

**1<sup>st</sup> Phytoplasma  
Workshop**  
**I° Incontro sul Fitoplasma**  
**I Taller de Fitoplasmas**



**CENSA, La Habana, Cuba**

**18-20 de Mayo de 2005**



### Introduction

Researchers, technicians, extension workers and farmers from a number of countries including the United Kingdom, Italy, Israel, Bolivia and Cuba, participated in the First Phytoplasma Workshop, organised by the National Centre for Animal and Plant Health (CENSA), San José de Las Lajas, Havana, Cuba, from 18-20 May 2005.

Phytoplasmas are noncultivable prokaryotes causing many important diseases of food, fibre and ornamental crops around the world. In Cuba, diseases of sugarcane and papaya, two important crops have been recently associated with these plant pathogens. The main objectives of the workshop were focused on the updating of knowledge on phytoplasma research, the exchange of experiences for the improvement of disease management, and the promotion of collaborative work amongst participant institutions in order to strength the subject in the country.

The meeting consisted of two Round Tables: "Overview of the current situation of phytoplasmas in Cuba and world-wide" and "Phytoplasma Vectors with 6 keynote and 14 offered papers. Topics covered included: The current situation of phytoplasma diseases in Cuba; emerging phytoplasma diseases in other parts of the world; advances in phytoplasma diagnostics; quarantine; vector identification; epidemiology and genomics, including the application of new technologies such as confocal microscopy, microarrays and real time PCR. Abstracts of papers are presented here.

### Introduzione

Ricercatori, tecnici, assistenti tecnici ed i coltivatori di un certo numero di paesi tra i quali il Regno Unito, Italia, Israele, Bolivia e Cuba, hanno partecipato al primo gruppo di lavoro sui Fitoplasmii, organizzato dal Centro Nazionale per gli animali e la fitopatologia (CENSA), a San José de Las Lajas, Avana, Cuba, il 18-20 maggio 2005. I Fitoplasmii sono Procarioti non coltivabili *in vitro* che causano molte malattie importanti nel mondo in particolare in piante alimentari da fibra e ornamentali. A Cuba, le malattie della canna da zucchero e della papaia, due colture importanti, sono state recentemente associate con questi agenti patogeni. Gli obiettivi principali della ricerca sono stati messi a fuoco aggiornando la conoscenza e la ricerca sui Fitoplasmii e scambiando esperienze sul miglioramento della gestione delle malattie promuovendo collaborazione di lavoro fra le istituzioni partecipanti, evidenziando il notevole interesse sull'argomento.

La riunione ha previsto due tavole rotonde: "Descrizione della situazione attuale sui Fitoplasmii e vettori dei Fitoplasmii in Cuba " e "dei vettori di Fitoplasmii" con 6 presentazioni generali e 14 lavori. Gli argomenti trattati hanno riguardato: La situazione attuale delle malattie di Fitoplasma a Cuba; e malattie da fitoplasmii emergenti in altre parti del mondo; avanzamenti nella diagnosi dei Fitoplasmii; quarantena; identificazione del vettore; l'epidemiologia genomica, compresa l'applicazione di nuove tecnologie quali microscopia confocale, microallineamenti e PCR in tempo reale. Gli atti sono presentati di seguito.

Dr Yaima Arocha CENSA, Havana Cuba



## PROGRAMMA

### May 18

#### Morning Session

- 9.30-10.30 Registration  
10.00-10.30 Coffee Break  
10.30-10.50 Official Workshop Presentation  
10.50-11.10 Conference Professor Phil Jones, United Kingdom  
**Current advances of phytoplasma research**  
11.15-11.45 Presentation Dr E. L. Peralta, Cuba  
**Phytoplasma diseases in Cuba: an overview**  
11.50-12.10 Presentation Dr Armando Garcia, Cuba  
**Phytoplasmas and Plant Quarantine in Cuba**  
12.15-12.35 Presentation Dr Marina Barba, Italy  
**Scientific strategies and phytosanitary roles for control of  
'Grapevine Yellows' in Italy Friuli Venezia Giulia**  
12.40-12.50 Visit to CENSA's Scale model  
12.55-13.25 Description of CENSA's scale model  
13.30 Welcome Cocktail and Lunch

#### Afternoon Session

- Round Table: Overview on current situation of phytoplasmas in Cuba  
and world-wide  
Chairmans: Professor Phil Jones (United Kingdom) and Dr Yaima  
Arocha (Cuba)
- 14.30-15.00 Conference Prof. Phil Jones, United Kingdom  
**New and emergent phytoplasmas diseases**  
15.05-15.30 Presentation Dr Pablo Franco, Bolivia  
**Incidence of phytoplasmas in potato and tomato cobs in Santa  
Cruz, Bolivia**  
15.35-16.05 Presentation Dr La Rosa, Italy  
**Grapevine Yellows diseases in central and Southern Italy**  
16.05-16.20 Presentation Dr Yaima Arocha, Cuba  
**New isolates, alternative hosts and vector candidates associated  
with phytoplasma diseases in Cuba**  
16.25 Coffee Break  
16.45 Bus Leaving

**May 19**

**Morning Session**

Round Table: Overview on current situation of phytoplasmas in Cuba and world-wide (cont.)

