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Biology and integrated management of leafhoppers and phytoplasma diseases in vineyards of eastern Canada

Technical Bulletin

DIVISION DE AGCORD

Canada

Biology and integrated management of leafhoppers and phytoplasma diseases in vineyards of eastern Canada

Authors:

- **Julien Saguez** (Ph.D.), Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada. saguezj@yahoo.com
- **Chrystel Olivier** (Ph.D.), Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada. chrystel.olivier@agr.gc.ca
- **Jacques Lasnier**, Président, Co-Lab R&D, div. Ag-Cord Inc., Granby, Quebec, Canada. jlasnier@translog.ca
- **Andy Hamilton** (Ph.D.), Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada. andy.hamilton@agr.gc.ca
- **Lorne Stobbs** (Ph.D.), Agriculture and Agri-Food Canada, Vineland, Ontario, Canada. lorne.stobbs@agr.gc.ca
- **Charles Vincent** (Ph.D.), Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada. charles.vincent@agr.gc.ca

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FOREWORD

Grape growers in eastern Canada are faced with a variety of insect pests and diseases that are still largely unfamiliar and that could have adverse effects on the yield of their vineyards. Leafhoppers, one of the many insect pests of grapevines, not only feed directly on grape foliage but also are vectors of pathogens that cause various diseases, including phytoplasma diseases.

This technical bulletin is designed to aid in the identification of the main species of leafhoppers and reservoir plants associated with eastern Canadian vineyards and in the detection of phytoplasma diseases. It presents methods for managing leafhopper populations and reducing the risk of phytoplasma transmission. It also briefly outlines rearing and analysis techniques to help the reader understand how the grapevine–leafhopper–phytoplasma system is being studied.

Table of contents

List of Figures

List of Tables

Introduction

In Canadian vineyards, leafhoppers can be serious pests, particularly when populations reach high densities. Since 2006, several entomologists from Agriculture and Agri-Food Canada have worked together under various research programs to assess leafhopper biodiversity and the prevalence of phytoplasma diseases in Canadian vineyards. This work was carried out with the collaboration of many vineyards and various private sector companies, notably Co-Lab R&D, a division of Ag-Cord Inc.

Leafhoppers may carry pathogens that can affect plant health, including phytoplasmas, which are bacteria-like plant pathogens in the class of Mollicutes. Phytoplasmas are essentially controlled using indirect methods, because there is currently no commercially available plant protection product registered for their control in plants in Canada. Phytoplasmas are difficult to study because their culture is almost unrealizable under laboratory conditions (except one experimental case recently reported). Phytoplasma diseases may affect many plant families. In grapevines they are named “grapevine yellows”.

This technical bulletin begins with a brief overview of eastern Canada’s grape-growing industry, followed by information on the life cycle and biology of leafhoppers in vineyards. Illustrated factsheets are presented for the most abundant and economically important leafhopper species of Eastern Canada. Next is a section on the damage caused by leafhoppers and the mode of transmission of phytoplasmas, with illustrations of their effects on plants. This bulletin also provides information on the methods of collecting insects, scouting plants that are potentially infected by phytoplasmas, and detecting phytoplasmas in leaf and insect samples. The bulletin includes a section on regulatory aspects and on phytosanitary measures to control phytoplasmas. Lastly, the bulletin discusses various techniques to aid in understanding the specificities and challenges involved in the study of the tritrophic grapevine–leafhopper–phytoplasma system (Saguez et al. 2009).

This technical bulletin, which is intended for grape growers, agronomists, sector stakeholders, technicians, and students, summarizes the results of studies conducted in recent years by the authors’ team (see also Olivier et al. 2010; Saguez et al. 2014; Vincent et al. 2015) and presents a number of developments in phytoplasma disease research in vineyards. It will address how to identify the main species of leafhoppers that are associated with Canadian vineyards and are known phytoplasma vectors, how to recognize plants that are phytoplasma reservoirs, and how to select methods used in scouting for leafhoppers and phytoplasma diseases in vineyards.

1. Viticulture in eastern Canada

In eastern Canada, grapes are grown in Ontario, Quebec, Nova Scotia, New Brunswick, and Prince Edward Island. In 2010, the total area planted to table and wine grapes was 8,249 ha, with a total marketed production of 64,027 tons and a farm gate value of $76.1 million (Table 1).

Table 1: Cultivated area, marketed production, and farm gate value of grapes in Canada (Source: Statistics Canada 2012)

	Cultivated area (ha)			Marketed production (tons)			Farm gate value ($M)		
Province	2000	2005	2010	2000	2005	2010	2000	2005	2010
Ontario	6 313	7 325	7 133	55 000	28 950	61 759	44,12	23,78	72,32
Quebec	107	227	612	575	663	1,303	0,75	0,44	2,45
Nova Scotia	—	111	466	—	575	904	—	0,75	1,24
New Brunswick	—	0	29	—	0	45	—	0	0,06
Prince Edward Island	—	0	9	—	0	16	—	0	—
Canada	6 420	7 663	8 249	55 575	30 188	64 027	44,87	24,97	76,07

From 2000 to 2010, cultivated area and production increased by 30%, and farm gate value doubled. These figures reflect growing consumer interest in and demand for locally produced wines. Grape production has expanded into specific regions of the various provinces. In Ontario, the main grape-growing regions are the Niagara Peninsula, the north shore of Lake Erie, Pelee Island, and Prince Edward County. In Quebec, most vineyards are in the southern part of the province, specifically the Eastern Townships and Montérégie. In Nova Scotia, vineyards are located on the Malagash Peninsula and in the Annapolis, LaHave River, and Bear River valleys. In New Brunswick, most of the wineries are located in the southern part of the province between Saint John and Moncton, and between the Bay of Fundy and the Atlantic coast.

1.1. Climate and terroir

Given the wide range of climatic and geographic conditions throughout eastern Canada, cultivars and cultural practices can vary from vineyard to vineyard and from province to province. Temperature, wind, sunlight, and precipitation vary depending on the exposure, altitude, and orientation of the vineyard. Soil structure and composition also vary greatly. All of these characteristics create a particular terroir (i.e., a combination of factors including soil, topography, and climate) for each vineyard. Grapevine rootstock and cultivar combinations differ greatly in their ability to adapt to the environment, their grape yield and quality, and their resistance and tolerance to pests and diseases (Creasy and Creasy 2009).

1.2. Vineyard cultural practices

Because climate and terroir differ from region to region, growers establish vineyards under different conditions, using different agricultural practices and grape varieties adapted to the various ecosystems. Grape is a hardy perennial plant that can grow and survive in many and varied climates and areas. However, in order to produce a sustainable commercial crop, grapevine requires well-drained soil, heat, sufficient water, and appropriate nutrients (Creasy and Creasy 2009). Cultural practices are important methods for not only protecting plants from harsh environmental conditions but also controlling pests and diseases. The following cultural practices can directly and indirectly impact leafhopper populations and the presence of phytoplasmas in vineyards.

Planting

Plant spacing within rows and between rows depends on the cultivar and results in a wide variety of canopy conditions and foliage densities. For example, in Ontario many growers space vines 1.2 m apart within rows and 2.4 m apart between rows. In Quebec, vines are planted more closely together (0.75–1.2 m), resulting in a denser canopy. Because leafhopper movement and dispersal are influenced by the planting layout, grapevine foliage density can be used as one of the control measures aimed at reducing leafhopper populations (Lessio and Alma 2004).

Irrigation

Owing to a dry climate, many viticulturists in the Okanagan Valley of British Columbia use spray irrigation. This practice can also protect vines from spring frost. Some viticulturists use regulated deficit irrigation (RDI), a practice for managing water stress in vines to reduce the size and enhance the quality of the grapes. Carefully lowering the amounts of water applied reduces vine vigour without reducing crop yield. A RDI system also helps reduce leafhopper density, fecundity, and adult dispersion (Daane and Williams 2003).

Tillage

Some viticulturists grow grass between vine rows (Fig. 1a), whereas others work the soil to remove all vegetation between rows (Fig. 1b). In some cases, maintenance of a season-long ground cover was associated with a 20% reduction in late-season leafhopper density (Costello and Daane 2003). The maintenance of soil cover and the choice of grasses may also impact the biodiversity of leafhoppers in vineyards, and some plants may act as reservoirs for phytoplasmas (see § 5.1. *Integrated weed management and maintenance of vineyards and adjacent areas*).

Fig. 1: Soil cover between rows: a) vineyard with ground cover (Ontario); b) vineyard without ground cover (Quebec); c) vineyard with ground cover (Nova Scotia).

Fall pre-pruning

Cane or spur pruning is an efficient agronomic practice used in Europe to control phytoplasma diseases, as it promotes recovery by exposing grapevines to abiotic stresses (Riedle-Bauer et al. 2010). In eastern Canada, some viticulturists carry out pre-pruning in the fall (Fig. 2). This practice involves removing a major part of the current-year canes. In the spring, pruning is done to remove canes damaged by frost over the winter. Even if done for other reasons, pruning may reduce the phytoplasma threshold in grapevines.

