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# Grapevine Yellows Diseases and Their Phytoplasma Agents Biology and Detection

 Springer

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# Grapevine Yellows Diseases and Their Phytoplasma Agents

Biology and Detection

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# Preface

Grapevine yellows diseases were reported in most viticultural regions worldwide even before their association with phytoplasmas had been established. Due to the significant losses in yields through widespread “bois noir” and several epidemics of the quarantine “flavescence dorée” in European vineyards, a lot of effort has been put recently into research on phytoplasmas associated with these two diseases. The knowledge that has been obtained considerably improves our understanding of their epidemiology, as well as their alternative host plants and the biology of their insect vectors. Moreover, new data have greatly contributed to further understanding the interaction between phytoplasmas and their hosts in general.

This book reflects the knowledge gained by the authors over many years of basic research and diagnostic practices on grapevine yellows diseases. Here we have examined all the disease aspects, including their worldwide distribution, the taxonomy of their agents, and the interactions between the host plant grapevine and phytoplasmas. The last chapter of the book presents the state-of-the-art diagnostic applications, with some promising ones that have not been generally used in routine practice. The presented topics, such as validation, measurement of uncertainty, and solutions that guarantee quality assurance, are of crucial importance for phytoplasma diagnostics.

The authors hope that this book will bring useful information to researchers and professionals at all levels and will even stimulate additional scientific work in the field of the still poorly understood phytoplasma world.

We would like to thank our colleagues at the National Institute of Biology: the *Alma Mater Studiorum*, University of Bologna; and AgriBio who have supported our research and our collaborators, with whom most of the new data on the phytoplasmas were obtained through partnerships in international projects. We also express our gratitude to Dr. Christopher Berrie for his linguistic touch.

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# Chapter 1

## Phytoplasmas – Dangerous and Intriguing Bacteria

**Abstract** Phytoplasmas were discovered almost 50 years ago and initially they were named mycoplasma-like organisms. These cell-wall lacking bacteria and members of the class Mollicutes inhabit plant phloem sieve elements and are transmitted and spread primarily by leafhoppers, plant hoppers and psyllids that feed on infected plants phloem. Phytoplasmas interact with their hosts in a strong manner, through manipulation of the morphological features of the plants, and in several cases, also of the biology of their insect vectors. Molecular genetics analyses have improved the understanding of phytoplasma taxonomy, and also enhanced the ability to identify phytoplasmas that are detected in hosts and insect vectors. In particular, it has been determined that, among the plant species infected by phytoplasmas, grapevine is one of those that are most severely affected, at a worldwide level. Molecular studies have provided considerable insights into phytoplasma molecular diversity and genetic relationships, taxonomic ranking has been achieved by using 16S ribosomal gene classification and other phytoplasma genes as epidemiologic molecular markers. On the other hand, the inability to fulfil Koch's postulates severely restricts the understanding of the real roles of phytoplasmas in diseases and in plant–insect interaction. Together with the new possibility to cultivate phytoplasmas in artificial media, molecular genetics studies are now opening possibilities for studying the best management of these bacteria that severely threaten worldwide agriculture, and in particular viticulture.

### 1.1 History and Biology

Phytoplasmas belong to the taxonomic domain Bacteria, but unlike most bacteria they lack a cell wall and are therefore obligate parasites that live in plant phloem and insect haemolymph. They can induce disease symptoms by sequestering metabolites produced by host cells and also by altering the expression of plant homeotic genes (Himeno et al. 2011). Phytoplasmas are introduced into plant sieve tube elements by vector insects during feeding, and they spread then systemically. In most cases, a specific insect vector in different geographic regions transmits distinct phytoplasmas. However, mixed phytoplasma infections are also common, although little is known in terms of mixed infections in insect vectors, and whether they occur as often as in plants. Mixed phytoplasma infections appear to be more common

where farming is intensive and mixed culture is routine practice. Under these conditions, polyphagous insect vectors can feed on non-host plants that can become infected, if they are susceptible to the phytoplasma carried by the visiting vectors. These phytoplasmas might then be exposed to a new group of insect vectors and begin to establish a new biologic and ecologic cycle that can quite often end in a new disease outbreak (Lee et al. 1998a). Transovarial transmission of phytoplasmas has also been reported in some insect vector–phytoplasma combinations (Alma et al. 1997; Kawakita et al. 2000; Hanboonsong et al. 2002; Tedeschi et al. 2006). However, one of the most important ways that phytoplasma spread in the field, and especially over long distances, is through the vegetative propagation methods that are used to multiply the plant material and that avoid sexual reproduction, such as grafting, cutting, and micropropagation.

Although phytoplasmas have the smallest genome among plant pathogenic bacteria, gene duplication and redundancy, and differences in chromosome size have been reported, with many core housekeeping genes present in multiple copies. In their divergence from Gram-positive bacteria, they have lost several metabolic pathways, and they were assigned to the ‘*Candidatus* Phytoplasma’ genus (IRPCM 2004) that comprise the organisms incompletely described (Murray and Stackebrandt 1995). The entire genome sequences have now been completed for two strains of aster yellows (‘*Candidatus* Phytoplasma asteris’), two strains of ‘*Ca. P. australiense*’, and one strain of ‘*Ca. P. mali*’ (Oshima et al. 2004; Bai et al. 2006; Tran-Nguyen et al. 2008; Kube et al. 2008; Andersen et al. 2013).

Symptoms of phytoplasma infection vary considerably among plant hosts according to a range of factors, such as concentration and localisation of phytoplasma in host tissues, seasonality of infection, and ultimately the metabolic interactions that occur between the phytoplasmas and the host species (Bertaccini 2007). In some perennial woody plant hosts, phytoplasmas can lay dormant through a season (Jarusch et al. 1999; Seemüller et al. 1984), or they can accumulate while remaining asymptomatic in some species that act as reservoirs for their further spread (Carraro et al. 1998). Finally, one phytoplasma strain can induce different symptoms among multiple hosts, and indeed some shared symptoms among infected hosts can arise from infections by different phytoplasmas and/or from other unrelated causes (Bertaccini et al. 2014). Laboratory-based methods for phytoplasma detection and identification are therefore prerequisites for the early control of infected hosts, for phytosanitary screening processes, and for biosecurity concerns regarding cross-border disease outbreaks that can result from the introduction of phytoplasma infected vectors and/or hosts.

The information achieved to date through full genome sequencing mainly relates to phytoplasma putative biochemical pathways. This information has shown that phytoplasmas are very special microorganisms, because they lack many relevant features of other bacteria, such as mobility and key enzymes. However, metabolic pathways allow phytoplasmas to have a ‘trans-kingdom’ life and interact with both plant and insect hosts, upon which they are dependent for survival. As many phytoplasma genes encode transporter systems, with some present in multiple copies, it has been suggested that they import many metabolites from their host cells, and this