Chairmans: Professor Phil Jones (United Kingdom) and Dr Yaima Arocha (Cuba)

9.30-10.00 Presentation Dr Marina Barba, Italy

**Fruit tree phytoplasmas: emergent diseases in Italy**

10.05-10.25 Presentation Dr La Rosa, Italy

**Phytoplasmas associated with Cacti**

10.30-10.50 Presentation Lic. Raixa Llauger, Cuba

**Current outbreaks of Coconut Lethal Yellowing and the use of diagnosis molecular techniques for phytoplasma detection**

10.55-11.15 Sugarcane Experimental Station, I.Juventud, Cuba

**Expression and damages of sugarcane yellow leaf syndrome (YLS)**

11.15-11.30 Coffee Break

11.35-11.55 Sugarcane Experimental Station, I. Juventud, Cuba

**Evaluation of quarantined sugarcane varieties to Yellow Leaf Syndrome (YLS)**

12.00-12.20 Sugarcane Experimental Station, I. Juventud, Cuba

**DAPI staining, an efficient technique for the diagnosis of sugarcane yellow leaf syndrome (YLS)**

12.30 Round Table "Phytoplasma Vectors"

**Chairmans: Dr Mike Wilson (United Kingdom) and Dr E. L. Peralta (Cuba)**

12.25-12.45 Presentation Dr Mike Wilson, United Kingdom

**What are know vectors and why: an introduction to Hemiptera classification**

12.50-13.15 Presentation Lic. Ariannys Roque, UNAH, Cuba

**Behaviour of spread percentage of the main vectors of papaya diseases in micro-propagated plants**

13.30 Lunch

**Afternoon Session**

14.30-14.55 Presentation Dr Mike Wilson, United Kingdom

**From field to laboratory: methods of insect sampling for phytoplasma detection**

15.00-15.25 Presentation Dr Phyllis Weintraub, Israel

**Phytoplasma Vector Management**

- 15.30-16.00 Presentation Dr Yaima Arocha, Cuba  
**Saccharosydne saccharivora in italic, *Saccharosydne saccharivora***  
**Leaf Syndrome (YLS)**
- 16.00-16.20 Coffee Break
- 16.45 Bus Leaving

**May, 20th**  
**One Day-Work Session CENSA/Foreign Delegation**



## CURRENT ADVANCES IN PHYTOPLASMA RESEARCH

**Phil Jones**

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Phytoplasmas are intracellular plant pathogens that have insect vectors but have never been cultivated *in vitro*. Since the advent of molecular technologies for the sequencing and manipulation of nucleic acids they are gradually beginning to give up their secrets. The ultimate goal for many research groups has been the elucidation of the complete genome sequence of a phytoplasma. This has recently been achieved by a Japanese group working with *Candidatus* Phytoplasma asteris strain OY. Sequencing of the genome has opened the way for studies in functional and comparative genomics which will lead to a better understanding of phytoplasma cell biology, phytoplasma - plant and phytoplasma - vector interactions. Examples of these will be discussed. Light and electron microscopy have been traditionally used as proof of phytoplasma aetiology. New imaging tools offer us greater scope to study the relationships of phytoplasmas *in situ* using unfixed tissues. Confocal laser scanning microscopy allows 3-dimensional reconstruction of live tissues while laser dissection microscopy can be used to isolate individual phloem cells for molecular studies. Phytoplasma diagnosis has reached new levels of specificity and sensitivity with the use of real time PCR, while the development of antibody based lateral flow techniques allows accurate diagnosis to be done on site. These techniques will be discussed using examples from current literature and research.

## **PHYTOPLASMA DISEASES IN CUBA: AN OVERVIEW**

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The study of diseases associated with phytoplasmas in Cuba is fairly new. The first references are about lethal yellowing (LY) of coconut palm a disease which has a high impact in the Eastern region of Cuba. New varieties have been introduced and their behavior is being evaluated in the field, electron microscopy studies have revealed the presence of pleomorphic phytoplasma bodies in ultra-thin sections of affected palm tissues. Since 1995 a number of different national projects have been developed for the study of phytoplasma diseases, covering three main fields: Biological and molecular characterization; improvement of the diagnosis and management and the development of diagnostic tools for exotic phytoplasma pathogens such as sugarcane grassy shoot, Ramu stunt and white leaf, Stolbur and Lime witches' broom, all diseases which could seriously threaten production in they gained access to Cuba. The first two fields have focused on the main phytoplasma diseases in Cuba: Sugarcane Yellow Leaf Syndrome (YLS) and LY. Some of the most relevant advances on research and diagnosis of such diseases will be presented. Also those needs of research will be discussed including pathogen genetic diversity, population variation, ribosomal gene characterization, improvement of diagnosis, and the co-infection and plant-pathogen-vector interaction; and those referring to the epidemiology of diseases: spread, new vectors, transmission, host range, rate of re-emergence and improvement of phytoplasma disease management.

## PHYTOPLASMAS AND PLANT QUARANTINE IN CUBA

**Armando García, Luis Pérez Vicente, Elizabeth Blanco**

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Phytoplasmas are phloem inhabiting-prokaryotes of the class *Mollicutes* which cause diseases in more than 500 plant species. They cause important losses for the national economies, reaching 70-100% in most of crops of economic interest.

In a vegetatively propagated crop like sugarcane, phytoplasmas can be readily spread to new locations through infected stem cuttings if suitable precautions are not taken. These can include cold water treatment and meristem tissue culture. Moreover, because their transmission is through leafhoppers, planthoppers and psyllids, and to date only one vector has been identified for those phytoplasma diseases present in Cuba, their management and control become difficult in field conditions. There is a need for new specific and more reliable diagnostics in order to develop sustainable control measures.

In this paper we summarize the main phytoplasma groups and diseases present in Cuba in weeds, sugarcane (Yellow Leaf Syndrome, YLS), papaya (Papaya Bunchy Top-like disease), and coconut (Lethal Yellowing).

