Assunta Bertaccini · Phyllis G Weintraub
Govind Pratap Rao · Nicola Mori   *Editors*

Phytoplasmas: Plant Pathogenic Bacteria - II

Transmission and Management of
Phytoplasma - Associated Diseases

Springer

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Editors
Assunta Bertaccini
Department of Agricultural and Food
Sciences
Alma Mater Studiorum – University of
Bologna
Bologna, Italy

Govind Pratap Rao
Division of Plant Pathology
Indian Agricultural Research Institute
New Delhi, Delhi, India

Phyllis G Weintraub
Department of Entomology
Agricultural Research Organization, Gilat
Research Center
D.N. Negev, Israel

Nicola Mori
Department of Agronomy, Food, Natural
resources, Animals and Environment
University of Padua
Padova, Italy

Preface

Phytoplasma-associated diseases are a major limiting factor to the quality and productivity of many ornamental, horticultural, and other economically important agriculture crops worldwide. Annual losses due to phytoplasma diseases vary in many crops, but under pathogen-favorable conditions, they always lead to disastrous consequences to farming communities. There is no effective cure for phytoplasma diseases; the management options emphasize pathogen exclusion, to minimize their spread by insect vectors and propagation materials, and development of host plant resistance. The scientific literature concerning transmission, epidemiology, and management of phytoplasma-associated diseases is growing at a fast pace. Significant advancements have been made on these perspectives in the last decade. Very few compilations are available to show the progress of phytoplasma research on epidemiology and management aspects hence, the major recent research findings are compiled in this book.

The book covers recent and updated information on epidemiology, means of transmission, and management of phytoplasma-associated diseases in 11 chapters contributed by experienced and recognized scientists.

We most sincerely acknowledge all the contributed authors for their earnest efforts in synthesizing the most updated reviews on the subjects. We also like to thank the support and input of the publisher, Springer Nature, for its effort to publish this book. We strongly hope that the book will be useful to everyone interested in phytoplasma research, plant pathology, microbiology, plant biology, and agriculture and serve as an exhaustive and up-to-date reference on the various applied aspects of phytoplasma-associated diseases studied during the past decades.

Bologna, Italy   Assunta Bertaccini
D.N. Negev, Israel   Phyllis G Weintraub
New Delhi, India   Govind Pratap Rao
Padova, Italy   Nicola Mori

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About the Editors

Assunta Bertaccini is Professor of Plant Pathology at the University of Bologna, Italy. In more than 40 years of research her major studies were devoted to plant diseases associated with phytoplasmas and bacteria, focusing on their biology and epidemiology. She was invited speaker at national and international meetings and seminars in 40 Countries around the globe. She has received numerous awards, including the Emmy Klienenberger-Nobel Award for distinguished research in mycoplasmology. She is author or coauthor of about 800 publications including books and book chapters. She is Editor-in-Chief of *Phytopathogenic Mollicutes*, Senior Editor of *Phytopathologia Mediterranea*, reviewer of international scientific journals and founder and head of the International Phytoplasmologist Working Group (IPWG).

Phyllis Gail Weintraub is Research Scientist in Entomology at Gilat Research Center, Nagev, Israel. Her research focuses primarily on applied and field studies in non-chemical pest control (physical and biological) in greenhouses and identification and characterization of phytoplasmas and their insect vectors. She has been an invited speaker at national and international meetings and seminars and is the author or coauthor of 160 publications, including books and book chapters. She is the Editor-in-Chief of the *Journal of Insect Science* and serves as a reviewer for numerous international scientific journals.

Govind P. Rao is working as a Principal Scientist in Plant Pathology at the Indian Agricultural Research Institute, New Delhi, India. He has 30 years of research experience in the field of plant pathology, especially sugarcane diseases, cereal viruses, and phytoplasmas. He has published over 140 research papers, 22 reviews, 48 book chapters and authored or edited 20 books. He also guided 20 MSc and PhD students on different aspects of plant pathology. He is Editor-in-Chief of *Phytopathogenic Mollicutes* journal and has received several national and international awards. He has visited 30 countries for invited talks, postdoc fellowships, research training, panel discussions, workshops, and conferences. Dr Rao is also a member of the scientific committee of the International Phytoplasmalogist Working Group (IPWG).

Nicola Mori is Professor of General and Applied Entomology in the Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Italy. His research focuses primarily on the epidemiology and transmission of phytopathogenic agents and integrated pest management. He is a consultant and adviser to the Phytosanitary Italian Service on phytoplasma insect vector control and the side effects of chemical control on agricultural ecosystems, author of more than 50 publications, and a reviewer for more than 10 international journals listed in WOS/SCOPUS databases, as well as for national and international institutions.

Chapter 1
Insects as Phytoplasma Vectors: Ecological and Epidemiological Aspects

Alberto Alma, Federico Lessio, and Herbert Nickel

Abstract The different aspects involved in the transmission of phytoplasmas by insect vectors (leafhoppers, planthoppers, and psyllids) are presented from an ecological point of view. The epidemiology of phytoplasma-associated diseases is a consequence of the vectors' ability in acquisition, inoculation, dispersal, survival, host range, and habitat colonization. Within the same vector species, acquisition efficiency may depend on the phytoplasma load in source plants and on the vectors' life instar (nymphs *versus* adults). Inoculation may occur earlier or later in the season, depending on the availability of phytoplasma sources and/or possible presence of transovarial transmission. Monophagous and oligophagous species are generally more efficient vectors than polyphagous ones. Among grass feeders, many vector species are considered oligotopic. Ecotones, plant patches, and plant architecture affect the movement and survival of vectors. Vectors' flight activity and spatial distribution, which may differ depending on gender, affect the spread of phytoplasmas and their epidemics may follow an open or a closed cycle. Five examples of diseases, with different phytoplasma cycles (open/closed) and one or more insect vectors involved, are presented: grapevine "flavescence dorée", Palatinate grapevine yellows, grapevine "bois noir" and maize redness in Europe; aster yellows in USA; sugarcane white leaf yellows in South-East Asia; and coconut lethal yellowing in North and Central America.

Keywords Transmission · Open and closed cycles · Host-range · Habitat · Dispersal

A. Alma (✉) · F. Lessio
Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Entomology unit, University of Torino, Grugliasco, Italy
e-mail: alberto.alma@unito.it

H. Nickel
Freelance scientist, Göttingen, Germany

1.1   Introduction

The epidemics of phytoplasma diseases are a consequence of the network formed by pathogens, plants, insect vectors, environmental factors, and farm management. However, although many other aspects of phytoplasma diseases have been widely covered in previous studies, the influence of the vector ecology has often been overlooked. Therefore, insect vectors of phytoplasmas in their ecological and epidemiological aspects are presented in this chapter. The key point will be the transmission process, from an epidemiological point of view, focusing on the sources and sinks of phytoplasmas in insects and/or plants, and on the environmental factors affecting vectors' populations and therefore phytoplasma spread. Five examples of phytoplasmas' epidemics which involve different host plants (e.g. mono and dicothyledons, trees *versus* herbaceous), different plant associations, in different parts of the world, and having different life cycles and behaviour of insect vectors will also be described.