Fig. 2: Fall pre-pruning: a) to c) vines; d) to f) various cutting operations; g) grapevine aspect after spring pruning in Ontario.

Hilling and geotextile protection

Hilling (Fig. 3) is a critical step in cold-climate vineyards. It is used primarily in Quebec to protect the vines of certain varieties from harsh winter conditions, particularly frost. Hilling consists of forming a mound roughly 50 cm high over the vines, using soil from between the rows. The operation is performed using very specialized farm machinery. As a result of this practice and the corresponding need for re-seeding each year, most rows between vines are not planted to grass. The mounds are removed in the spring after the last frost.

In recent years, with climate warming and less severe winters, some growers have been using geotextile fabrics to protect their vines from the cold. After pre-pruning, the vines are covered with a geotextile fabric that moderates the effects of cold temperatures and frost on the vines (Fig. 4).

Fig. 3: Hilling: a) and b) farm machinery used for hilling in Quebec; c) hilled vines; d) hilled rows at the beginning of winter (ice wine variety – grape clusters in nets); e) unhilled rows in winter in a vineyard of Nova Scotia.

Fig. 4: Protection of grapevines with geotextile fabrics in Quebec: a) and b) general views; c) geotextile being covered with soil.

1.3. Grapevine cultivars grown in each province

The cultivars grown in Ontario, Quebec and Nova Scotia (Tables 2 to 4) differ depending on the climate in the province and also, to some degree, on consumer preferences. Nevertheless, resistance to phytoplasma diseases varies greatly from one cultivar to another. For example, in Europe, Chardonnay and Riesling are more severely affected by phytoplasma diseases (Constable 2010), whereas in Canada, the cultivars Sauvignon Blanc, Cabernet Franc, Shiraz/Syrah, and Cabernet Sauvignon appear to be more susceptible (Olivier et al. 2014). Consequently, depending on the cultivar, the relative abundance of leafhoppers and the risk of phytoplasma infections can vary between and within provinces.

Table 2: Main grapevine varieties grown in Ontario
(Source: Grape Growers of Ontario 2014)

White	Red
Auxerrois	Baco noir
Chardonnay	Cabernet franc
Gewürztraminer	Cabernet Sauvignon
Pinot blanc	Chambourcin
Pinot gris	Gamay noir
Riesling	Maréchal Foch
Sauvignon blanc	Merlot
Sémillon	Pinot noir
Seyval blanc	Shiraz/Syrah
Vidal	Zweigeltrebe
Viognier	

Table 3: Main grapevine varieties grown in Quebec
(Source: Association des vignerons du Québec 2014)

White	Red
Adalmiina	Baco noir
Cayuga blanc	Baltica
Chardonnay	Beta
Delisle	Chambourcin
Eona	Chancellor
Frontenac gris	De Chaunac
Geisenheim	Frontenac
Hibernal	Gamay
Kay Gray	Léon Millot
La Crescent	Lucy Kuhlmann
Louise Swenson	Maréchal Foch
Osceola Muscat	Marquette
New York Muscat	Petite perle
Prairie Star	Pionnier
Riesling	Radisson
Saint Cliche	Sabrevois
St. Pépin	St. Croix
Seyval blanc	Seyval noir
Swenson blanc	Skandia
Traminette	
Vandal-Cliche	
Vidal	

Table 4: Main grapevine varieties grown in Nova Scotia (Source: Winery Association of Nova Scotia 2014)

White	Red
Acadie blanc	Baco noir
Chardonnay	Léon Millot
New York Muscat	Lucy Kuhlmann
Ortega	Maréchal Foch
Riesling	
Seyval blanc	
Vidal	

2. Leafhoppers

Leafhoppers (Hemiptera: Cicadellidæ) constitute a group of piercing-sucking insects that includes about 22,000 species around the world. About 110 species have been found in Canadian vineyards (Bostanian et al. 2003; Saguez et al. 2014).

2.1. Biology

Leafhoppers are typically found on the underside of leaves (Fig. 5). Females lay their eggs under the epidermal layer of the leaves. Eggs can be laid in clusters (Fig. 5a, b) or singly (Fig. 5c, d). For a skilled eye or with the aid of a hand lens, the eggs of many species can be detected by the presence of a whitish, translucent veil that covers them (Fig. 5a, b, d). In some leafhopper species such as *Erythroneura vitis*, eggs are laid singly along the leaf veins and are covered with an opaque, dark brown protective coating (Fig. 5c). Because eggs are transparent, it is often possible to see the developing leafhoppers' pairs of eyes (Fig. 6), which are aligned in the same direction. In heavy infestations, the leaves become discoloured, except at the oviposition site (Fig. 5a, b).

Embryonic development in the eggs lasts between 10 and 15 days. Following egg hatch, complete nymphal development lasts an average of 21 days (Wells and Cone 1989; Olsen et al. 1998; Saguez and Vincent 2011), and adults typically live for up to a month.

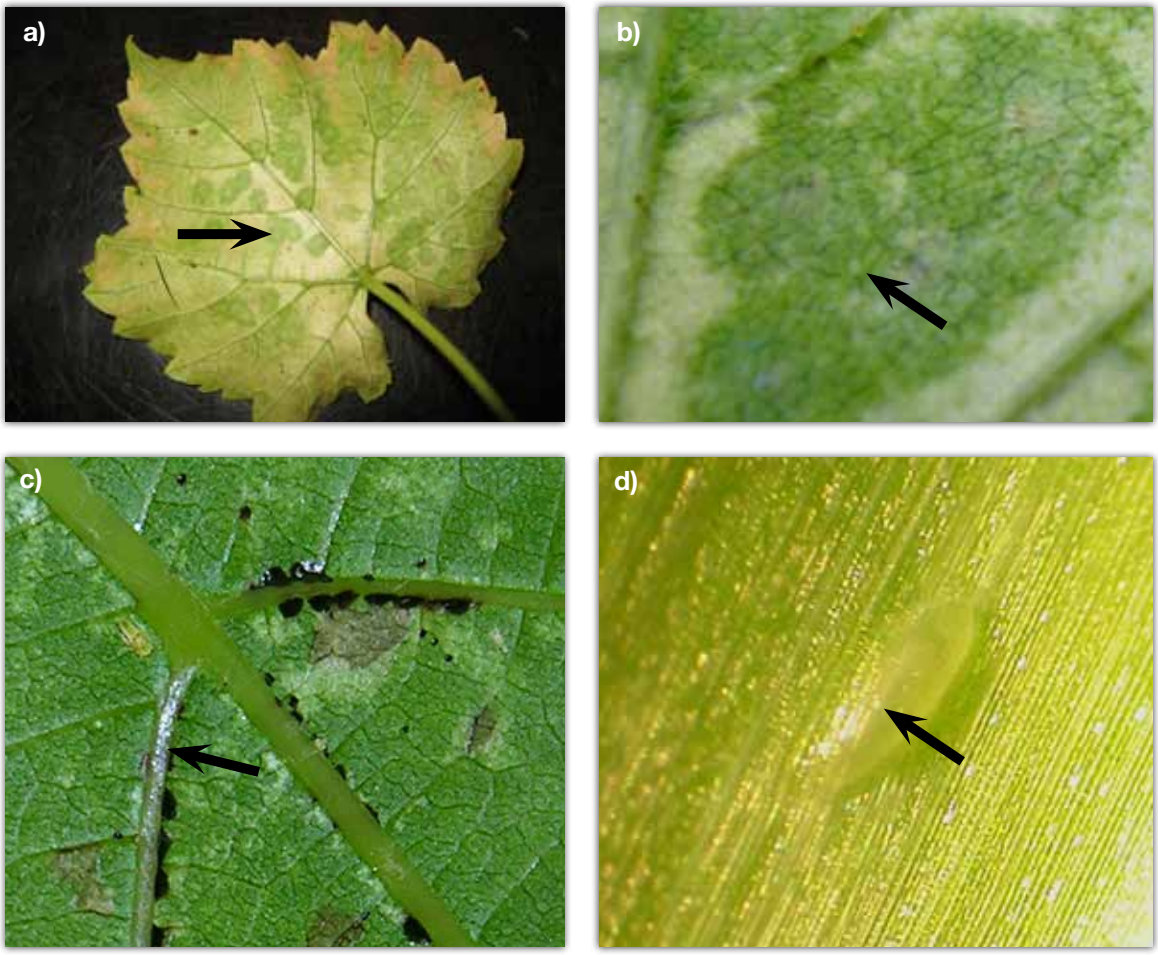

Fig. 5: Different types of leafhopper eggs: a) and b) eggs of *Erythroneura ziczac* laid in clusters; c) eggs of *Erythroneura vitis* laid singly along the veins; d) egg of *Macrosteles quadrilineatus* laid singly under the epidermis. Arrows indicate eggs.