We also give an overview of quarantined and exotic phytoplasma isolates, the state of current diagnostics for the main phytoplasma diseases present in the country and the national quarantine regulations established, as well as recommendations in to improve their control and management.

**SCIENTIFIC STRATEGIES AND PHYTOSANITARY ROLES TO  
CONTROL "GRAPEVINE YELLOWS" IN ITALY**

**Marina Barba**

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Flavescence dorée (FD) one of the grapevine yellows (GY) group of diseases was first observed in Italy at the end of '60 years ago in the Oltrepo' pavese (Lombardia region, northern Italy). Later, FD occurred in other regions including Veneto, Liguria, Friuli Venezia Giulia, Piemonte, Emilia and Trentino where severe damage to the plants and serious production crop losses for the production were recorded: in the same areas, high populations of *Scaphoideus titanus*, the vector of the disease, were observed.

The phytoplasmas detected in FD- affected plants belong to the 16SrV elm yellows group and in particular to the subgroups 16SrV-C and 16SrV-D. Bois noir (BN), a more slowly spreading form of grapevine yellows GY, is widely distributed in Italy: it is associated with a phytoplasma belonging to the 16SrXII subgroup -A but occasionally is generally associated with diseased grapevines. Seldom phytoplasmas of the 16SR 1 Aster yellows phytoplasmas group have been found associated with GY in some Italian regions.

FD is present in almost all the areas of northern Italy where grapevine is cultivated, while BN occurs in all the Italian regions but is of much lesser importance; moreover, *Hyalestes obsoletus*, the vector of BN is only found in the central and southern Italian regions.

To reduce the economic losses due to the spread of GY the following actions have been adopted by the Italian Ministry of Agriculture:

1. The publication of a ministerial decree for the compulsory eradication of grapevines affected by FD (DM 31-05-2000); the areas cultivated with grapevine have been split in three categories:
  - zona focolaio (foci area): an area where both FD phytoplasma and its vector *S. titanus* are present but the number of infected plants is limited allowing the control of the disease through roguing of infected plants;
  - zona di insediamento (settled area): an area where FD and its vector are present in very high numbers so that the elimination of infected plants is not possible. In this case the control of the disease is mainly based on the use of specific pesticides to reduce the vector population. In this area no nursery activity is allowed;

- zona indenne (immune area): an area where FD and its vector are not present. An accurate monitoring of vineyards is a prerequisite to prevent the introduction of the disease.
- 2. The promotion of a ring test to establish a diagnostic protocol suitable for the discrimination of the different phytoplasmas associated with GY. Italian researchers, belonging to 6 research groups have compared different protocols for DNA extraction, PCR amplification and RFLP analysis. The results of the ring test, held in Rome at ISPaVe, have allowed us to define the most efficient and simple protocols that will discriminate the FD from the BN phytoplasma and enable an evaluation of the phytosanitary status of large numbers of plants during field surveys.
- 3. Finance the national research project “*Grapevine yellows: a limiting factor of grapevine production*” to harmonize all different studies carried out in research Institutes throughout Italy. The project, coordinated by Dr. Marina Barba of ISPaVe brings together 10 groups (Tab. 1) of pathologists and entomologists and covers the following aspects:
  - Define the spread, in the open field, of GY caused by phytoplasmas; with particular attention to the characterization of different strains of phytoplasmas. All researchers responsible of the field survey will use common protocols for detection and characterization of the phytoplasmas.
  - Define the spread of those vectors of GY; vector population is monitored in infected vineyards, using previously defined protocols, particular attention is given to the identification of new potential vectors of the disease.
  - Control of the disease through the i) identification of natural reservoirs of phytoplasmas (weeds, forest trees), ii) the evaluation of efficacy of different pesticides to control vector population in the respect of integrated and/or organic production, iii) the study of the physiological basis of the recovery phenomenon.

Table 1 - Research Units participating to the finalized project "Grapevine yellows: a limiting factor of grapevine production" (coordinated by M. Barba).

INSTITUTION	RESPONSABLE	RESEARCH TITLE
C.R.A. Istituto Sperimentale per la Patologia Vegetale - Roma	G. Pasquini	Diffusion and characterization of phytoplasmas associated to grapevine yellows in central Italy
Istituto di Virologia Vegetale CNR, Torino	C. Marzachi	Diffusion, characterization and individuation of alternative hosts of FD and BN
C.R.A. - Istituto Sperimentale per la Viticoltura Conegliano Veneto (TV)	M. Borgo	Strategies to contain economic losses caused by Flavescence doree in grapevine
Istituto di Patologia Vegetale Università degli Studi di Milano	P. A. Bianco	Grapevine yellows in Lombardia Region
Dipartimento di Biologia applicata alla difesa delle piante Università degli Studi di Udine	R. Osler	Grapevine yellows: study finalized to symptom regression
Dipartimento di Biologia applicata alla difesa delle piante Università degli Studi di Udine	F. Pavan	Comparison of different strategies to control the vector <i>Scaphoideus titanus</i>
DI.VA.P.R.A. - Entomologia e zoologia applicate all'ambiente "Carlo Vidano" Facoltà di agraria -Torino	A. Alma	Influence of bio-ethology and vectors epidemiology in the spreading of grapevine yellows.
DiSTA, Patologia vegetale Alma Mater Studiorum, Università di Bologna	A. Bertaccini	Molecular biodiversity and epidemiology of grapevine yellows
C.R.A. - Istituto Sperimentale per la Zoologia Agraria - Firenze	B. Bagnoli	Bioecology and strategies control of <i>S. titanus</i> , <i>H. obsoletus</i> and other auchenor - rinchichi vectors of phytoplasmas in grapevine
Dipartimento di Scienze e Tecnologie Fitosanitarie (DISTEF), Facoltà di Agraria, Università degli Studi di Catania	R. La Rosa	Monitoring of grapevine yellows and their vectors in Sicily
Unità di ricerca sui Fitoplasmidi dell'INRA, Università di Bourgogne, Dijon (Francia)	E. Boudon-Padieu	Maintenance and transmission of grapevine yellows identified in weeds and/or in potential vectors
Department of Plant Physiology and Biotechnology del National Institute of Biology di Lubiana (Slovenia)		Physiological studies of recovery phenomenon
Dipartimento di Agrochimica e Agrobiologia, Università degli studi Mediterranea di Reggio Calabria	G. Albanese	Monitoring of grapevine yellows and their vectors in Calabria
Istituto Sperimentale per la Patologia Vegetale - Roma	M. Barba	Coordination of the Project