1.2   The Transmission Process: Acquisition, Latency and Inoculation

The typical transmission process of phtyoplasmas consists of three phases: (i) acquisition access period (AAP) when phytoplasmas are sucked from the phloem sieve tubes by the vector's mouth parts (insects are infected); (ii) latency period (LP, or LAP), necessary for phytoplasma multiplication and circulation inside the insect body, including the salivary glands; and (iii) inoculation access period (IAP), when phytoplasmas are injected into the host plant (insects are infective). Therefore, each species has a typical AAP, LP, IAP. Phytoplasmas are transmitted by insect vectors in a persistent-propagative manner, requiring short AAP (a few days), long LP (weeks) and medium-short IAP (Alma et al. 2015). The pathways of phytoplasmas inside the vectors, their influence on vectors' physiology, the possibility of transovarial transmission, the mechanisms regulating the vectors' specificity, the physiology of feeding strategies, have been widely investigated (Weintraub and Beanland 2006; Wilson and Weintraub 2007; Bosco and D'Amelio 2010; Bosco and Tedeschi 2013; Alma et al. 2015) and will be covered in dedicated chapters of the present volume.

Acquisition The acquisition is mainly performed by nymphs, which hatch from the egg on host plants which are already infected (Alma et al. 2015). Nymphs of vectors (leafhoppers, planthoppers, and psyllids) are generally sedentary, moving from plant to plant only by walking or jumping. In some cases (e.g. Cixiidae) they are born and develop underground on the roots, therefore, acquisition may be successful only if adults are laying eggs directly on infected plants. A manipulation of phytoplasma infection on attracting vectors has been observed in *Scaphoideus titanus* Ball, the main vector of "flavescence dorée" (FD) phytoplasmas (16SrV-C and –D): both nymphs and adults are more frequently found on grapevine

FD-infected leaves compared to healthy ones (Chuche et al. 2016). It is not clear if the cues involved are olfactory or visual, although leafhoppers in all stages are more attracted by yellow rather than green. Acquisition may have different efficiency depending on the life instar. Fifth-instar nymphs of *Euscelidius variegatus* (Kirschbaum) are more efficient in acquiring 16SrI-B phytoplasmas from infected daisies than first instar ones, whereas no differences were found in *Macrosteles quadripunctulatus* (Kirschbaum) (Palermo et al. 2001). Nymphs of *S. titanus* are capable of acquiring FD phytoplasmas from grapevine only from the third instar on (Chuche and Thiéry 2014). This important aspect when dealing with pest management strategies may be due also to a lower phytoplasma load in grapevine plants in the early season. In fact, the application of insecticides, particularly insect growth regulators (IGR) require a good timing depending on the life cycle of *S. titanus,* in order to prevent nymphs from acquiring FD phytoplasmas from infected grapevines (Rigamonti et al. 2011; Chuche and Thiéry 2014). However, in certain environmental situations, this could be less important because of infective adults coming from external sources (Lessio et al. 2007b, 2014, 2015) (Example 1). Recent findings suggest that *S. titanus* adults may be capable of acquiring phytoplasmas and transmitting them within just 2 weeks, making pest management of nymphs with IGRs or other active ingredients less important (Alma et al. 2018). In fact, the ability of adult vectors performing AAP has been seldom tested. *M. quadripunctulatus* and *E. variegatus* acquiring the 16SrI-B phytoplasma agents of chrysanthemum yellows (CY) from infected daisy represents another case: for both species, acquisition was successful after 7 days AAP, whereas it was significantly reduced when AAP lasted only 1 day (Palermo et al. 2001). In other cases, it is not clear if acquisition is made by adults or nymphs. For instance *Haplaxius crudus* (van Duzee), the vector of coconut lethal yellowing in Florida and Mexico, is supposed to acquire the phytoplasmas in the adult stage feeding on infected coconut palms, but this aspect has not been clarified yet. The length of AAP varies depending on phytoplasmas, host plant source, and insect vector species. In *S. titanus* the AAP of FD phytoplasmas by nymphs from infected grapevine (*Vitis vinifera* L.) and broadbean (*Vicia faba* L.) is known to last approximately 7 days (Chuche and Thiéry 2014). However, the same species is able of acquiring (at the nymph stage) also 16SrI-B phytoplasmas from infected grapevines with an AAP of 3 days, and from infected daisies (*Chrysanthemum carinatum* L.) with an AAP of 1–3 days (Alma et al. 2001). Sometimes, nymphs stay overtime on the same host plant and therefore the AAP is difficult to measure, but also it is not so important from an epidemiological point of view. For instance, nymphs of *Hyalesthes obsoletus* Signoret feed and overwinter on roots of stinging nettle (*Urtica dioica* L.), where they are able to acquire '*Candidatus* Phytoplasma solani' ("stolbur") (Lessio et al. 2007a), however the length of AAP has never been investigated. Acquisition efficiency also depends on other factors. One of the most important is the phytoplasma load in host plants. In *S. titanus* nymphs, AAP's efficiency increases along with phytoplasma load in grapevine, depending on the season (Galetto et al. 2014; Roggia et al. 2014), the cultivar (Roggia et al. 2014; Bressan et al. 2005; Galetto et al. 2016), and the status of the disease (e.g. recovered plants are a poor phytoplasma source) (Roggia et al. 2014). Acquisition of more than one

phytoplasma strain is possible, at least from a physiological point of view (Alma et al. 2015). For instance, *E. variegatus* is able of acquiring and inoculating both FD and chrysanthemum yellows (CY, 16SrI-B) phytoplasmas feeding on different sources, with little competition on salivary glands colonization and no competition on transmission efficiency (Rashidi et al. 2014). However, in nature it seems more difficult for a single insect vector (especially in the nymph stage) to acquire different phytoplasmas from different plant sources. Therefore, mixed infections in the same insect under field conditions may not result in inoculation ability of both phytoplasmas, but may be due to random acquisition possibly by adults moving from one plant to another.

