr. Gérard Labonne , INRA, France
  - Dr. Mariano Cambra, IVIA, Spain
  - Dr. Maria-Luisa Badenes, IVIA, Spain
  - Dr. Juan-Antonio Garcia, CSIC-CNB, Spain
  - Dr. Donato Boscia, CNR-IVV, Italy
  - Dr. Lech Michalzcuk, ISK, Poland

Press Conference speakers

Prof. Mariano Cambra, research plant virologist and epidemiologist since 1977, expert in the design of serological and molecular diagnostic methods. Centre of Plant Protection and Biotechnology Virology and Immunology.

- **Organisation: Instituto Valenciano de Investigaciones Agrarias**

IVIA is a public research institution dedicated to agricultural sciences. The Institution is organized in seven research units. In the SharCo project two research units are involved: Plant Protection and Biotechnology (PPB) and Fruit Production (FP). The Department of Virology and Immunology from the PPB Centre is the Reference Laboratory of the Spanish Ministry of Agriculture for woody plant viruses. It has 30-years research experience on detection and characterisation of plant viruses infecting woody species. The Department of Fruit Breeding from FP is in charge of breeding stone fruits resistant to PPV and has expertise in molecular genetics. Both research units are leading the applied research and management of sharka in Spain and have participated in international projects, expert-groups, cooperation and consultancies related to this topic.

Dr Miroslav GLASA, scientist specialised in molecular biology, pathogen diagnosis, plant pathology and epidemiology of fruit tree viruses, leader of several national projects

- **Organisation: Institute of Virology, Slovak Academy of Sciences**

The Department of Plant Virology, founded in 1953, is a part of the Institute of Virology, a public basic research institute of the Slovak Academy of Sciences,. It is the only laboratory dealing with the basic research in the field of plant virology in Slovakia. The main current topics include the study of molecular variability, epidemiology and detection of potyviruses, especially Plum pox virus. Several national and international bilateral projects have been succesfully managed by the research team in the last years. The Department has a close contact to agricultural research institutes, fruit breeders and nurseries.

Dr. Véronique DECROOCQ, plant molecular biologist and geneticist, specialised in the understanding of the interactions of PPV with its hosts, *Prunus* species and *Arabidopsis thaliana*. She has been coordinating regional, national and bilateral international projects focusing on resistance to sharka disease.

- **Organisation: Institut National de la Recherche Agronomique**

INRA is a public research institute dedicated to agricultural, environment and food sciences with multidisciplinary expertise in plant and animal disciplines. INRA undertakes most of French research on sharka, from virus characterisation to stone fruit tree breeding programmes. INRA is represented in SharCo by two divisions addressing the two main areas of the project: virology and molecular genetics.

▪ **Other INRA researchers involved in SharCo :**

- INRA's Genomics and Pathogenic Properties Development joint research unit (GDPP), INRA Bordeaux

  Thierry CANDRESSE: molecular virologist specialised in the study of viral diversity and in the identification of new viruses. **Michel RAVELONANDRO**: virologist specialised in molecular virology and plant biotechnology for resistence to virus.

- INRA's Biology and Genetic of Plant/Microbe Interactions for Integrated Protection (BGPI) joint research unit, INRA Montpellier.

  Gérard LABONNE: Specialized on virus vectors (aphids), **Sylvie DALLOT**: plant virologist expert in *Plum pox virus* epidemiology, **Gaël THEBAUD**: Epidemiologist with modelling and simulation skills.

- INRA, Fruits and Legumes Genetics and Breeding unit (GAFL), INRA Avignon.

  J-M AUDERGON is responsible of the apricot breeding programme at INRA, he assumes the animation of the European Apricot working group. **Thierry PASCAL** is responsible of the peach breeding programme at INRA. **Patrick LAMBERT** is in charge of the *Prunus* genomic plateform comprising marker assisted selection in apricot and peach.

Concept and objectives

Concept

The concept of SharCo is to combine prophylactic and genetic solutions to prevent or limit the spread of the sharka disease (caused by PPV). The project scope covers the entire chain from planting materiel (seedlings, scions…) production to orchard management. It addresses all concerned stakeholders, breeders, nurserymen, fruit producers, and plant protection services with relevant outcomes including respectively resistant varieties, management guidelines, cultivation guidelines, optimised survey and detection methods and tools.

Strategic and scientific and technological objectives

The entry of countries from Central Europe and the Balkans into the European Union (EU) will considerably increase the threat caused by the sharka disease as these regions are known as endemic centres of PPV, making crucial the development of new ways to controll the spread of this virus and ti improve the resistance of cultivated crops to this disease.