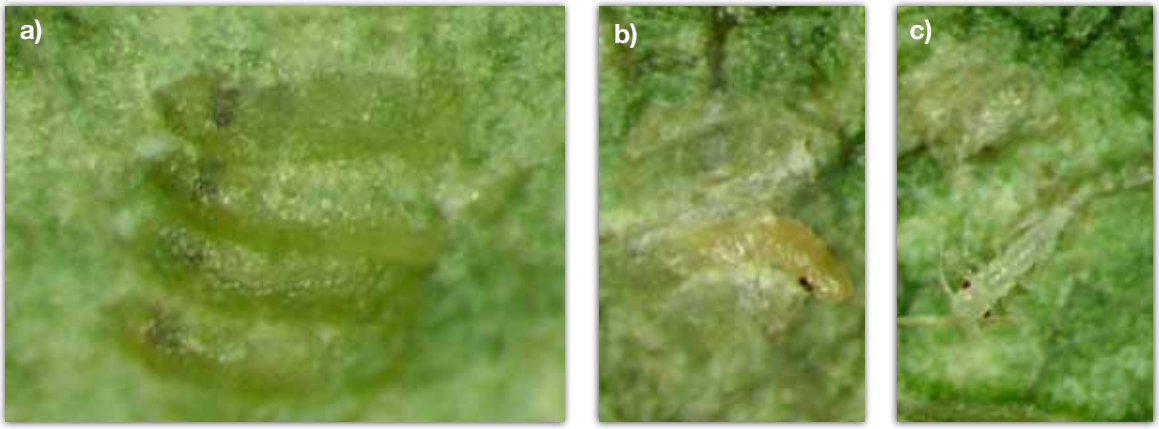

Fig. 6: Leafhopper egg hatching stages: a) egg in epidermis; b) hatching; c) neonate nymph.

Newly hatched nymphs (Fig. 6c) measure less than 1 mm and are very vulnerable to desiccation and natural enemies.

Leafhoppers grow by successive molts. The cast skins (exuviæ) are left on the host plants (Fig. 7). Leafhoppers develop through five nymphal instars (immature stages) (Fig. 8) before reaching the adult stage. Wing pads increase in size between each nymphal stage, and the antennæ become smaller. After the final molt (Fig. 9), winged adults emerge.

Fig. 7: Leafhopper exuviæ.

Fig. 8: 1st, 2nd, and 3rd instar nymphs of *Erythroneura vitis*.

Fig. 9: Imaginal molt.

Adult coloration appears during the freshly molted (teneral) phase and is fully developed several hours after the final (imaginal) molt.

Adults average 3 to 4 mm in length, and their sex can be determined by observing structural differences in genitalia (Fig. 10). Females have an ovipositor (Fig. 10a), whereas males do not. Mating of individuals of the same species occurs approximately 7 to 10 days after emergence (Fig. 11), and egg laying follows (Fig. 12).

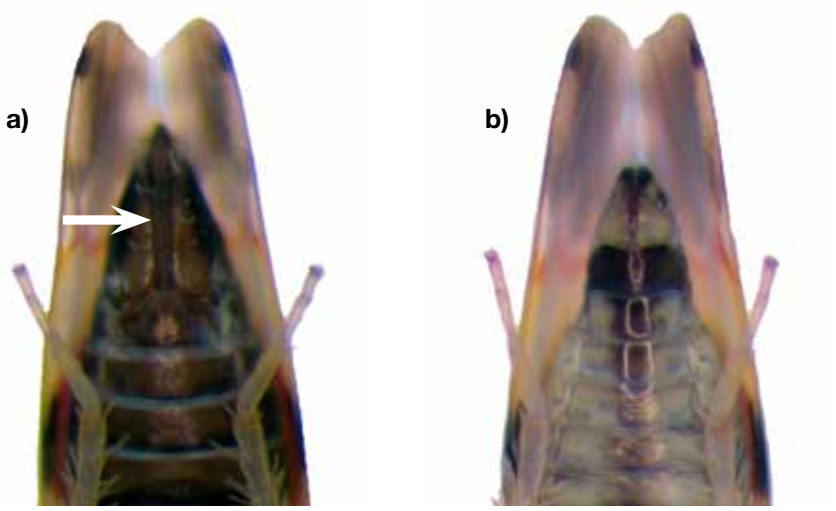

Fig. 10: Leafhopper genitalia: a) female; b) male. White arrow indicates the ovipositor.

Fig. 11: Mating.

Fig. 12: Leafhoppers laying their eggs.

Leafhopper development depends largely on environmental conditions. Accumulated degree-days can be used to estimate the development time of a population and also help predict the earliest date of arrival in vineyards of leafhopper species that overwinter in warmer regions. Leafhoppers can have two to three generations a year, depending on the species, and can overwinter in various forms. *Scaphoideus titanus* deposits its eggs in the bark of shoots and canes on its host plants (Vidano 1964; Claridge and Howse 1968). Species of the genus *Erythroneura* overwinter as adults under dry leaf debris (Wells and Cone 1989; Olsen et al. 1998). Some species do not overwinter in Canada and migrate every year. For example, *Empoasca fabæ* migrates to Canada from the northern United States in the spring.

In eastern Canada, the dates of arrival of various leafhopper populations in vineyards can be modelled and predicted (Hardman 2012). Bostanian et al. (2006) used a model based on accumulated degree-days to predict leafhopper abundance. Their model was developed using species of the genus *Erythroneura* and a threshold temperature of 8°C (starting on March 1). According to their model, monitoring should be initiated at 630 degree-days and can be terminated at 1,140 degree-days. Maximum abundance is between 850 and 860 degree-days.

Bressan et al. (2006) developed a degree-day model to improve control decisions in infected vineyards. This model predicts 1) the proportion of eggs of *Scaphoideus titanus* that will hatch, 2) the proportion of leafhoppers that will be infected by the grapevine phytoplasma disease Flavescence Dorée (see § 4.2. *Major phytoplasma diseases*), 3) the latency period prior to phytoplasma transmission, and 4) the proportion of leafhoppers that could infect grapevines.

Degree-day models can also be developed to study leafhopper population dynamics (Cerutti et al. 1992) and to predict the risk of infestation of crops by leafhoppers, as was done for the vector of Pierce's disease (Hoddle 2004).

2.2. Main leafhopper species associated with Canadian vineyards

About 110 species of leafhoppers have been found in Canadian vineyards. Bostanian et al. (2003) reported about 60 species associated with Quebec vineyards. Saguez et al. (2014) listed 110 species and presented 72 colour photographs of adults of the main species that are found in Canadian vineyards and that feed on grapevines or are associated with weeds and grasses. These photographs may help growers recognize species found in their vineyards, before formal identification is done by specialists. Identification of immatures is often difficult, because several species have the same morphological appearance, especially at the early nymphal stages. In some cases, it is therefore advisable to rear the specimens to identify them as adults (see § 6.1. *Leafhopper rearing*).

Table 5: Most abundant leafhopper species associated with vineyards in eastern Canada.

Leafhopper species	Ontario	Quebec	New Brunswick	Nova Scotia	Prince Edward Island
Empoasca fabæ	X	X	X	X	X
Erythroneura bistrata	X				
Erythroneura coloradensis	X				
Erythroneura comes	X	X			
Erythroneura elegantula	X	X			
Erythroneura nigra	X	X			
Erythroneura tricincta	X	X			
Erythroneura vitifex	X	X			
Erythroneura vitis	X	X			
Erythroneura vulnerata	X	X			
Erythroneura ziczac	X	X	X		
Fieberiella florii	X				
Macrosteles fascifrons	X	X	X	X	
Macrosteles quadrilineatus	X	X	X	X	X
Neokolla hieroglyphica	X	X	X	X	X
Norvellina chenopodii	X	X			
Scaphoideus carinatus	X	X	X	X	
Scaphoideus cinerosus	X	X			
Scaphoideus cylindratus	X	X		X	X
Scaphoideus major	X	X			
Scaphoideus melanotus	X	X			
Scaphoideus opalinus	X	X			
Scaphoideus titanus	X	X		X	
Scaphytopius acutus	X	X	X	X	X
Xestocephalus superbus	X	X	X	X	

In a given year and vineyard, leafhopper diversity and abundance will vary depending on several factors, including cultivar, year, environmental conditions, and cultural practices (see above). Table 5 presents a list of the 25 most abundant leafhopper species that were found between 2006 and 2009 in vineyards in Ontario, and Quebec and potentially present in Atlantic provinces and that use grapevine as a primary or alternative host. Most of these species are reported to be vectors of grapevine diseases. Based on published information (Beirne 1956; Maw et al. 2000) and on unpublished data from Andy Hamilton, the Canadian wine-growing regions where these species are found or could potentially be found are indicated.

The ten factsheets on the following pages describe the most important leafhopper species in Canadian vineyards. Where possible, each entry features photos (Figs. 13–22) as well as information on location, host plants, and risks associated with each species.

Empoasca fabæ

Common name: Potato leafhopper.

Description: Pale green, sometimes yellowish. This species causes “hopper burn” in grapevines and is easily confused with *Empoasca vitis*, a species that is present in Europe and has the same colour.

Adult size: 3–4 mm long.