Latency The length of latency period depends on the multiplication kinetics of phytoplasmas in the vector's body. For instance, it has been demonstrated that 16SrI-B (CY) phytoplasma multiplies faster in *M. quadripunctulatus* (LP = 18 days) than in *E. variegatus* (LP = 30 days) (Bosco et al. 2007). Factors influencing the LP include temperature and carbon dioxide (Galetto et al. 2011) and this may result in shorter/longer LP depending on the season, with consequences on the diseases' outbreak. Recently, it has been demonstrated that adults of *S. titanus* are capable of acquiring phytoplasmas from infected broad beans, and of transmitting them to healthy broad beans after short LP (Alma et al. 2018). In this case under lab conditions, adults acquired FD phytoplasmas (subgroup 16SrV-C, FD-C) after an AAP = 7 days on experimentally infected broad bean plants, and inoculated it after only 7 days of IAP on healthy broad bean plants, with overlapping of AAP, LP and IAP. Actually, LP lasted at least 14 days including AAP and IAP (AAP + LP + IAP ≤ 14 days), whereas previously it was thought that *S. titanus* required a LP of 35–42 days, or a minimum of 21 days under laboratory conditions (Chuche and Thiéry 2014). This shorter LP may be due to many different factors; for instance, an AAP performed by adults may have permitted a higher phytoplasma intake, or temperature may have accelerated phytoplasma multiplication.

Inoculation is generally made by adults. Nymphs cannot fly and are unable to move from an infected to a healthy host plant; moreover, usually they become adults during LP. Inoculation may occur in different moments of the season, depending on the biology of vectors and their infective status. Early-season inoculation happens when adult vectors arriving into crop fields are already infective. This is possible, for instance, in migratory species like the aster yellows vector leafhopper, *Macrosteles quadrilineatus* (Forbes), which arrives in Ohio from Southern States as migrating infective females responsible of triggering the epidemics in lettuce crops (Hoy et al. 1999). Another chance of early inoculation occurs when phytoplasmas are transmitted to the progeny. Transovarial transmission allows the vector to maintain a source of inoculum throughout generations, without relying on host plants as sources. This is particularly important for phytoplasmas affecting annual crops. One of the most important examples is the sugarcane white leaf disease: phytoplasmas (16SrXI-B group) are maintained transovarically by *Matsumuratettix hiroglyphicus* (Mastumura). On the other hand, when phytoplasmas are acquired by insect vectors

within the growing season, the starting of inoculation depends mainly the length of latency period. For instance, when *S. titanus* acquires FD phytoplasma in the nymph stage, it takes generally 4 weeks to complete LP and to turn into adults capable of flying and spreading the disease (Chuche and Thiéry 2014). Inoculation takes place from the end of July and is more frequent in August and September because of the higher phytoplasma load in insects. However, since new findings have demonstrated that under laboratory conditions *S. titanus* adults are capable of completing AAP + LP + IAP on broad beans within 2 weeks (Alma et al. 2018), inoculation may occur following AAP by adults on grapevine regardless of the growing season. The major concerns are about late summer and beginning of autumn, before harvest, when no or little insecticides are sprayed.

1.3 Host Plants of Insect Vectors

Host range and host plant species The degree of specialization or plasticity of vectors with respect to their host plants drives their ability of spreading phytoplasma diseases. According to Nickel and Remane (2002) and Nickel (2003), Hemiptera are classified as follows depending on their host range: first degree monophagous (m1): 1 plant species; second degree monophagous (m2): 1 plant genus; first degree oligophagous (o1): 1 plant family; second degree oligophagous (o2): 2–5 plant families, or up to 5 plant species; polyphagous (p): other. Monophagous species (m1 and m2) are often more efficient vectors than polyphagous ones, leading to a closed epidemiological cycle (Constable 2010; Alma et al. 2015). One of the most important cases is *S. titanus*, a grapevine specialist that may be considered as m2 since its host plants include *V. vinifera*, and other *Vitis* species as well as rootstock hybrids (Chuche and Thiéry 2014). Generally, specialist insects are more likely to be reduced by increased management pressure e.g. pesticides and/or mowing, whereas polyphagous species are more likely to adapt. This goes for instance about *Psammotettix alienus* (Dahlbom), another vector of CY (16SrI-B) (Alma et al. 2015), which becomes dominating in low biodiversity habitats, whereas *H. obsoletus* and *S. titanus* are negatively influenced by it (Trivellone et al. 2012).

After Nickel (2003) who analysed the Auchenorrhyncha fauna on the Central European flora, species-rich plant families such as Poaceae, Cyperaceae, Rosaceae and Asteraceae also, tend to have more Auchenorrhyncha species than species-poor plant families, which may be explained by a facilitated insect radiation on closely-related host plants. Even more important is the host plant size, with trees generally holding a higher load of insects than most herbaceous plants. The proportion of monophagous species (first and and second degree together) is highest on Pinaceae, Salicaceae, Cyperaceae, and Poaceae. This pattern of host specialisation is difficult to interpret and may be caused by different factors for different plant families, e.g. secondary compounds in the former two and neural constraints (Bernays 2001) in the latter.

According to the "Resource Concentration" hypothesis, an insect should tend to remain in dense stands of its host plant (Root 1973). Therefore, specialists rely on plants that are capable of dominating, whereas generalists may be more likely of exploiting assemblages of diverse host plants. Concerning vectors, the majority of oligo- and polyphagous species are associated to herbaceous hosts. Some species live on weeds, although adults may occasionally move to trees and shrubs: this happens with *H. obsoletus* moving from nettle and bindweed (*Convolvolus arvensis* L.) to grapevine (Alma et al. 1988; Weber and Maixner 1998; Lessio et al. 2007a). The same behavior may be observed in *Dictyophara europaea* (L.), an occasional vector of 16SrV phytoplasmas to grapevine (Filippin et al. 2009), which lives on many weeds including pigweed, *Amaranthus retroflexus* L., and occasionally moves onto grapevines (Lessio and Alma 2008; Krstic et al. 2016). *Euscelis incisus* (Kirschbaum) is polyphagous on grasses and weeds, but it transmits 16SrIII-B phytoplasmas following an open epidemiological cycle from *Lathyrus* spp. (source) to *Cirsium arvense* L. (sink) (Jakovljevic et al. 2015). Another species, *Neoaliturus fenestratus* (Herrich-Schaeffer) transmits 16SrII phytoplasmas to *Picris hieracioides* L. plant-to-plant (closed epidemiological cycle), but only the second generation seems to have vector ability (Mitrovic et al. 2012). *N. fenestratus* may be considered a first-degree oligophagous (o1), feeding and breeding mainly on species in the family Asteraceae (Nickel and Remane 2002; Minuz et al. 2013; Lessio et al. 2017b). Oligophagous species seem to be quite sensitive to plant, and particularly to sward, architecture (Blake et al. 2011), and their fluctuations in time may be due to changes in vegetation height and cover. An exception is *Orientus ishidae* (Matsumura), a polyphagous species related to many trees and shrubs (Lessio et al. 2016). To date, there is no evidence that populations of *O. ishidae* are different from one host to another. In fact, it may be found in great numbers on single trees or shrubs, provided that food resources remain available. *Fieberiella florii* (Stål), an occasional vector of the apple proliferation phytoplasma (16SrX-A) (Tedeschi and Alma 2006) is also considered polyphagous, feeding and breeding on trees and bushes of many broad-leaf species, including especially Rosaceae (Nickel and Remane 2002). Both *O. ishidae* and *F. florii*, however, are considered as occasional vector species.