The strategic objective of SharCo is to provide the EU with novel methods and tools to face this menace. On that purpose, the project will :

- In the field of epidemiology, develop new methods for monitoring and fighting the PPV spread by:
  - Identifying the driving factors of the PPV spread and diversification
  - Developing novel systems for detecting, assessing and warning sharka outbreaks
- In the field of biology, develop new genetic tools for breeding to contribute to improve the resistance of orchards cultivated plants, by:
  - Identifying molecular markers linked to resistance
  - Implementing marker assisted selection to speed up the breeding of resistant fruit trees.
  - Reinforcing plant resistance by combining new and complementary resistant genetic mechanisms
- In the field of agricultural management, help the end-users – notably but not exclusively breeders, nursery gardeners and fruit producers – to take advantage of the project outcomes by:
  - Establishing guidelines aimed at minimising the virus spread likelihood at the levels of nurseries and orchards
  - Delivering a risk management system designed to minimise the exposure resulting from the entry of new variants from accessing EU countries

Relevance to the objectives of the addressed call

SharCo is all about developing knowledge – PPV epidemiology and stone fruit genetics – with the aim to respond to the requirements of end-users (fruit producers and authorities in a broad sens). To the former, the project will propose new PPV resistant varieties, best practices for reducing contamination and spread of the disease in nurseries and orchards, and decision support system aimed at helping them face outbreaks in the most appropriate way. To the later ones, the project will submit guidelines for stone fruit cultivation and trade with policy makers and plant protection services as particular stakeholders.

Achievements of the project

Introduction to PPV dissemination

Transport of infected plant material is the main way by which sharka disease spreads over long distances. More locally, PPV is transmitted by aphid vectors in a non-persistent manner. It is a brief event (time-range of a few minutes) resulting from the probing behaviour shared by all aphid species. As for other non-persistently transmitted viruses, over twenty species of aphids, including many ones which are not hosted by *Prunus*, can act as vectors of the virus. It should be stressed that in this type of transmission the infectivity is lost through the aphid's moults. Thus, the winged aphids are non-viruliferous when they take off for their first long distance emigration flight even if they where on an infected plant (and unless they have probed on their host before departing, which is not reported as a normal behaviour). This has two consequences on the virus dissemination: (i) the aphid species hosted by *Prunus* trees are not necessarily the main vectors, and (ii) the dissemination is mainly due to aphid visitors, i.e. aphids that perform short range flights in their search for a suitable host plant. Consequently, long distance (more than 1000 meters away from the source of inoculum) dissemination by aphids is thought to be very unlikely. Due to these aphid transmission characteristics, it can be assumed that introduction of the disease into new territories away from existing infection loci is the result of the human trading activities, while local spreading can be due to both aphid activity and to agricultural practices. After its introduction, a key parameter for the spread of the virus is the quality of the inoculum sources available for aphids, the prevalence of the disease and the vector activity in a given ecological area.

- **Identification of the pathogen diversity and spreading driving forces**

After PPV introduction in an orchard, the installation of sharka disease varies depending on the identity of the PPV strain and the *Prunus* species involved. Epidemic properties at a larger scale are still under speculation. Moreover, the recent discovery of highly divergent PPV isolates in neighbouring EU countries (Moldavia, Turkey, Serbia) indicates that much remains to be unravelled about PPV distribution and diversity. This is a key issue in the elaboration of a PPV risk management system. In this context, the project will focus on the diversity of the virus and on the vector-virus-host interaction properties:

- Characterisation of the emergence of new PPV variants. This will be achieved through the development of high throughput typing tools for PPV isolates and the understanding of the occurrence and origin of new PPV variants. Models of PPV emergence will be proposed and discussed.

- Characterisation of PPV outbreak mechanisms through the mapping of PPV diversity and characterisation of epidemic properties. Models and different scenarios of PPV outbreak will be analysed and tested.

- **Identification of genes and loci controlling resistance to the sharka disease**

  Breeding for resistance to PPV encounters the usual problems of breeding perennial plants together with the difficult procedure of screening for PPV resistance that hinder the programmes. In SharCo, we will achieve the development of molecular tools that will be used to accelerate the selection of seedling progenies in the first steps of breeding programmes, on seedlings. In apricots, implementation of marker assisted selection for resistance to PPV will be achieved in the EU-member states, by transferring tools and knowledge to breeding stations in Romania, Bulgaria, Serbia and Turkey.

  However, although natural sources of resistance have been identified in some *Prunus* species, they are limited in number. In order to diversify the mechanisms of resistance to sharka disease, we will test new biotechnological strategies during the SharCo project. We will achieve the identification of *Prunus* plant proteins necessary to the virus and develop strategies (natural mutants, RNAi constructs) which interfere with PPV infection. Still-unknown host proteins restricting viral infection will be identified in the model plants *Arabidopsis thaliana* or *Nicotiana benthamiana* and will be transferred to *Prunus* to assess their impact of resistance. Complementary strategies will be evaluated, from the expression of recombinant antibodies or virus-derived constructs to the interference in glycosylation or phosphorylation of PPV proteins. The results expected from this part of SharCo will thus be the development of traditional and innovative approaches for a durable resistance to sharka disease.