Habitat/host plants: *Empoasca fabæ* is highly polyphagous and attacks many plant species. Grapevine is a secondary host; the primary host is potato.

Detection period: Does not survive the winter in Canada; migrates from the northern United States to Canada every year in the spring, starting in mid-June; present all summer.

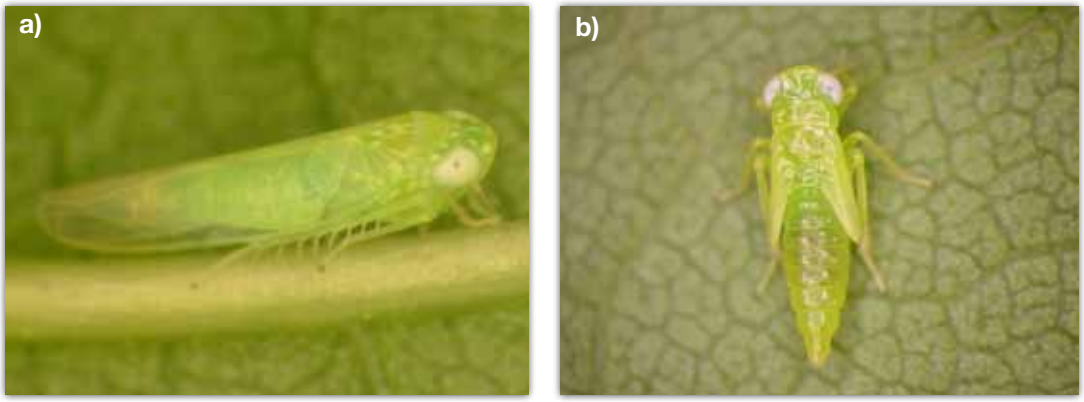

Fig. 13: *Empoasca fabæ*: a) adult; b) nymph.

Erythroneura comes

Common name: Eastern grape leafhopper.

Description: The adult has orange or red marks on a yellowish background, similar to those of *Erythroneura vitifex*. The largest and darkest spots are near the bases of the forewings, and the smaller spots are towards the tips. There is no way to differentiate between *Erythroneura comes* and *Erythroneura vitifex*, except by observing male genitalia.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Essentially grapevines.

Detection period: Early in the spring to late in the season.

Fig. 14: *Erythroneura comes* adult (Photo © Yurika Alexander).

Erythroneura elegantula

Common name: Western grape leafhopper.

Description: Pale body (light yellow or white) with yellow-orange markings; two darker spots on the thorax. The nymph is easily confused with that of the three-banded leafhopper, *Erythroneura tricincta*. Note that *Erythroneura elegantula* has only two black spots on the hindwing pads, whereas *Erythroneura tricincta* has spots on the forewing pads, hindwing pads, and thorax. The two species also differ in eye colour: *Erythroneura elegantula* has white eyes, whereas *Erythroneura tricincta* has reddish-brown eyes. The adult of *Erythroneura elegantula* could also be confused with that of *Erythroneura comes* because of the orange wing patterns, but the dark thoracic spots of *Erythroneura elegantula* are characteristic.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Grapevine, wild grape.

Detection period: Summer to early fall.

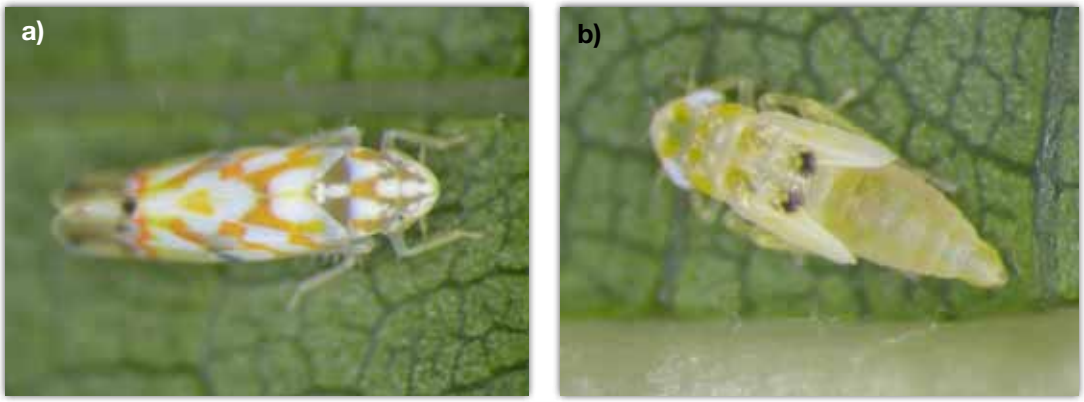

Fig. 15: *Erythroneura elegantula*: a) adult; b) nymph.

Erythroneura tricincta

Common name: Three-banded leafhopper.

Description: Yellow with three brown or black bands; red eyes. The adult can be easily confused with that of the grapevine leafhopper (*Erythroneura vitis*). *Erythroneura tricincta* is yellower with narrower bands, and the first band does not extend onto the forewings. The nymph can be easily confused with that of the Western grape leafhopper (*Erythroneura elegantula*), although that species has only two black spots on the hindwing pads, whereas *Erythroneura tricincta* has spots on the forewing pads, hindwing pads, and thorax. The two species also differ in eye colour: *Erythroneura elegantula* has white eyes, whereas *Erythroneura tricincta* has reddish-brown eyes.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Grapevine, wild grape.

Detection period: Late in the spring to the end of the summer.

Fig. 16: *Erythroneura tricincta*: a) adult; b), c), and d) 2nd, 3rd, and 5th instar nymphs, respectively.

Erythroneura vitifex

Common name: Vine leafhopper.

Description: The adult has bold, interconnected orange-to-red lines on the forewings like those of *Erythroneura elegantula*, but without black spots on the thorax. May be confused with *Erythroneura comes*. There is no way to identify all specimens of *Erythroneura vitifex* except by observing the male genitalia.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Essentially grapevines.

Detection period: Early spring to late in the growing season.

Fig. 17: *Erythroneura vitifex* adult.

Erythroneura vitis

Common name: Grapevine leafhopper.

Description: The adult is yellowish with three large bands perpendicular to the long axis of the body: one band on the thorax, one in the middle of the abdomen, and a darker one at the tip of the wings. Coloration appears gradually, first forming an orange U on the thorax in young nymphs and then a brown square in the last nymphal stage. The name of this species is easily confused with that of *Empoasca vitis*, a species that occurs in Europe. The names of these two species are often abbreviated to "*E. vitis*" in the literature. The adult *Erythroneura vitis* is easily confused with that of the three-banded leafhopper (*Erythroneura tricincta*). In *Erythroneura vitis*, the bands are wider, and the first band extends onto the forewings.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Grapevine, wild grape.

Detection period: June to the end of September.

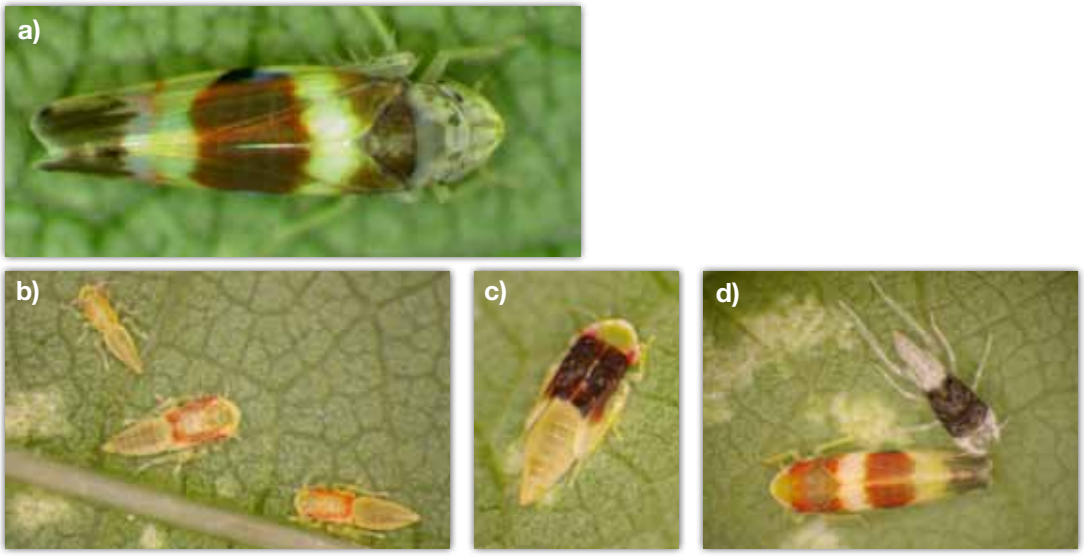

Fig. 18: *Erythroneura vitis*: a) adult; b) 2nd and 3rd instar nymphs; c) 5th instar nymph; d) young adult with its exuviæ.

Erythroneura vulnerata

Common name: Wounded leafhopper.

Description: The body of the nymph is completely brown except for the legs, which are yellowish. The adult is usually brownish or the colour of dried blood, and its colour pattern includes three white lines on the head.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Grapevine, wild grape, Virginia creeper.