Pioneers, aurytopic, oligotopic and stenotopic species Regarding their life strategies, grassland leafhoppers and allies can be divided into four subgroups, respectively: (i) pioneer species (highly mobile generalists, polyphagous or broadly oligophagous), (ii) eurytopic species (widespread generalists, usually oligophagous), (iii) oligotopic species (specialists of certain habitats, usually oligophagous), and (iv) stenotopic species (specialists of habitats, monophagous) (Nickel and Achtziger 2005). Among vectors, oligotopic species include *H. obsoletus*, whereas *E. incisus* is eurytopic and *P. alienus*, *Macrosteles cristatus* (Ribaut) and *M. laevis* (Ribaut) are pioneers (Trivellone et al. 2012; Nickel and Achtziger 2005). On the other hand, *Macrosteles septemnotatus* (Fallén) is reported as stenotopic (Nickel and Achtziger 2005), however its vector ability has not been proven. It is possible that few or no stenotopic species are phytoplasma vectors because of their strict host and environmental needs. From an evolutionary point of view, this may result in

little vector efficiency as stenotopic species are less likely to build up great populations, which makes it difficult to spread phytoplasmas.

1.4   Movement and Dispersal

Ecotones and movement of insect vectors among habitats   Phytoplasma diseases are not restricted to a single host plant or crop. Because of the movement of vectors, the same phytoplasma may be carried from one plant species to another, and from one patch to another within the same ecosystem or even – through aerial drift – across long distances up to hundreds and thousands of kilometres. The proximity of habitats may influence the movements of vectors between crops or from spontaneous vegetation to crops. This happens sometimes for tree feeders moving between different host plants depending on their degree of specialization/plasticity. It is the case of *S. titanus* which moves from wild to cultivated grapevine (Lessio et al. 2014), of *O. ishidae* from hazelnut or willow to grapevine (Lessio et al. 2016), and of *Oncopsis alni* (Schrank) moving from alder to grapevine (Maixner and Reinert 1999). A more specialized alternation of tree hosts is observed in many psyllids. For instance, *Cacopsylla melanoneura* (Foerster) and *C. picta* (Foerster), vectors of apple proliferation, overwinter on pine trees (family Pinaceae) and move to apple (*Malus* spp.: *C. melanoneura* and *C. picta*) and/or hawthorn (*Crataegus* spp.: only *C. melanoneura*) trees for feeding and breeding (Lauterer 1999; Tedeschi et al. 2009; Alma et al. 2015). On the other hand, *Cacopsylla pyricola* (Foerster) and *Cacopsylla pyri* (L.) are considered monophagous (m2), being related only to pear trees (*Pyrus* spp.) (Lauterer 1999; Tedeschi et al. 2009; Alma et al. 2015). In many other cases, feeders of grasses and herbs are compelled to move on trees or shrubs when the herb layer dries up during the hot season. In Israel, *H. obsoletus* completes two generations per year, and the adults of the second one move onto grapevine because the weeds disappear due to drought stress (Orenstein et al. 2003). As well, in Europe it moves from nettle to grapevine (Mori et al. 2015) or from clary sage (*Salvia sclarea* L.) to lavender (Hossard et al. 2018) although in the latter case there is no grass/tree host alternation. *H. crudus* in the nymph stage lives on the roots of grasses within coconut plantations, whereas the adults move on palm leaves to feed and mate. In this case, it seems however that the movement of adults on palm trees is compulsory for this species to feed and mate. Many other species are known to feed occasionally on grapevine in the adult stage, apart from the already-cited *D. europaea* (Lessio and Alma 2008), and *N. fenestratus* (Bosco et al. 1997; Minuz et al. 2013). *P. alienus* relies mainly on monocotyledons (Gramineae) (Lindblad and Areno 2002; Nickel and Remane 2002; Manurung et al. 2005; Landi et al. 2013). Since soil coverage of vineyards influences and enhances the presence of some leafhopper species (Mazzoni 2006), *P. alienus* is more abundant in vineyards with a grassy (monocotyledon) ground cover. *E. variegatus* is also highly polyphagous (Nickel and Remane 2002) and develops on a wide range of host plants either in field margins or in the vineyard interrow (Lessio et al. 2017b). However, both *E. variegatus* and *P. alienus* are not caught in great numbers on the grapevine canopy

(Bosco et al. 1997). Some species, on the other hand, lay eggs on woody plants but nymphs move onto grasses. This is the case of *Anoplotettix fuscovenosus* (Ferrari) which lays eggs under the bark of grapevine but nymphs move on grasses in the interrow lanes (Alma 1995; Alma et al. 2015). Recently, it has been demonstrated that *S. titanus* nymphs may leave grapevine and move on weeds, especially *Trifolium repens* L. and *Ranunculus acris* L., in the inter-row (Trivellone et al. 2013) that may however, not be considered as host plants since no oviposition occurs. Habitat diversification has an influence on the dispersal and abundance of vectors: for instance, the aster leafhopper *M. quadrilineatus* is more abundant in carrot fields when surrounded by spelt (*Triticum spelta* L.) or other cereals with respect to broadleaf weeds (Szendrei 2012), and *Psammotettix* spp. becomes a dominant species due to a loss of habitat conservation (Hollier et al. 2005; Trivellone et al. 2012).

The influence of plant patches and plant architecture Other important factors affecting vectors' populations are the size and shape of plant patches and the diversification of vegetation layers. In other terms, they are influenced by both horizontal and vertical distribution of their host plants. Species that lay eggs into the ground are usually favored by patches of bare soil, alternated to plant swards. This is the case of *D. europaea*, polyphagous and feeding on many weeds (Lessio and Alma 2008; Krstic et al. 2016). This species is common in xerothermic habitats with isolated grass patches and portions of bare soil, used for laying eggs (Nickel and Remane 2002), and may move on trees and shrubs such as wild and cultivated grapevine (Lessio and Alma 2008) and *Clematis vitalba* L. (Krstic et al. 2016) during the dry season. Other important factors influencing leafhoppers and planthoppers communities are the diversification of the vegetation layer, and the structure of plant swards, the latter having an influence especially on oligophagous species (Blake et al. 2011). For instance, in pastures, *M. laevis* and *E. incisus* respond positively to sheep grazing as they rely mainly on short sward architecture (Brown et al. 1992). On the other hand, structural complexity of plants promotes diversity of associated insect guilds. For instance most tussock grasses have more leafhoppers than non-tussock grasses and tall tree species have more than smaller ones (Nickel 2003).