## **NEW AND EMERGING PHYTOPLASMA DISEASES**

**Phil Jones**

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Phytoplasma diseases have demonstrated their potential to cause devastation of the environment and serious economic losses of food and cash crops amongst some of the poorest rural communities in Africa and South East Asia. Epidemics of Cape St Paul wilt and other lethal yellowing-type phytoplasma diseases of coconut in Africa continue to wreak havoc in Ghana, Tanzania and Mozambique. In South East Asia sugarcane white leaf and grassy shoot disease have seriously affected rural incomes and national production targets. In Oman lime witches' broom has almost wiped out an entire industry. Newly recognised phytoplasma diseases such as sugarcane yellow leaf and Napier grass stunt in Africa, and the coconut wilts in Indonesia present new challenges to national research bodies, funding headaches for governments and NGOs, and dilemmas for the international exchange of germplasm. This presentation will review the emergence of phytoplasma diseases on crops in Africa, South East Asia and the Pacific Ocean islands.

**INCIDENCE OF PHYTOPLASMAS IN POTATO AND  
TOMATO CROPS IN SANTA CRUZ, BOLIVIA**

**Antezana Olivia<sup>1</sup>, Franco Pablo<sup>1</sup>, Jones Phil.<sup>2</sup>, Montellano Ernesto<sup>1</sup>,  
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Potato and tomato are the main crops of smallholder farmers in Santa Cruz, Bolivia. Two new phytoplasma diseases 'Brotos Grandes' (Big Bud) and 'Hoja de Perejil' (Parsley Leaf) affecting potato and tomato, respectively, have been reported recently from Santa Cruz province and are associated with phytoplasmas. In 2000, a survey was carried out for potato pests in the province M. M. Caballero, Santa Cruz, Bolivia. Out of 42 farmer's fields, 14 showed had plants with symptoms of 'Brotos Grandes'. A survey was also carried out to determine the prevalence of 'Hoja de Perejil' in tomato, the spread of the disease and damage to the crop. All tomato fields surveyed had 'Hoja de Perejil' symptoms and 77% of tomato plants were affected. Eight *Cicadellidae* insects were found in the valleys and identified as possible vectors. Some fields where measures had been applied to control the vectors saw a 50% reduction of disease incidence. The use of tomato hybrids together with appropriate advice on the use of insecticides, contributed to lower crop losses.

## GRAPEVINE YELLOWS DISEASES IN CENTRAL AND SOUTHERN ITALY

**Rosa La Rosa<sup>1</sup>, Carmelo Rapisarda<sup>1</sup>, Matilde Tessitori<sup>1</sup>, F. Ruta<sup>1</sup>,  
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Grapevine yellows disease (GY) is distributed worldwide and poses a serious limitation on grapevine production. The causal agents of GY are phytoplasmas which belong to a number of different taxonomic groups but which induce similar symptoms on the vines.

The most dangerous and destructive of the grapevine phytoplasmas is that of Flavescence dorée (FD) belonging to the 16Sr-V Elm Yellows group. The disease is very efficiently transmitted by the monophagous leafhopper *Scaphoideus titanus* Ball. and spreads rapidly in the vineyards. Another important GY disease, widely distributed in Europe, is Bois Noir (BN) caused by a phytoplasma belonging to the Stolbur, 16Sr XII group. The low transmission efficiency of this phytoplasma makes BN less of a threat than FD. The possible vector is the polyphagous *Hyalesthes obsoletus* Signoret.

In Italy FD is controlled by a mandatory law and it is present, together with the vector, in the northern area, causing serious economical damage on grapevine; in central-southern regions BN is the most widespread GY disease, it's endemic but here but although it can cause severe symptoms it seems to be not pose such a high level of threat.

To improve our knowledge of the FD and BN phytoplasmas and to avoid the spread of FD and/or a recurrence of BN many investigations have been carried out or are still in progress. A major national research program titled "Grapevine yellows: a limiting factor of grapevine production - GIAVI" which involves researchers from all Italian grapevine areas, and which is financed by the Ministry of Agriculture.

Recently FD has been occasionally reported from some regions of central Italy: Tuscany and Marche and *S. titanus* has been found in central-southern regions, Basilicata, Campania and Tuscany. By molecular analysis a phytoplasma of the 16Sr XII Stolbur group has been identified from FD-affected vines in the following regions: Apulia, Basilicata, Calabria, Campania, Latium, Sardinia, Sicily,

Tuscany and Umbria. In addition phytoplasmas belonging to the 16Sr III X disease group and the 16Sr I aster yellows group, are sporadically detected while in the regions of Marche and Sardinia phytoplasma of the 16Sr XII Stolbur group are found.