Detection period: Beginning of July to the end of August.

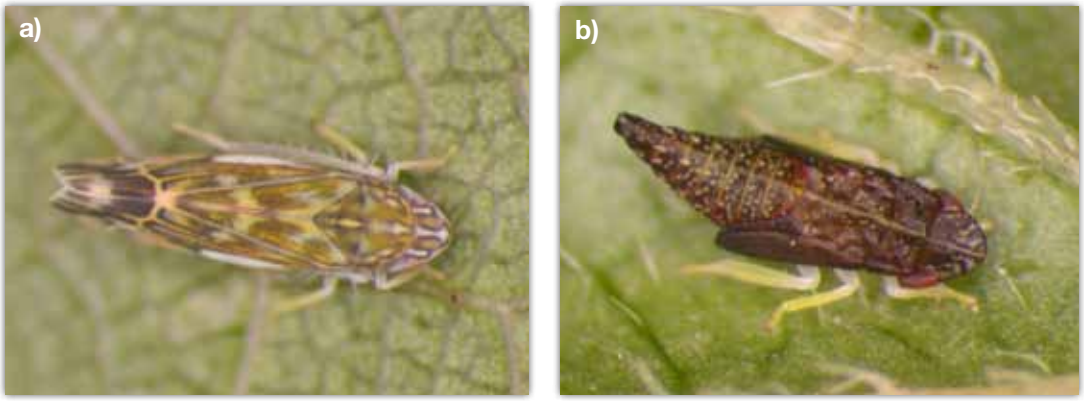

Fig. 19: *Erythroneura vulnerata*: a) adult; b) nymph.

Erythroneura ziczac

Common name: Virginia creeper leafhopper.

Description: The nymph is ivory with red spots on the prothorax and brown spots between the wing pads. The adult is yellowish with brown zigzag patterns along the back.

Adult size: 2.5–3.5 mm long.

Habitat/host plants: Virginia creeper, wild grape, grapevine.

Detection period: Summer.

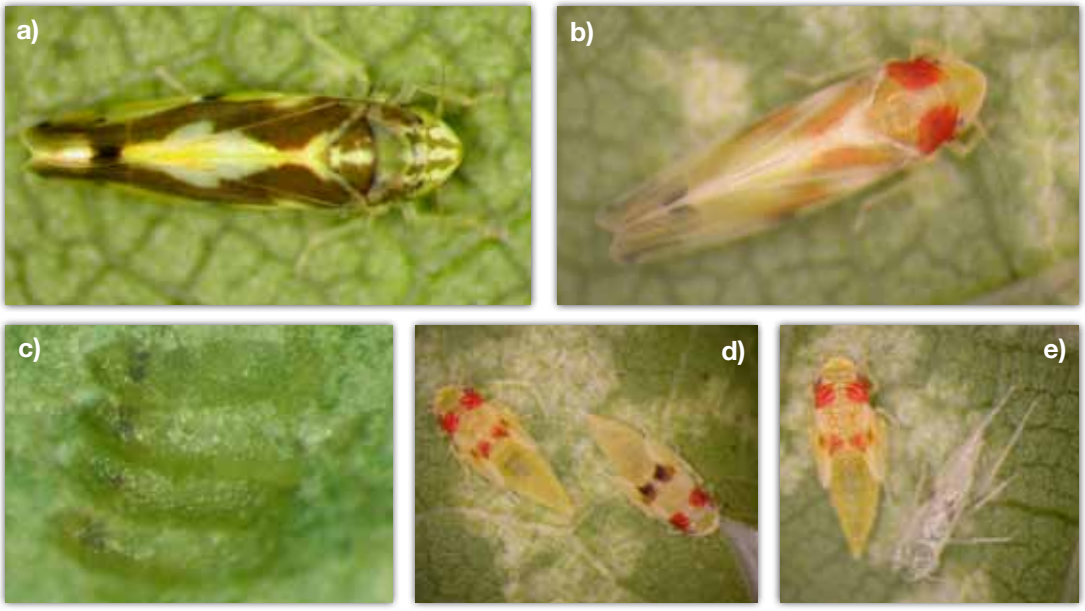

Fig. 20: *Erythroneura ziczac*: a) adult; b) young adult with incomplete colours; c) eggs; d) 3rd instar nymphs with different colour patterns; e) 4th instar nymph with its exuviæ.

Macrosteles fascifrons / Macrosteles quadrilineatus

Common name: Aster leafhopper.

Description: *Macrosteles quadrilineatus*, the vector of aster yellows phytoplasmas, has longer wings than *Macrosteles fascifrons* does (>4 times as long as they are wide). The nymphs are yellow, and the adults appear dark green with transparent wings and a black abdomen.

Adult size: 3.5–4.5 mm long.

Habitat/host plants: Highly polyphagous, these two similar species have many hosts that differ depending on the species. The preferred host of *Macrosteles fascifrons* is toad rush (*Juncus bufonius*), and the preferred host of *Macrosteles quadrilineatus* is aster. These leafhopper species are frequently found in vineyards, especially in grass buffers and along the edges of vineyards.

Detection period: End of August to mid-October.

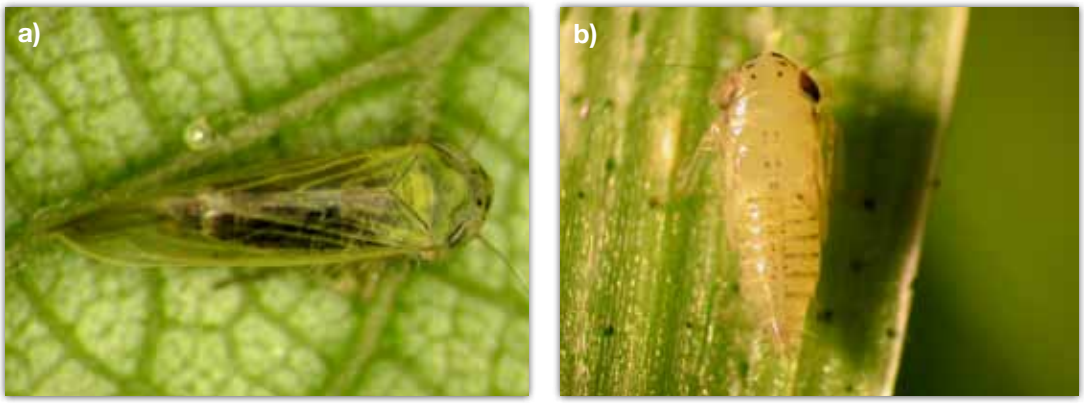

Fig. 21: *Macrosteles quadrilineatus*: a) adult; b) nymph.

Scaphoideus titanus (formerly *Scaphoideus amplus*)

Common name: American grapevine leafhopper.

Description: The nymph hops. The adult is tan, with brown markings, brownish elytra, and dark spots. The adult has twisted cells on the wing tips similar to those of other species in this genus. Native to North America, *Scaphoideus titanus* is the vector of Flavescence Dorée in Europe. Although present in Canadian vineyards, this species has not yet been found positive for Flavescence Dorée phytoplasmas. However, the presence of this leafhopper requires increased monitoring.

Adult size: 4.5–5.5 mm long.

Habitat/host plants: Wild and cutivated grapevines.

Detection period: Summer.

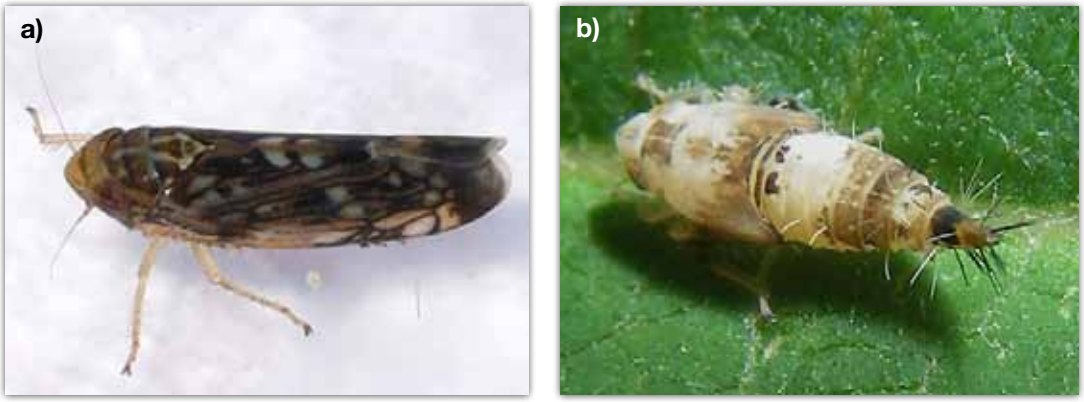

Fig. 22: *Scaphoideus titanus*: a) adult (Photo© Ilona Loser); b) nymph (Photo © Kenneth E. Barnett).