- **Tools and strategies for outbreak containment**

  The spread of the non-persistently transmitted viruses is not affected by the use of pesticide treatments against aphids. In some instances, insecticides may increase, rather than suppress the spread of virus transmission by destructing the predators and parasitoids targeting aphids or by causing increased vector activity. Another control measure, the biological control of the vector, is also not an effective strategy to reduce the spread of non-persistently transmitted plant viruses. Control strategies other than conventional treatments have thus to be developed either by the plantation of resistant material (see above), the exclusive propagation and commercialisation of PPV-free nursery plants accurately checked by reliable diagnostic methods, or the use of innovative strategies reducing PPV incidence in nurseries and orchards. One objective of this project will be to develop standards for PPV testing and certification of nursery stock among EU countries. A portion of the research effort is directed at understanding how PPV spreads in nursery blocks, and from orchard to orchard within growing regions. This information will be used to develop of warning systems, to monitor the arrival of the first PPV-viruliferous aphids, of epidemiological models and of disease management systems. New strategies for PPV control in nurseries and orchards will be tested alone or in combination. This will be the case of oil treatments in nurseries. Mineral oils have been shown to interfere efficiently with non-persistently transmitted viruses by altering the transmission process. Two teams involved in the SharCo consortium have performed preliminary experiments with encouraging results. Successfull new strategies will be part of the PPV risk management system proposed at the end of the project.

- **Formulation of EU policies for sharka containment**

  The final practical achievement of SharCo will be therefore the formulation of guidelines for stone fruit tree cultivation and trade and the transfer of the knowledge and propositions to stakeholders: EU policy makers, plant protection services, extension personnel, nurserymen, fruit growers, and fruit industry representatives. Likewise, certification standards at the European level are needed to guarantee PPV-free production, transport, and sale of nursery stock and budwood; they will be discussed and improved with the help of the relevant agricultural extension services.

Synergy with other European initiatives :

✓Project FP6-**ENDURE** (http://www.endure-network.eu/accueil5.html). ENDURE network's objectives are to define research priorities on pest control and reduction at the European level, to gather knowledge, facilities and human resources according to the needs of agricultural extension, industry, and the non-profit sector and become a source of reference satisfying farmer needs and societal expectations. If appropriate, ENDURE members are expected to advise us on European agricultural extension, farmer needs and feedbacks on research on aphids control would be welcome. ENDURE coordinator belongs to the same organisation as SharCo coordinator, INRA. (Contact : **Pierre Ricci**, INRA Sophia-Antipolis).

✓Project FP7-**PRATIQUE**. This project is intended to develop sustainable, integrated plant health management strategies and enhance effective policy and decision-making by better assessing and managing plant health risks. Close collaboration with PRATIQUE network is expected to help us to develop sustainable PPV management strategies with integrated technical support for policy development in the case of emergency situations/PPV outbreaks. Coordinator : CSL, UK. (Contact in France : **Alain Roques**, INRA Orléans).

✓ Projetc **ResistVir** (coordinated by INRA Avignon) aims to improve coordination of research on genetic resistance to plant pathogenic viruses and vectors in European crops. The main long-term goal is to provide a vision of European excellence while linking the stakeholders involved in the development and sustainable use of genetic resistance as a tool to improve food quality and safety. This will ultimately result in new sources and mechanisms of sustainable resistance to plant viruses and vectors, being used in conventional and GM crops, and in decreased pesticide usage. Several research groups are involved both in SharCo and ResistVir. The ResistVir web site and database of European research activities on resistance to viruses in plants can be found at http://www.resistvir-db.org. (Contact : **Carole Caranta**, INRA Avignon).

17 partners in ShaCo consortium

Number	Name	Short name	Country
1.	Institut national de la recherche agronomique	INRA	France
2.	AgroBio institute	ABI	Bulgaria
3.	Universitatea de Stiinte Agronomice si Medicina Veterinara Bucuresti	USAMV	Romania
4.	Consejo Superior de Investigaciones Científicas	CSIC	Spain
5.	Consiglio Nazionale delle Ricerche-Istituto di Virologia Vegetale	CNR-IVV	Italy
6.	Instituto Valenciano de Investigaciones Agrarias	IVIA	Spain
7.	Instytut Sadownictwa i Kwiaciarstwa Skierniewice	ISK	Poland
8.	Mustafa Kemal University	MKU	Turkey
9.	Crop Research Institute	CRI	Czech Republic
10.	Technische Universität München	TUM	Germany
11.	SAVBA, Institute of Virology	SAVBA	Slovakia
12.	Fruit Research Institute	FRI	Serbia
13.	Agricultural Research Service	ARS	United States of America
14.	Fruit Growing Institute	FGI	Bulgaria
15.	Statiunea de Cercetare-Dezvoltare pentru Pomicultura Bistrita	SCDP	Romania
16.	Università degli Studi di Milano	UMIL	Italy
17.	Mendel University of Agriculture and Forestry	MUAF	Czech Republic