FD and *S. titanus* presence in some central and southern regions needs more attention o to be paid to the disease in these areas.

The movement of *S. titanus* with its associated threat of the spread of FD and the increase of BN in central and southern Italian regions needs preventive measures to control the disease in this area.

**NEW ISOLATES, HOSTS AND PUTATIVE VECTORS  
OF PHYTOPLASMA DISEASES IN CUBA**

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Yellow leaf syndrome (YLS) is one of five major diseases affecting Cuban sugarcane production causing more than 30% loss. *Saccharosydne saccharivora* (Westwood) a delphacid planthopper has been recently identified as the vector of YLS and its associated phytoplasmas. Papaya (*Carica papaya* L.) identified for development as an export crop with an estimated production of 55 000-60 000 t per year is affected by a disease similar to Papaya Bunchy Top (PBT) which is spreading throughout the country and is associated with phytoplasmas, but no putative insect vectors have yet been identified.

During 2003-2004, surveys of YLS and PBT-like disease were done on plantations in Havana province, to determine the roles which weeds and *Auchenorrhyncha* insects play in their epidemiology. Over 250 plant and insect samples were collected and indexed by a nested PCR (nPCR) for phytoplasma 16S rDNA using generic primers P1/P7 and R16F2n/R16R2. A new species of derbid of the genus *Cedusa*, captured from sugarcane plantations was identified. Nested PCR products were further characterised by restriction RFLP using HaeIII, *AluI*, *Sau3AI*, *Tru9I*, *HpaII*, *HhaI* and *TaqI*, giving patterns which clearly distinguished them from those of reference phytoplasma controls. Phylogenetic analysis of 16S rRNA sequences identified the phytoplasmas present in sugarcane, Bermudagrass (*Cynodon dactylon* L.), Canadian horseweed (*Conyza canadensis* L. Cronq.), (Asteraceae), Johnsongrass (*Sorghum halepense* L. Pers) (Fabaceae), phasey bean (*Macroptilium lathyroides* L. Urb.) (Fabaceae), *Cedusa* sp., *Saccharosydne saccharivora* (Westwood) and those in papaya and *Empoasca* sp., as two new phytoplasma species, which form two new 16Sr phytoplasma groups with the proposed names *Candidatus Phytoplasma graminis* (16SrXVI group) and *Candidatus Phytoplasma caricae* (16SrXVII group), respectively.

Results indicate the need to carry out further studies on the epidemiology of both YLS and PBT-like diseases, in order to determine the possible roles and impact that putative insect vectors and alternative phytoplasma hosts identified here could play in the spread and management of these important diseases.

The Genbank/EMBL/DDBJ accession numbers of 16S rDNA gene sequence of phytoplasmas associated with sugarcane, *Cynodon dactylon*, *Conyza canadensis*, *Macroptilium lathyroides*, *Sorghum halepense*, *Saccharosydne saccharivora*, *Cedusa* sp., papaya and *Empoasca* sp. are AY725228, AY742327, AY742328, AY742329, AY742330, AY725229, AY744944, AY725234 and AY725235.

## **FRUIT TREE PHYTOPLASMAS: EMERGING DISEASES IN ITALY**

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Phytoplasmas are systemic pathogens causing severe damages to fruit trees where ever over they are cultivated. They are spread mainly by the use of infected planting material (providing the primary source for natural spread by vectors in the field).

In order to minimize the risk of distribution of these pathogens, many governments, including Italy, have issued a set of rules (quarantine, certification) aimed at guaranteeing the phytosanitary status of materials for vegetative propagation.

The most important fruit tree diseases, caused by phytoplasmas, covered by the Italian phytosanitary regulation are:

1. European stone fruit yellows (ESFY). Decline diseases associated with phytoplasmas are known to affect different *Prunus species* in Europe. Among them, the economically and most important are apricot chlorotic leaf roll (ACLR) on apricot, plum leptonecrosis (PLN) on Japanese plums, yellowing and decline on almond and peach.

Phylogenetic studies, based on molecular characterization of the 16S ribosomal RNA gene, has shown that the phytoplasmas associated with naturally infected apricot, plum, almond and peach have similar restriction profile confirming that they are very closely related.

At an early stage of infection the plant shows symptoms very irregularly distributed on the branch inoculated by the insect vector. The pathogen requires two or more years to completely colonize an adult tree. The highest potential of infection is in late summer (August-September) when symptoms are particularly evident on the foliage. Symptom expression is influenced by different factors such as strain virulence, genotype susceptibility of rootstocks and/or peach variety.

- Apricots show interveinal foliar yellowing, leaves smaller than normal with conical rolling along the longitudinal axis.
- Japanese plums are very sensitive: leaves are smaller than normal, cylindrically rolled, slightly chlorotic, later brownish-red. Diseased trees show anticipated foliage before flowering. Extended phloem necroses and decline of branches or entire trees can be observed.

– Peach and nectarine show yellowing or reddening of leaves that are rolled and curled. Leaves of infected branches are smaller in size and are thicker and more brittle than normal. The leaf midribs and lateral veins are enlarged and affected leaves abscise earlier than healthy ones. The tree has a less vigorous aspect, is more sensitive to frost in winter and gradually decline. Productivity of infected trees is reduced. Early emergence of flowers and leaves can occur during winter on diseased trees that are easily distinguished from healthy ones.

The causal agent of the disease is a well characterized phytoplasma belonging to the 16Sr X Apple Proliferation phytoplasma group which includes phytoplasmas infecting pear (Pear decline, PD) and apple (Apple proliferation, AP) trees in Europe.