The flight boundary layer A definition of the "flight boundary" layer in insects, especially leafhoppers, was given by Taylor (1974), as "a layer of air where the flight speed of insects is faster than wind speed", and therefore insects are capable of active movement. Species relying on grasses and weeds, usually are not caught in great numbers higher than ground level. This has been observed on grapevine for *N. fenestratus, H. obsoletus, Euscelis* sp. and *P. alienus* (Minuz et al. 2013), and also for *D. europaea* (Lessio and Alma 2008).

Movement of the insect vectors is one of the most important factors influencing epidemiology of phytoplasma diseases. Leafhoppers and planthoppers tend to disperse mainly in the adult stage. Two main patterns may be distinguished: "free-air"

movement, and "road map" movement. The former happens when a vector species disperses in any direction, regardless of the environmental constrictions. It is typical for grass-feeding species, and for small species that tend to rely on wind. On the other hand, tree-dwellers are not likely to be often carried by the wind, and rely mainly on active movements. In particular, they need the presence of ecological corridors to move along. It is the case of *S. titanus*, that needs a network of wild grapevine for moving along grapevine-growing areas (Lessio et al. 2014). As well, the intercropping lettuce/endive or lettuce/escarole limits the movement of *M. quadrilineatus* with respect to lettuce monoculture (Zhou et al. 2003).

Spatial patterns of plants and insect vectors Spatial distribution of vectors often reflects the displacement of their own host plants. *S. titanus* adults are generally aggregated at the edges of vineyards, because of specimens moving from neighbouring stands of wild grapevines (Lessio et al. 2014; Pavan et al. 2012). Moreover, generally they are more likely to infest the same rows within the vineyard rather than moving from row to row (Lessio et al. 2009a). As well, *O. ishidae* adults are more concentrated at the edges of vineyards because they are moving from host plants outside e.g. willow and hazelnut. However, in this case fewer individuals may be found within the vineyard because grapevine is not a favourite host plant (Lessio et al. 2016). Other species which have a spatial distribution matching that of their own host plants are those living on grasses and weeds: *N. fenestratus,* and *P. alienus* (Minuz et al. 2013), and also *H. obsoletus* (Mori et al. 2012; Minuz et al. 2013).

1.5 The Influence of Vector Sex Ratio on Phytoplasma Epidemics

A different feeding and/or dispersal behaviour between genders may have an influence on the epidemics of phytoplasma diseases. Generally males of leafhoppers and planthoppers hatch earlier in the season than females. In *S. titanus,* the *sex ratio* is more male-biased at the beginning of the season, whereas in late summer and autumn many more females are found (Lessio et al. 2009b). Since the phytoplasma load in grapevines increases with the growing season, late born nymphs and/or late emerged adults (mainly females) are more likely acquiring them. Moreover, females have a longer lifespan compared to males (70 *versus* 50 days) (A. Alma, unpublished), consequently they are capable of transmitting for a longer period of time. Females of *M. quadrilineatus* are more likely transmitting agents of aster yellows diseases (16SrI) with respect to males; however, they spread less than males in lettuce fields, and this results in a more clustered infection pattern in crops (Beanland et al. 1999). In particular, mated females are much more sedentary, whereas unmated ones are able of performing vertical flights, and males are more engaged in local movements (Hoy et al. 1999).

1.6 Modelling the Epidemics

The challenge of modelling epidemics of phytoplasma diseases and their vectors has been faced many times, with respect both to space (spatial models, diffusion models, etc.) and time (phenology models, outbreak models, etc.). Post-embryonic development of *S. titanus* has been modelled as a function of temperature, in order to make pest management more efficient (Rigamonti et al. 2011; Falzoi et al. 2014). A similar model was applied to *C. pyri* (Schaub et al. 2005). An attempt was also made to predict infestations of *S. titanus* during different seasons (Maggi et al. 2013). Other models have been elaborated to forecast FD epidemiology. A deterministic model taking into account the influence of different factors such as recovery, plant replacement, insecticidal sprays, and the presence of hotbeds has been developed. In this model, the insect vector is not present explicitly, but has been introduced as a coupling factor between healthy and infected grapevines (Lessio et al. 2015). A stochastic epidemiological model was implemented taking into account the variations of *S. titanus* within the vineyard due to survival from one year to another, but without considering immigration sources (Maggi et al. 2014). The infection of carrots by aster yellows has been modelled too, as a function of the abundance and infectivity of *M. quadrilineatus*, the main vector of this phytoplasma in the study area (Frost et al. 2013). Finally, another important issue is the forecast of the potential spread of a vector when introduced into a new geographical area. Models of this kind have been proposed about the spread of *S. titanus* in China (Ge and Wen 2006) and Chile (Quiroga et al. 2017).

1.7 Open *versus* Closed Cycle

The epidemiology of phytoplasmas may follow either an open or a closed cycle. Open cycles occur when the phytoplasmas are transmitted by vectors from one plant species (source) to another (sink, or dead-end), and not the other way round. Conversely, closed cycles occur when the phytoplasmas are transmitted always between host plants of the same species (Constable 2010; Alma et al. 2015). However, a phytoplasma rarely follows just one type of cycle: in many cases, both cycles are present, although they may rely on different insect vectors. Feeding specialization has an influence on vector ability. Generally, monophagous vectors lead to a closed epidemiological system of the pathogen, with AAP, LP and IAP spent on the same plant species, whereas polyphagous species are more likely to become occasional vectors, within the frame of an open epidemiological system (Alma et al. 2015). Five examples are presented below about phytoplasma epidemics. A summary of phytoplasma groups and vectors, type of cycle, overwintering strategy of phytoplasmas, and plant source is given in Table 1.1.

Example 1 "Flavescence dorée" phytoplasmas and other phytoplasmas of group 16SrV in grapevine: one vector for closed cycle and three vectors for open cycle.