3. Leafhopper damage

Leafhoppers are piercing-sucking insects. Using mouthparts called stylets (Fig. 23), leafhoppers pierce the leaves and suck the xylem or phlœm sap from plants. These insects can also remove the contents of mesophyll cells.

Fig. 23: Piercing-sucking mouthparts of three different leafhopper species. Stylets are indicated by black arrows.

3.1. Direct injury

Some species are exclusively xylem feeders (they feed on xylem sap, the crude sap of plants), whereas others are exclusively phlœm feeders (they feed on phlœm sap, the elaborated sap of plants). Still other species feed by removing the contents of mesophyll cells. A number of species use several feeding strategies to find the food resources and nutrients that they need for their development.

Punctures

The feeding of many leafhopper species causes white stippling of leaves (Fig. 24). This stippling is due to a loss of pigmentation of the leaves associated with the removal of chloroplasts and chlorophyll from leaf cells.

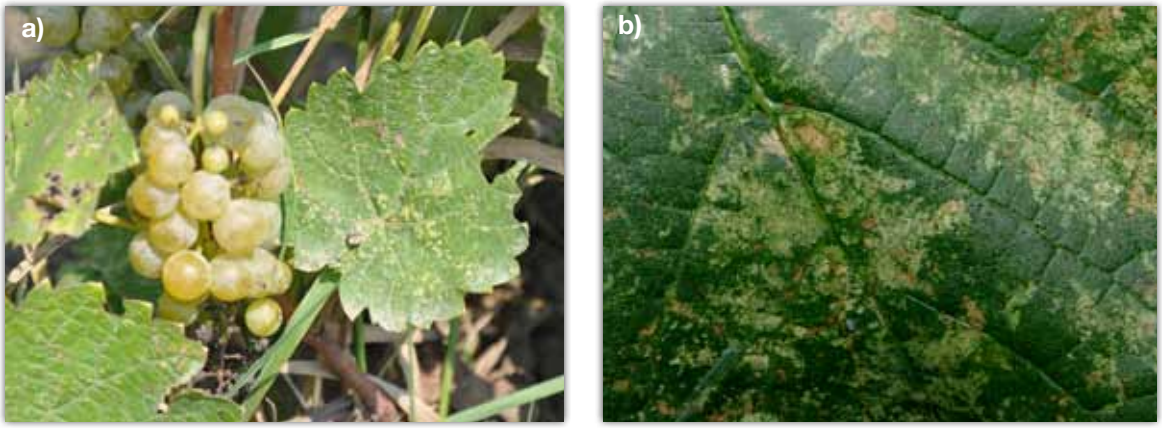

Fig. 24: Damage to grapevine leaves on two cultivars: a) Vidal; b) Seyval Blanc.

Hopper burn

Damage can also result from the plant's reaction to leafhopper feeding. Leaves attacked by leafhoppers take on a blistered appearance that is characteristic of hopper burn (Fig. 25) and results from the hypertrophy of certain cells.

Fig. 25: Hopper burn on grapevine leaves.

Punctures generally appear on the lower parts of the plant and close to the grapes, whereas hopper burn is seen mainly at the top of the grapevine canopy.

3.2. Indirect injury: disease transmission

During feeding, leafhoppers can acquire and transmit viruses, bacteria, and phytoplasmas present in plant vascular tissues. Acquisition occurs essentially during sap ingestion (Fig. 26a). Some pathogens, such as the bacterium causing Pierce's disease, do not circulate in the body of the leafhopper but rather attach themselves to its mouthparts. Viruses and phytoplasmas, for their part, circulate in the leafhopper's body. They penetrate the insect's digestive tract, cross the intestinal barrier, reach the hemolymph, and migrate to the salivary glands (Fig. 26b). Although some viruses do not multiply in the leafhopper's organs, other viruses and phytoplasmas multiply actively. As the insect feeds on plant tissues, the pathogens are transmitted to the plant through the injection of contaminated saliva (Fig. 26c).

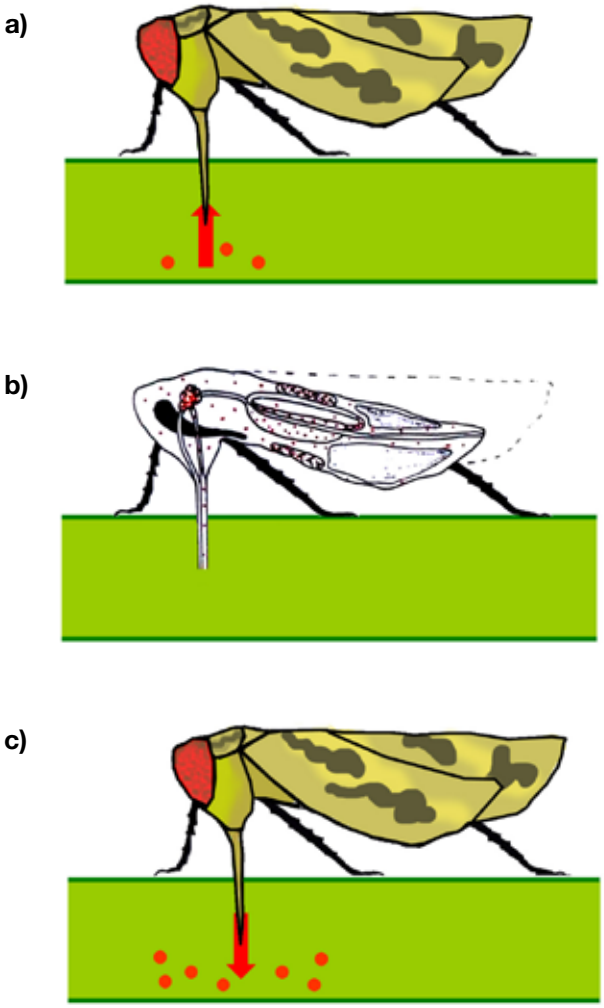

Fig. 26: Cycle of phytoplasmas (red dots): a) acquisition; b) spread in an insect; c) transmission. Arrows indicate the flow of phytoplasmas between the leafhopper and plant tissues.

Phytoplasmas are essentially transmitted by insect vectors and notably leafhoppers. Phytoplasmas can also be transmitted during grafting, because either the rootstock or the cane can be contaminated. The long-distance dissemination of several important phytoplasma diseases such as Flavescence Dorée and Bois Noir has occurred in Europe as well as in Canada through phytoplasma-infected propagative material (Rott et al. 2007; Constable 2010). Therefore, prevention programs should include the use of clean propagative materials (see § 4.2. *Major phytoplasma diseases* and § 5.3. *Prevention*).

A third mechanism that could induce phytoplasma transmission involves parasitic plants (Contaldo et al. 2012).

4. Phytoplasma diseases of grapevine

4.1. What are phytoplasmas?

Phytoplasmas (Fig. 27) are prokaryotic bacteria belonging to the class of Mollicutes, deprived of a cell wall, having pleomorphic shape (that varies depending on the environmental conditions), with a small size (diameter < 1µm) and a small genome (size of 680 to 1600 kb) (Bertaccini and Duduk, 2009). Phytoplasmas are obligate parasites that need hosts to multiply, notably insects (e.g. leafhoppers and also psyllids) and plants (grapevine is a final host). Phytoplasmas are very difficult to cultivate in vitro, sensitive to high temperatures and few antibiotics (notably tetracyclines). Phytoplasmas live and move primarily in the phlœm tissues of plants and are essentially transmitted by phlœm-feeding insects and are therefore not transmitted by wind, water, or soil. These pathogens overwinter in the bodies of insect vectors, in the roots and dormant wood of many perennials, and in the buds of some trees (Bertaccini and Duduk 2009). Phytoplasmas manipulate the genome of their animal and plant hosts, causing physiological and behavioural changes in insects and plants, such as the conversion of flower buds to leaf buds in plants (Hogenhout et al. 2008).

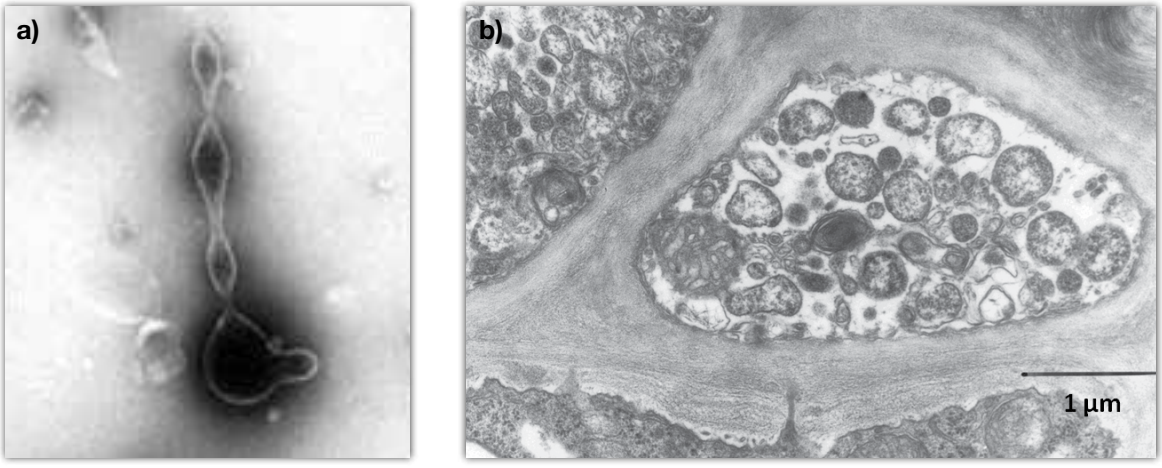

Fig. 27: Phytoplasmas observed by electron microscope: a) isolated phytoplasma (Photo Caudwell et al., 1982); b) phytoplasmas in phlœm vessels. (Photo © INRA Dijon).