*Cacopsylla pruni* is the main natural vector in the field of ESFY phytoplasma. Infected psylla transmit phytoplasmas in a persistent manner: the insect acquires the pathogen by feeding on infected trees for 2-4 days and retains the infectivity for life. A latency period of 2-3 weeks between acquisition and transmission phase has been reported.

Several wild *Prunus species* (*P. spinosa*, *P. cerasifera*, *P. domestica*) are natural hosts of the ESFY phytoplasma and can act as natural reservoir of the pathogen. The role that they play in the diffusion of the disease is very important because they host also the vector *C. pruni* allowing endemic establishment of the ESFY phytoplasma independently from the presence of infected cultivated stone fruit trees.

2. Pear decline (PD) is one of the most economically important diseases in pear, mainly because its natural spreading by *Psylla pyricola* is very efficient and quick. *Psylla* acquires the phytoplasma in few hours feeding on infected plants and remains infective at least for three weeks or for all its life. The integrated control of other pests, with consequent reduction of chemical treatments in pear orchards, determined the increasing of psylla population and, as direct consequence, of pear decline. Symptoms differ according to the rootstock and the variety. Leaves of infected trees are few, small and may become reddish in fall. They roll upward along the longitudinal axis and drop earlier than normal leaves. The tree may live for many years or die within a few years.
3. Apple proliferation (AP) is mainly present in north Italy where in the last years is causing severe economic losses in productive orchards of Val d'Aosta and Trentino Regions. It causes symptom on shoots, leaves and fruits. The first sign of infection are witches' brooms as consequence of the lack of apical dominance in affected shoots. The fruits have longer peduncle, are smaller than normal, no well ripen and no uniformly coloured, the taste is poor and the quality very low: leaves show also enlarged stipules. Golden delicious, Renetta del Canadà and Jonathan are the most sensitive cultivar. *Cacopsylla costalis* is the vector of phytoplasmas associated with AP.

As with other systemic pathogens, preventive measures, as the use of phytosanitary certified propagation material and the respect of quarantine measures, are the most important approaches to reduce the risk of diffusion of phytoplasma epidemics in areas where the disease is still not present.

The control of the local vector population, the elimination of woody and/or herbaceous plants that could act as reservoirs of the phytoplasmas or that could allow the vector multiplication and over wintering, are good agricultural practices that reduce the risk of contamination.

Obviously effective control of the spread of phytoplasmas is based on reliable diagnostic methods. Until a few years ago their detection was mainly based on biological indexing on experimental host-plants (periwinkle - *Catharanthus roseus*), on E.M. observation of ultra thin sections of diseased tissue or on DAPI (4',6'-diamidino-2-phenylindole, 2HCl) staining of infected tissue followed by epifluorescence microscopy observations. The main disadvantage of these techniques lies in the fact they are non-specific and do not allow the identification of the pathogen.

Serological methods, using monoclonal antibodies, allowed more rapid detection but the big revolution has been the introduction of molecular techniques (molecular hybridization using specific probes, polymerase chain reaction -PCR). PCR technology, using cluster-specific primers followed by RFLP analysis, can be considered a sensitive and reliable test for the diagnosis and identification of the phytoplasmas infecting fruit trees.

Routine diagnosis of this class of pathogens must take in consideration the fact that they generally occur in relatively low titres in infected tissues, are uneven distributed within the phloem of the tree and their presence in different parts of the tree is varies according to the season. For all these reasons diagnostic protocols should specify for each phytoplasma the best vegetative period and tissue sample to use for reliable detection.

## PHYTOPLASMA ASSOCIATED WITH CACTI

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Mexico is the world's main producer of cactus pear, while Italy is the most important producer in the Mediterranean basin. Phytoplasmas have been detected in ultra-thin sections of opuntia tuna and by PCR of total DNA from *O. linguiformis* with witches broom symptoms. Phytoplasma diseases have not been reported in *Opuntia ficus-indica*. In 2003 three plants of cactus pear showing abnormal growth were observed in the DISTEF collection at the University of catanmia in Sicily. The plants showed severe proliferation of cladodes with a lack of flower, fruit and spine production.

Total DNA was extracted from the plants with and from two without symptoms by the method of Cai *et al.*, (2002) and used as templates for the amplification of phytoplasma specific 16S rDNA with three universal primer pairs (P1/P7, R16f2/r2, fU5/rU3) DNA from phytoplasma reference strains maintained in periwinkle was used as a positive control. DNA from this first amplification was used as template in a nested PCR using primers R16f2/r2. RFLP analysis was performed with *AluI*, *HhaI*, *HpaII*, *MseI*, *TaqI* on these amplicons No PCR products were observed in samples from healthy cactus.

Phytoplasma specific PCR products were observed in all samples with symptoms using P1/P7 and fU5/rU3 primers in direct PCR and with R16f2/r2 in nested PCR. Identical RFLP patterns were produced from the 2 plants with symptoms corresponding to that of faba bean phyllody phytoplasma, a member of the 16Sr II-C subgroup for which a "*Candidatus* Phytoplasma" species has not yet been proposed.

This is the first report of a phytoplasma infecting *O. ficus-indica*. These data are in agreement with previous finding in Christmas cactus and *Opuntia* spp., in Italy.