Table 1.1 Epidemiological cycles of selected plant diseases associated with phytoplasma presence

Phytoplasma ribosomal group/ subgroup	Insect vector	Epidemiological cycle	Overwintering sources	Plant sources
16SrI-B	*Macrosteles quadrilineatus*	Closed	Migrating females	Lettuce and carrots
16SrIV	*Haplaxius crudus*	Closed	Unknown	Coconut palm trees
16SrV-C/−D	*Scaphoideus titanus*	Closed	*Vitis* spp.	
	Dictyophara europaea	Open	*Clematis vitalba*	
	Orientus ishidae	Open	Broadleaf trees?	
16SrXI-B	*Matsumuratettix hiroglyphicus Yamatotettix flavovittatus*	Closed	Transovarial transmission	Sugarcane
16SrXII-A	*Hyalesthes obsoletus*	Open	Nymphs on weeds' roots	Nettle, bindweed and other weeds
	Reptalus spp.	Open		Unknown

FD is associated with the presence of phytoplasmas enclosed in the 16SrV ribosomal group (subgroups 16SrV-C and 16SrV-D) (Martini et al. 1999). The main vector is *S. titanus* (Fig. 1.1), which has been introduced into Europe from North America in 1958 (Chuche and Thiéry 2014). It is a second-degree monophagous (m2) species, feeding and breeding on *Vitis* spp. including *V. vinifera* (European grapevine), American grapevines such as *Vitis labrusca* (L.), *Vitis riparia* Michx, and *Vitis berlandieri* Planchon, as well as rootstock hybrids (Chuche and Thiéry 2014), which are also a source of FD phytoplasmas although they might not show any symptoms (Lessio et al. 2007b). *S. titanus* is monovoltine (Fig. 1.2), and overwinters in the egg stage laid under the bark (Chuche and Thiéry 2014). During summer, adults may move from wild grapevines into vineyards (Pavan et al. 2012; Lessio et al. 2014). This behaviour has been demonstrated with mark-capture techniques, by applying a protein marker (chicken egg whites, or milk) directly on wild grapevine stands and placing traps at different distances from them (Lessio et al. 2014). The majority of adults were captured within 20 m from the source, however some individuals have covered distances up to 350 m (Lessio et al. 2014). Successive experiments showed that in a few cases *S. titanus* may travel at distances up to 2.5 km. Immigrant adults may be already infected, having acquired phytoplasmas on wild grapevines (Lessio et al. 2007b). Moreover, a recent research carried out under laboratory conditions suggested that they may acquire phytoplasmas directly from infected cultivated grapevines (Alma et al. 2018). Either way, *S. titanus* transmits FD phytoplasmas only grapevine-to-grapevine (closed cycle) (Chuche and Thiéry 2014). However, as *S. titanus* has been introduced into Europe from North America, where FD is not known to occur, it has been suggested that other vectors

Fig. 1.1 *Scaphoideus titanus*: second instar nymph (left), fifth instar nymph (middle) and adult (right) (DISAFA, Entomology unit, University of Torino, Italy)
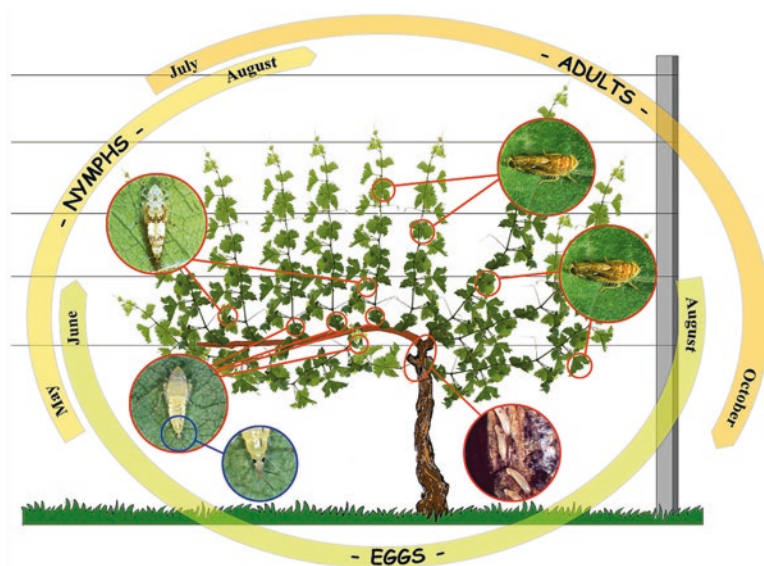
Fig. 1.2 *Scaphoideus titanus*: life cycle (Artwork L. Picciau)

had a role in introducing the phytoplasma from wild plants to grapevine (Belli et al. 2010). In fact, FD phytoplasma may also rely on insect vector species that are bound to other host plants but are able of moving and feeding on grapevines too. These are the cases of *O. alni* (Maixner et al. 2000), *D. europaea* (Filippin et al. 2009), and *O. ishidae* (Lessio et al. 2016). Just like *S. titanus,* all these species are monovoltine and overwinter in the egg stage.

O. alni (Fig. 1.3) lives on alder maintaining alder yellows phytoplasma (AldY) in a closed cycle, but is able to move and feed on grapevines where in Germany the phytoplasma is associated with Palatinate grapevine yellows disease (PGY) (Maixner and Reinert 1999). However, this movement is not so frequent since *O. alni* is a second-degree monophagous species (m2) being strictly associated with alder (Nickel and Remane 2002), therefore it is likely that other vectors are involved in transmitting PGY from alder to grapevine. The genetic variability of AldY has been recently reviewed by Hotz et al. (2016), and a recent survey (Jarausch et al. 2017) has demonstrated that phytoplasmas in the 16SrV group were found at high rates (65%) in *Allygus* spp. and *O. ishidae*.

D. europaea (Fig. 1.4) is able to transmit 16SrV-C phytoplasmas from *C. vitalba* to grapevine under experimental conditions (Filippin et al. 2009). In Piedmont (North West Italy), it was found infected mainly by 16SrV-C phytoplasmas (Gonella et al. 2017). However, adults do not move very often onto grapevine (Lessio and Alma 2008; Lessio et al. 2017b). Eggs are laid into the soil, especially in presence of bare ground patches, and host plants include many grasses and weeds e.g. *A. retroflexus, Solidago canadensis* L., and *Urtica dioica* L. (Lessio and Alma 2008; Krstic et al. 2016). *D. europaea* is considered therefore as a polyphagous species (Nickel and Remane 2002). When placing yellow sticky traps on the grapevines' canopy, few adults are captured (Lessio and Alma 2008). A recent research on

Fig. 1.3 *Oncopsis alni* (Schrank): nymph (left) and adult (right) (Courtesy of G. Kunz)

Fig. 1.4 *Dictyophara europaea* (L.): nymph (left) and adult (right) (Courtesy of G. Kunz)

leafhopper fauna of foraging strip crops (a mix of alfalfa and other legumes) close to vineyards demonstrated that *D. europaea* is abundant only if the strip is poorly covered by the seed mix, and infested by weeds; on the other hand, it is absent or irrelevant when the seed mix provides a good cover of the strip (Lessio et al. 2017b). For these reasons, *D. europaea* may not be considered as an important vector of FD phytoplasmas to grapevine.