In Canada, except for the hot water treatment that imported plants must undergo (see § 5.3. *Prevention*), there is no direct control method available for phytoplasmas, and no product registered or commercially marketed to control these pathogens. The most common techniques used to control phytoplasma diseases are chemical treatments targeting insect vectors. Prevention programs, including monitoring of crops, destruction of diseased plants and reservoirs, and certification of imported plants, are also very important (Weintraub and Wilson 2010; Olivier et al. 2012). Several strains of phytoplasmas were reported in different species (Olivier et al. 2009a). New strains have been recently identified in Canadian vineyards (Olivier et al. 2014).

Phytoplasmas cause diseases called yellows and can affect a very large number of plant species, including grapevines. There are many different grapevine yellows diseases, and they are present in most grape-growing regions around the world. Grapevine yellows diseases are caused by a dozen different phytoplasmas (Constable 2010). Flavescence Dorée and Bois Noir are two economically important epidemic diseases caused by phytoplasmas in Europe. Those diseases are also considered quarantine diseases in Canada.

In Canada, aster yellows phytoplasmas, which cause the disease known as aster yellows, and X-disease phytoplasmas have been detected in grapevines. No specific name has been given to these two grapevine diseases in Canada. In the United States, grapevines infected by aster yellows phytoplasmas, X-disease phytoplasmas, or both are reported to be infected by North American grapevine yellows, or NAGY (Wolf et al. 1994; Olivier et al. 2012).

In vineyards, the symptoms of yellows are identical regardless of the causal phytoplasma. Symptoms are observable on foliage, flowers, fruits, and canes (Figs. 28, 29) and include yellowing of the leaf blade in white varieties (Fig. 28) or reddening of the leaf blade in red varieties (Fig. 29), rolling of leaves, poor wood maturation, drying out of inflorescences, yield loss, stunting, and in many cases, the mortality of infected plants.

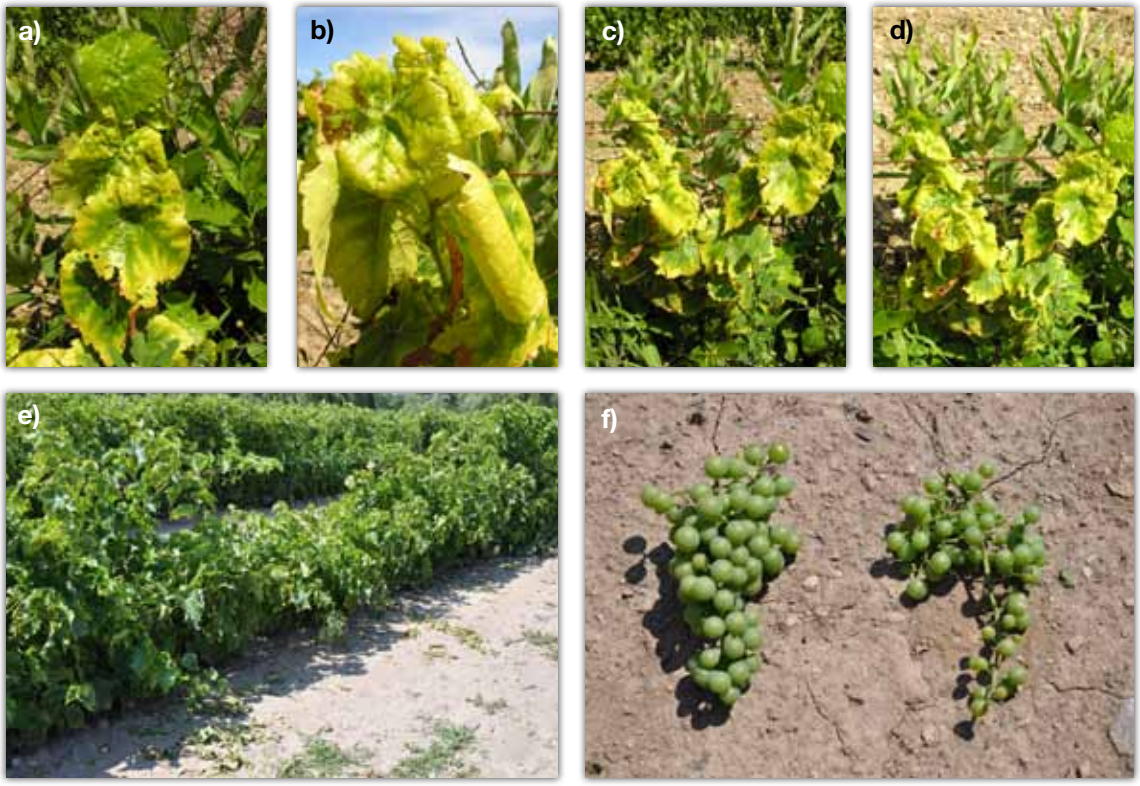

Fig. 28: Symptoms of yellows in white grape cultivars: a) to d) symptoms on leaves; e) general aspect; f) symptoms on grapes (left: normal; right: phytoplasma-infected).

Fig. 29: Symptoms of yellows in red grape cultivars: a) to e) different aspects on leaves.

4.2. Major phytoplasma diseases

Flavescence Dorée

Flavescence Dorée, or FD, appeared in France in the 1950s and is now epidemic in most European vineyards. It is transmitted by the leafhopper *Scaphoideus titanus*, which is native to North America (Constable 2010). In Europe, Flavescence Dorée is listed as a quarantine disease and has been subject to mandatory control regulated by ministerial orders since the late 1980s, owing to the serious economic consequences of the disease for European viticulture (Rouzet et al. 1989). As well, Flavescence Dorée is a notifiable disease that must be declared to government authorities if it is present or suspected. Although the vector, *Scaphoideus titanus*, is present in Canada, there have been no reports of Flavescence Dorée phytoplasmas in leafhoppers or vineyards in Canada to date.

Caution is nevertheless advised in Canada, and growers are asked to carry out increased monitoring efforts for this disease. The Canadian Food Inspection Agency (CFIA) has enforced strict regulations regarding the importation of plants and the declaration of infected vines or parts of vineyards (see § 5.3. *Prevention*). Flavescence Dorée phytoplasmas can be introduced in propagative material contaminated with the pathogen. The eggs of *Scaphoideus titanus*, which are laid beneath the bark, can also be imported in propagative material. Laboratory experiments have shown that aster yellows phytoplasmas can be transmitted by the eggs of *Scaphoideus titanus* (Alma et al. 1997), but such transmission has not been reported for Flavescence Dorée.

Bois Noir

Bois Noir, or BN, is widespread in Europe, particularly in the Mediterranean area, and is the second most serious phytoplasma disease after Flavescence Dorée. In some regions, Bois Noir has developed into a major disease in vineyards (Constable 2010). This disease is transmitted by a cixiid planthopper, *Hyalesthes obsoletus*, that is not present in Canada.

In 2006, the CFIA found in British Columbia and Ontario a number of plants imported from Europe that were infected with Bois Noir (Rott et al. 2007). The infected plants and the areas of vineyards in which they were detected were systematically destroyed. Later studies did not detect Bois Noir phytoplasmas in these vineyards.

In Canada, there is concern about the presence of weeds known to be major reservoirs of Bois Noir and about the identification of potential new vectors. Endemic in several weed species in Europe, Bois Noir can be transmitted from these host plants to grapevines, which are a final host (i.e., phytoplasmas cannot be transmitted from grapevines to other plants).

Aster yellows

Aster yellows, or AY, phytoplasmas are the most widespread phytoplasmas in Canada. They can infect over 250 species of plants, and some 20 species of leafhoppers are known vectors of aster yellows. The disease is present in vineyards in British Columbia, Ontario, and Quebec (Olivier et al. 2009b). The prevalence of grapevines infected by aster yellows is very low in British Columbia (<1% of symptomatic plants) and higher in Quebec and Ontario (approximately 5% of symptomatic plants). However, one of the characteristics of grapevine infection by aster yellows is the presence of a large proportion of asymptomatic infected plants. Although the main vector of aster yellows is the aster leafhopper, *Macrosteles quadrilineatus*, several other leafhopper species have been identified in vineyards as potential aster yellows vectors (Olivier et al. 2014).