**CURRENT OUTBREAKS OF LETHAL YELLOWING  
IN CUBA AND THE USE OF MOLECULAR DIAGNOSTIC  
TECHNIQUES IN PHYTOPLASMA DETECTION**

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Lethal Yellowing (LY) disease of coconut palm (*Cocos nucifera* L) in Cuba has been reported since the end of the 19<sup>th</sup> century. Currently the disease has been spreading throughout the country. Polymerase Chain Reaction (PCR) assays were used to detect phytoplasmas in coconuts from Cuba in different areas where plants showing typical disease symptoms were found. The PCR using “universal” phytoplasma ribosomal primers pair P1/P7 and non-ribosomal Florida LY-specific primers LY1F/LY1R, were used to detect the LY phytoplasma. PCR products of 1.8 kb in size were amplified from most of the DNA samples of LY-diseased palms from Cuba and from the positive control originating from Jamaica, Honduras, Ghana and Mozambique when the P1/P7 primers pair was used. LY1F/LY1R primers yielded PCR products of approximately 1 kb in size from some but not all DNA samples of palms. Presence of positive samples with P1/P7 primers which did not amplified when LYF/LYR primers were used, suggests that there could be at least two groups of LY phytoplasmas in Cuba, one of them different from the Florida group. The results of diagnosis confirmed the existence of the disease in many places across the Island, with variations in incidence between locations. Different pathogen distribution in plants were also determined.

**EXPRESSION AND DAMAGES OF SUGARCANE  
YELLOW LEAF SYNDROME (YLS)**

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Yellow Leaf Syndrome (YLS) was first reported in 1969, since then has been reported in most of sugarcane growing countries causing losses of more than 50% of susceptible varieties. The aetiology of the disease has been controversial; however, phytoplasmas have been consistently associated with YLS in Cuba, South Africa and Mauritius. Latent infections commonly occur in YLS affected cane plants, so it is difficult the diagnosis of the disease.

Because the presence of the disease in Quarantine areas, the identification of the pathogen associated and evaluation of varieties and clones and their behaviour have been objectives of our study. Brix measures and laboratory methods are needed for the identification of phytoplasmas associated. DAPI and Dienes staining are quick and not expensive compared with those of electronic microscopy, and can be generalized throughout the whole quarantine network, as confirmative tool. From our study, YLS phytoplasma causes decreasing of yield sugarcane production affecting the plant growth, physiology and development and has been found in plants with and without YLS symptoms.

Considering the control methods for YLS are not efficient enough and the main mission of Quarantine is to avoid the entrance of the disease in the country and the releasing of affected varieties, we recommend introducing DAPI and Dienes staining in the entire Quarantine network.

## **EVALUATION OF QUARANTINED SUGARCANE VARIETIES TO YELLOW LEAF SYNDROME (YLS)**

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Phytoplasmas cause many diseases in sugarcane crops, including grassy shoot (SGS), white leaf (SCWL) and yellow leaf syndrome (YLS). YLS can cause yield losses of 60% in susceptible varieties. Field diagnosis by monitoring of brix values in leaves have recently been reported, as a useful indicator for YLS without the need for laboratory tests.

40 quarantined sugarcane varieties were evaluated in the Quarantine Station of Isla de la Juventud, by using the brix measuring method from sugarcane leaf midrib juice. Sugarcane plants with and without YLS symptoms were indexed, and brix values below 8 (4.7-7.74) were mainly obtained for plants without symptoms, some varieties showed values over 8 which may indicate an incubating infection. All YLS plants with symptoms showed brix values over 8. No direct correlation was found between the intensity of YLS symptoms and the brix value. Electron microscopy analysis showed the presence of characteristic phytoplasma pleomorphic bodies from ultra thin phloem sections of YLS but not from symptomless plants.

Measures of brix values can be used for the field diagnosis of YLS if there are no other laboratory tools suitable to evidence the presence of phytoplasmas associated with the disease.

For quarantine purposes the values of brix as an indicator for YLS should not be relied on until validation of the results has been done.

## **DAPI STAINING, AN EFFICIENT TECHNIQUE FOR THE DIAGNOSIS OF SUGARCANE YELLOW LEAF SYNDROME**

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All sugarcane countries exchange germplasm in accordance with their agreed quarantine regulations. Phytosanitary protection authorities are responsible for the development and improvement of appropriate diagnostic tools. Methods used for the identification and detection of phytoplasmas in the Quarantine Station are Dienes staining, brix measures and transmission electron microscopy. Recently, we have introduced DAPI (4'-6 diamino 2-phenylindol) staining, which is based on the binding of DAPI to the phytoplasma DNA, forming a fluorescent complex in the phloem cells.

Fifty varieties with YLS symptoms and 17 without were analyzed by DAPI. All the YLS symptom plants showed the presence of fluorescent spots in the phloem cells, as did the apparently healthy canes with brix values from 15-18. No fluorescence was observed in those plants without symptoms and which had brix values below 15.

These results have shown DAPI staining to be an excellent technique to complement field diagnosis of YLS based on brix values. This technique is especially useful for the early detection of infection in apparently healthy plants. Hence, we recommend that DAPI staining should be used by our national diagnostic system.

**WHAT ARE KNOWN VECTORS AND WHY:  
AN INTRODUCTION TO HEMIPTERA CLASSIFICATION**

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The majority of known vectors of phytoplasma are found within the insect order Hemiptera. Within this group, which comprises the true bugs, leafhoppers and planthoppers, spittlebugs and psyllids, the vectors are confined to just a few sub-families and even tribes.

An introduction will be given to the classification of the Hemiptera and to highlight the biology of those groups in which vectors have been found.