Finally, *O. ishidae* (Fig. 1.5) is an Asian species, introduced into the USA in the early nineteenth century, probably along with ornamental plants of the genus *Aralia* sp., and reported for the first time in Europe (Switzerland) in 2002 (Guglielmino 2005). Its importance has been overlooked until some specimens were found to be positive to 16SrV phytoplasmas in Slovenia (Mehle et al. 2010), and in northern Italy (Gaffuri et al. 2011; Zambon et al. 2018). Recently its ability of transmitting 16SrV-C phytoplasmas from broad bean to grapevine under laboratory conditions has been demonstrated (Lessio et al. 2016). At the moment, sources of phytoplasma inoculum for *O. ishidae* in nature are still uncertain. Nymphs are able of acquiring FD phytoplasmas from infected grapevine, too (Lessio et al. 2016), and they have also hatched from eggs laid under the bark of wild and cultivated grapevine under laboratory conditions (Lessio et al. 2016, 2017a). However, nymphs were seldom found on grapevine leaves (Lessio et al. 2017a), which makes an acquisition of FD phytoplasmas less likely from this source. This leafhopper is polyphagous, relying on many trees and shrubs including willow, hazelnut, birch, hornbeam, apple, plum, bramble, and so on (Lessio et al. 2016) (Fig. 1.6). Recently, the same phytoplasmas have been detected both in specimens of *O. ishidae* and in willow and hazelnut (Casati et al. 2017). High populations of *O. ishidae* were also found on *Ailanthus altissima* L., which is also a host for 16SrV phytoplasmas (Filippin et al. 2011; Forte et al. 2013). It is therefore likely that this leafhopper is responsible for an open epidemiological cycle of FD phytoplasmas, by an AAP from other plant sources and IAP to grapevine, although further studies are needed to confirm this hypothesis.

All these species (apart from *O. ishidae*, which is not native from Europe) may have triggered the infection on grapevines moving from their own host plants. Then, when *S. titanus* appeared in European vineyards, the newly-born vector-pathogen association led to heavy disease outbreaks.


Fig. 1.5 *Orientus ishidae*: nymph (left) and adult (right) (Courtesy of G. Kunz)
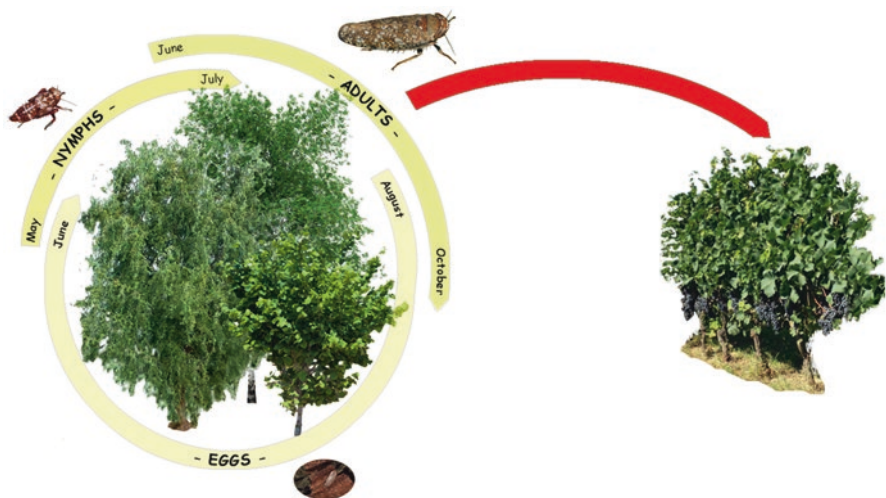
Fig. 1.6 *Orientus ishidae*: life cycle (Artwork L. Picciau)

Example 2 "Bois noir" (BN) in grapevine: same vector(s), open and closed cycle on different host plants. In other cases, the same vector is responsible for maintaining the same phytoplasma in both open and closed cycles. This happens with *H. obsoletus* (Fig. 1.7) and "stolbur" phytoplasmas (16SrXII-A), transmitted plant-to-plant to bindweed and to stinging nettle (closed cycles), and occasionally from the former host plants to grapevine (open cycle) (Sforza et al. 1998; Weber and Maixner 1998; Lessio et al. 2007a). Hence, weeds may be a reservoir for BN phytoplasma, favouring its diffusion in vineyards, and grapevine is a dead-end host for this pathogen. In Israel, where no other sources of BN phytoplasmas have been detected, grapevines are supposed to act as reservoirs (Sharon et al. 2015). However, no current evidence of grapevines being a source of inoculum for *H. obsoletus* are available. Indeed, while nettle, bindweed, and also lavender (Hossard et al. 2018) are the main host plants for *H. obsoletus* in Europe, in Israel this planthopper builds up great populations on *Vitex agnus-castus* L. (Sharon et al. 2005; Zahavi et al. 2007). However, this plant is not a source of phytoplasmas and may also be used in push-and-pull strategies for controlling *H. obsoletus* (Zahavi et al. 2007). BN may also be transmitted by *Reptalus panzeri* (Löw) and *Reptalus quinquecostatus* (Dufour) (Fig. 1.8) (Alma et al. 2015). However, to date the host plants for nymphs of these species are still little known. Adults are found on many trees and shrubs including willow, hornbeam, elm (Picciau et al. 2008), but the sources of inoculum remain unclear. An intermediate kind of cycle concerns maize redness (MR), associated with the presence of the same phytoplasma. In Serbia, MR is transmitted by *R. panzeri* which relies on the rotation between corn and wheat. Infective adult planthoppers feed and lay eggs on corn during summer, and nymphs perform AAP on roots of infected plants. However, when corn is harvested, it is replaced by wheat, which provides food for nymphs performing LP. Adults emerge at the end of the spring and the cycle starts again (Jovic et al. 2009).
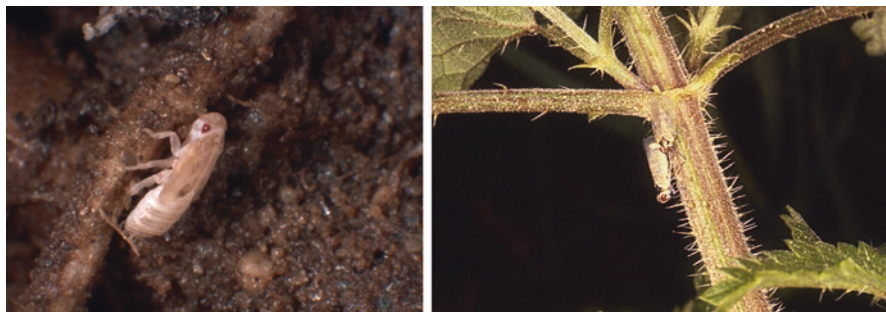
Fig. 1.7 *Hyalesthes obsoletus*: nymph (left) and adults (right) (DISAFA, Entomology unit, University of Torino, Italy)

Fig. 1.8 *Reptalus panzeri*: nymph (left) and adult (middle), and *Reptalus quinquecostatus* adult (right) (Courtesy of G. Kunz)