**INCIDENCE OF PAPAYA BUNCHY TOP-LIKE DISEASES IN  
PLANTATIONS: A COMPARISON OF NORMAL AND MICRO-  
PROPAGATED PLANTS**

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Papaya is versatile tropical fruit which is being developed as an export crop in Cuba. Nevertheless, yields have decreased in the last years from 19.73 t.ha<sup>-1</sup> in 1998 to 17.95 t.ha<sup>-1</sup> in 2003, due to a variety of pests and diseases in particular viruses and phytoplasmas, which have been associated with a disease complex that causes losses between 50 and 100% of trees in plantations. To control these growers use pesticides that affect the environment and increase the cost of crop production. Tissue culture is one of the most effective treatment methods to eliminate such diseases.

We evaluated the dynamics of vector spread percentage in the crop from the beginning of the plantation up to 12 months using the Pest Signal and Prognostic methodology, and the molecular diagnosis of pathogens either in control or micro-propagated plants established in two agroecosystems. This allowed us to determine variations in the incidence of the main vectors of papaya diseases between the evaluated treatments, where micropropagated plants showed pest incidence percentages lower than control plants for both agroecosystems.

**FROM FIELD TO LABORATORY: METHODS OF INSECT  
SAMPLING FOR PHYTOPLASMA DETECTION**

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An introduction is given to the various techniques commonly used to sample insects from plants, with comments on the advantages and problems with each technique. The techniques include, direct searching from plants, sweep net, yellow sticky traps, Malaise traps, and light traps. Information on the data required to accompany collection is also given. Comments are also made on the biology of leafhoppers and planthoppers.

## **PHYTOPLASMA VECTOR MANAGEMENT**

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Phytoplasmas are non-culturable, degenerate gram-positive prokaryotes, closely related to mycoplasmas and spiroplasmas. Plant diseases caused by, or associated with, phytoplasmas occur in hundreds of commercial and native plants, causing minor to extensive damage. Insect vectors, primarily leafhoppers, planthoppers and psyllids, have been identified for relatively few phytoplasma diseases, limiting the capacity of managers to make informed decisions to protect crops and endangered indigenous plants. Since even the fastest-acting insecticides are relatively slow, compared to the time required for pathogen transmission, other methods of control must be developed to properly manage phytoplasma transmitting insects.

Vegetation composition, habitat diversity and the nature of ecotones in and near a phytoplasma-vulnerable crop can have profound effects on the presence and dispersal of vectors, their natural enemies and other insects. As such, cultural control methods can play a significant role in managing phytoplasma diseases. These methods include grafting crop plants on resistant rootstock, removing non-crop plants that serve either as a reservoir for the phytoplasma or as breeding sites for vector insects, and mulching. In orchards, infected plants should be rogued after treating with insecticides to prevent dispersal of infective insects.

As insecticides have served as the standard form of management of vector species in the past, there will probably be reliance on genetically engineered plants in the future. Genetic modifications may include enhancement of genes naturally present within the plant that code for defensive compounds, for instance, or the introduction of alien genes into crop plants. Rice genetically engineered to express the snowdrop lectin (GNA), causes a reduction in the leaf- and planthopper vectors of phytoplasmas and viruses, a deduction in the number of diseased plants and a reduction in the amount of honeydew excreted by these hoppers.

**SACCHAROSYDNE SACCHARIVORA, A NEW VECTOR  
OF SUGARCANE YELLOW LEAF SYNDROME**

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Phytoplasmas are prokaryote plant pathogens of the class *Mollicutes*, which cause diseases of crops, ornamentals and weeds from the temperate to tropical regions but are still not cultivable in vitro. They are vectored by certain phloem-feeding leafhoppers, planthoppers and psyllids in a persistent manner, however, members of the *Cicadellidae* and several planthopper families (Fulgoromorpha) are amongst the most common vectors of phytoplasma diseases. *Saccharosydne saccharivora* (Westwood) is the only known *Saccharosydne* species to breed on sugarcane, and can be found in India, Central America and the Caribbean. Although *S. saccharivora* has never been reported as a disease vector, observations of high population densities during the periods December 2001 to March 2002 and December 2002 to March 2003, in sugarcane plantations located in the western and central regions of the country, led us to investigate its role in the epidemiology of yellow leaf syndrome.

From surveys, the delphacid planthopper *S. saccharivora* was the most prevalent of the *Auchenorrhyncha* fauna. Individuals of *S. saccharivora* collected tested positive for the sugarcane yellow leaf phytoplasma (SCYLP). *S. saccharivora* were reared in cages and used for experimental transmission studies of SCYLP. The *S. saccharivora* were given acquisition access feeds of 72 hours on SCYLP infected cane collected from the field followed by an inoculation access period of 15 days on healthy sugarcane seedlings. 24/36 plants developed symptoms of yellow leaf syndrome between 7 and 12 months post-inoculation confirming YLS symptoms were well reproduced to healthy sugarcane plants and phytoplasmas as the causal agent of the disease. No (0/36) healthy seedlings that were inoculated with *S. saccharivo* -

*ra* fed on phytoplasma-free sugarcane developed symptoms. All phytoplasma positive sugarcane and *S. saccharivora* samples showed identical RFLP patterns and had 99.89% similarity in their 16S/23S spacer region sequences but only 92.6% - 93.6% with other phytoplasmas. Sequences have been deposited with GenBank (Accession numbers: AY725237 (*S. saccharivora*), and AY257548 (sugarcane).

Our phylogenetic analysis suggests that the phytoplasmas from sugarcane and *S. saccharivora* are putative members of a new 16Sr phytoplasma group. This is the first report of the vector transmission of a phytoplasma associated with sugarcane yellow leaf syndrome and the first time that *S. saccharivora* has been shown to vector a phytoplasma.

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