Example 3 Aster yellows phytoplasmas: a migrating vector. Aster yellows phytoplasmas (AY), belonging to 16SrI ribosomal group, include a huge number of subgroups and are responsible for infecting a wide number of host plants (Duduk and Bertaccini 2009; Alma et al. 2015). They are transmitted worldwide by many different insect vectors. In the European and the Mediterranean area, ten or more species are listed including members of the genera *Macrosteles, Euscelidius, Euscelis* (Alma et al. 2001, 2015)*, N. fenestratus, Psammotettix* spp. (Alma et al. 2015), as well as *Empoasca decipiens* Paoli and *S. titanus* under laboratory conditions, the former with a very low transmission efficiency and possibly without any significance in the field (Alma et al. 2001, 2015). However in the USA the main vector appears to be *M. quadrilineatus*, which is absent in Europe. *M. quadrilineatus* (Fig. 1.9) is a migratory species: overwintering females, bearing AY phytoplasmas, move from Texas and other south States to Ohio, and are responsible for a primary transmission on vegetable crops such as carrot and lettuce (Hoy et al. 1992, 1999;

Fig. 1.9 *Macrosteles quadrilineatus* adult (Courtesy of T. Murray)

Beanland et al. 2005). Immigrant females are clustered at the edges of crop fields, according to patterns of AY infected plants in early spring (Beanland et al. 2005). Moreover, females appear to be more efficient vectors than males at least under laboratory conditions (Beanland et al. 1999). Meanwhile, resident populations over-winter in Ohio in the egg stage, taking advantage of winter cover crops (small grains) (Hoy et al. 1992). These populations, however, do not bear phytoplasmas as no transovarial transmission occurs. Afterwards, insects from the incoming generations start transmitting AY phytoplasmas plant-to-plant. In this case, LP is shorter and permits a closed epidemiological transmission cycle. A similar result has been noted in *M.quadripunctulatus*, which has a LP of 18 days for AY phytoplasma whereas *E. variegatus*, another vector, has a LP of 30 days (Bosco et al. 2007).

Example 4 Sugarcane white leaf phytoplasmas, an insect vector with transovarial transmission and a synergy between two vectors. Sugarcane white leaf disease (SCWL or SWL) is one of the most threatening diseases of sugarcane (*Saccharum officinarum* L.) in South-East Asia. It is associated with the presence of phytoplasmas in the 16SrXI-B ribosomal group (rice yellow dwarf) (Soufi et al. 2013; Zhang et al. 2016). Demonstrated insect vectors are the two leafhoppers *Matsumuratettix hiroglyphicus* (Matsumura) (Hanboonsong et al. 2002) and *Yamatotettix flavovittatus* Matsumura (Hanboonsong et al. 2006) (Fig. 1.10). Transmission seems to occur plant-to-plant in sugarcanes, without alternative hosts since phytoplasmas identified in alternative host plants (e.g. grasses) are genetically different. Therefore, the epidemiology of SCWL must be considered as a closed cycle. However, since sugarcane has an annual cycle, the survival of inoculum must take place elsewhere. In fact, phytoplasma agents of SCWL are transmitted to the progeny of *M. hiroglyphicus* (Hanboonsong et al. 2002). The two insect vector species act somehow in synergy. In Thailand, the flight peak of *M. hiroglyphicus* in sugarcane fields occurs approximately a month earlier with respect to *Y. flavovittatus* (April *versus* May).

Although both species share similar efficiencies of SCWL transmission (55% *versus* 45%), the former is more capable of inoculation to young sugarcane plants than the latter. Therefore, SCWL epidemics in sugarcane plantations is triggered by *M. hiroglyphicus,* but is continued by *Y. flavovittatus* (Hanboonsong et al. 2006).
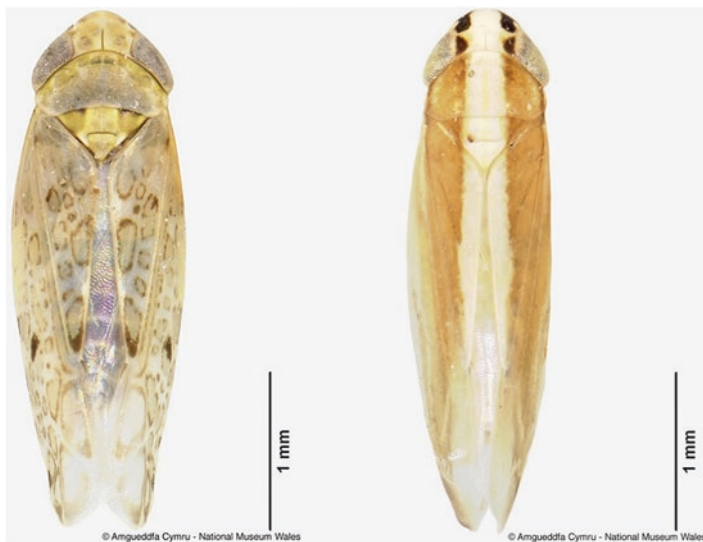
Fig. 1.10 *Matsumuratettix hiroglyphicus* (left), and *Yamatotettix flavovittatus* (right) adults (Courtesy of A. Cymru)

Dispersal is also different between the two vector species: *Y. flavovittatus* is able to travel downwind at twofold distances than *M. hiroglyphicus* (380 *versus* 160 m) (Thein et al. 2012). Many other leafhopper species are known to bear SCWL phytoplasmas, but their vector ability has not been proven, besides the fact that their biology e.g. abundance in sugarcane crops do not make them likely to be putative vectors. *Exitianus indicus* (Distant) was the only tested species (along with the two proven vectors), and did not transmit SCWL phytoplasmas in Thailand (Hanboonsong et al. 2006).

Example 5 Coconut lethal yellowing: closed cycle for phytoplasmas, different host plants for the insect vector. Phytoplasma-associated diseases in palms have been detected in the Southern States of the USA (Texas, Florida, and Louisiana), Mexico and Caribbean, Western and Eastern Africa, Australasia and Oceania (Gurr et al. 2016; Bander et al. 2017; Harrison et al. 2008; Harrison and Elliott 2016; Myrie et al. 2007). Phytoplasma diseases of coconut palm, *Cocos nucifera* L., belong mainly to subgroups in 16SrIV ribosomal group (Brown et al. 2007; Gurr et al. 2016). Among different phytoplasma diseases of palm trees, the most widely studied is coconut lethal yellowing (CLY) for which the only reported insect vector is *H. crudus* (Fig. 1.11). This planthopper is heterovoltine: nymphs live on roots of grasses such as Bermudagrass (*Cynodon dactylon* L.) and especially St. Augustine grass [*Stenotaphrum secondatum* (Walt.)]] within coconut plantations, whereas adults move onto the leaves of palms for feeding and mating, and females go back to grasses for egg laying (Tsai and Kirsch 1978). In this case, grasses do not seem to be a reservoir for phytoplasmas. It is therefore possible that AAP is performed by