X-disease

X-disease is common in Canada and affects many species of fruit trees, including cherry and peach. It exhibits a 10-to-15 year epidemic cycle (high incidence for 4 to 6 years followed by a 5- to 9 year period of remission). X-disease is transmitted by at least eight leafhopper species (Davis et al. 2013) but has been detected in only a few grapevines in Canada. One concern for Canadian growers is the presence of very large numbers of chokecherry trees (*Prunus virginiana*) around vineyards. Chokecherry can be an important reservoir of the X-disease phytoplasma and its vectors (Rosenberger and Jones 1978).

Pierce's disease

Not a phytoplasma disease. Pierce's disease is caused by the proteobacterium *Xylella fastidiosa*, which attacks the xylem of the plant. The disease is transmitted by xylem feeders, including certain species of leafhoppers and spittlebugs (Mizell et al. 2012). The bacterium attaches itself to the walls of the cibarial pump (the organ between the esophagus and the mouthparts of the insect) during initial feeding and can be re-injected into the xylem during a subsequent feeding, resulting in the rapid spread of the disease. Pierce's disease can infect 100 species of plants, including grapevine and certain fruit trees. This disease is widespread in the United States but cannot survive in cold climates. Pierce's disease has been detected in southern Ontario and Alberta (on maple, elm, sycamore, and other tree species), but little is known about its vectors in Canada (Chatterjee et al. 2008).

4.3. Detection and identification of phytoplasma diseases

Diseases caused by phytoplasmas are not easy to diagnose, because infected plants can be asymptomatic. Phytoplasmas may be found in only certain parts or tissues of the plant and not throughout the plant. A single shoot may contain phytoplasmas only in certain sections. Molecular screening techniques are therefore needed to detect and confirm infection by phytoplasma diseases.

All phytoplasma diseases have similar symptoms. Early detection methods were based on the use of non-specific dyes such as aniline blue, which binds to callose (a polysaccharide that forms a cap in the sap vessels) deposited as a result of the presence of phytoplasmas. Fluorescent markers such DAPI (4',6-diamidino-2-phenylindole) can also be used to easily locate phytoplasma DNA in plant sieve tubes, which normally do not contain DNA. These relatively non-specific methods are unable to detect phytoplasmas present in small quantities, identify the various phytoplasma strains, or distinguish phytoplasmas from certain other pathogens.

Various techniques are now used to detect, identify, and characterize phytoplasmas in leaf or insect samples (Dickinson and Hodgetts 2013). Such techniques include ELISA (enzyme-linked immunosorbent assay) and PCR (polymerase chain reaction) with RFLP (restriction fragment length polymorphisms).

The ELISA method uses antibodies to detect specific membrane proteins on phytoplasmas (Batlle et al. 1997). The presence of phytoplasmas in a sample tested with ELISA triggers a colorimetric reaction. Although ELISA can give a rapid answer concerning the occurrence of phytoplasmas in samples, that information is generally qualitative (i.e., presence/absence) and does not always allow the identification of the strain. Detection is also difficult when the samples contain a small concentration of phytoplasmas (as is generally the case in grapevines) or when the membrane proteins are not easily accessible by antibodies (Fig. 30). If the concentration of phytoplasmas in a sample is too low, the test could produce a negative result, even though phytoplasmas are present. However, this method has been used with considerable success in association with optical and electron microscopy to locate phytoplasmas in plant and insect tissues (Lherminier et al. 1990).

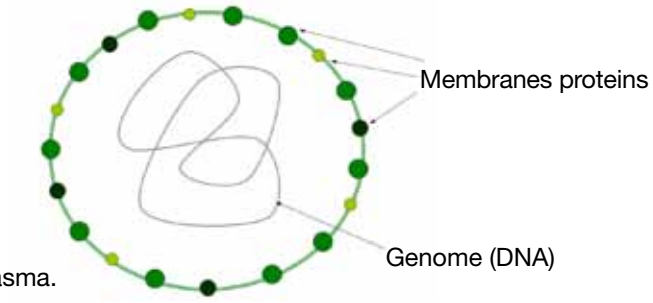

Fig. 30: Schematic drawing of a phytoplasma.

Other methods used to detect and characterize phytoplasmas in samples are molecular-based techniques, such as PCR and RFLP analysis. These techniques allow amplification, detection, and sequencing of the genome (Fig. 30) of phytoplasma strains present in DNA extracts from insects and grapevine samples.

The PCR method is based on a succession of chemical reactions at different temperatures to allow the amplification of specific DNA fragments of the phytoplasma genome using primers designed to recognize and bind to the specific DNA fragments to be amplified. After amplification, DNA fragments are separated on agarose gels. The presence of phytoplasmas in the sample is revealed by the appearance of specific bands on the gel (Fig. 31). Several variants of PCR-based techniques are available. On average, PCR assays require two to three days of work.

Once PCR assays have been conducted, the amplified DNA fragments are cloned and sequenced to identify the phytoplasma strain present in the sample. Sequences are compared with known sequences from phytoplasmas stored in gene banks in order to conduct phylogenetic analyses to determine the relatedness of the phytoplasma strains (Olivier et al. 2014). The phytoplasmas found in insects and plants can thus be compared to determine if they belong to the same strains.

Sequences are also virtually cut into fragments using various enzymes (RFLP) and visualized on virtual gel. The patterns of the cut DNA sequences obtained on the gel are specific to the phytoplasma species. The phylogenetic and RFLP analyses identify the phytoplasma species and can require up to a week of work. All these techniques could be used to identify new phytoplasma strains in Canadian vineyards (Olivier et al. 2014).

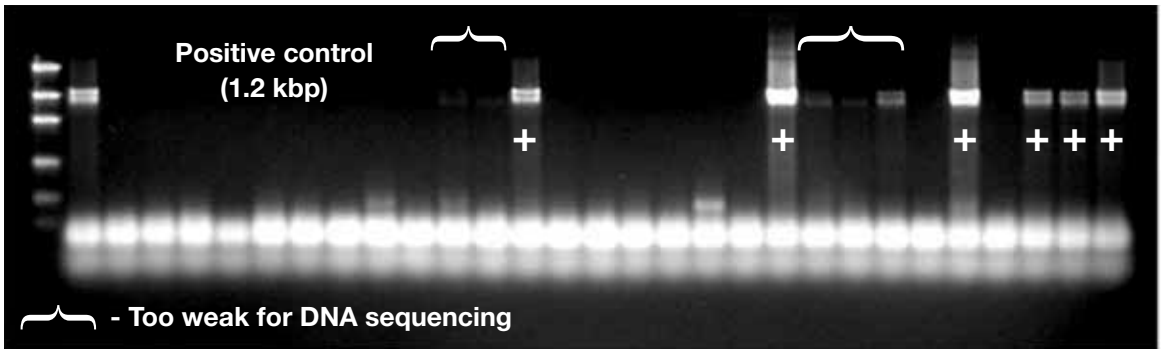

Fig. 31: Detection of phytoplasma DNA on agarose gel.

Techniques used to detect phytoplasmas continuously evolve and new methods and more efficient tests could be available in the coming years (Dickinson and Hodgetts 2013).

4.4. Other causes of yellowing

The symptoms of yellows can be confused with several other problems, as described below.

Mineral deficiency or toxicity

A number of mineral deficiencies and toxicities can result in foliar yellowing in grapevines. Iron deficiency can cause yellowing and stunting of leaves. Yellowing may occur on young leaves, particularly between the veins before the leaves dry up. Boron deficiency causes the growth of a dense mass of shoots from a single point (witch's broom) as well as the appearance of discoloured areas along the edge of the leaf blade. Manganese deficiency causes yellowing or reddening of the leaf blade with a marbled pattern. Potassium or magnesium deficiency can also cause discoloration of the leaves. Conversely, excess boron or manganese can be toxic to grapevines and can cause leaf rolling with a risk of necrosis and premature leaf drop.

Phytotoxicity associated with herbicide treatments

The exposure of grapevines to herbicides can cause the appearance of chlorosis (discoloration) similar to that caused by phytoplasmas on grapevine leaves. However, where herbicides are involved, chlorosis is not limited to one or a few plants but rather can affect several grapevines in one or more rows.

Mechanical injury

Hedging and pruning of grapevines can cause leaf injuries, and the leaves can turn red. In such cases, it is important to determine whether there are lesions on the leaves. When mechanical injuries occur, symptoms are found on several plants in the same rows and across the vineyard, in comparison with phytoplasma symptoms, which are sporadic in part of a vineyard.

Attack by other pests (phytophagous mites, thrips)

Phytophagous mites, such as the two-spotted spider mite (*Tetranychus urticæ*) (Fig. 32) and the European red mite, feed on grapevines by piercing and sucking the plant tissues. Additionally, mite saliva contains toxins that cause leaf rolling and discoloration (Fig. 33). These mites can be easily detected, because they weave webs on attacked leaves. Thrips can also have phytotoxic effects but do not weave webs. A hand lens may be necessary to identify these species.

Fig. 32: Two-spotted spider mite (*Tetranychus urticae*) (Photo Jacques Lasnier).

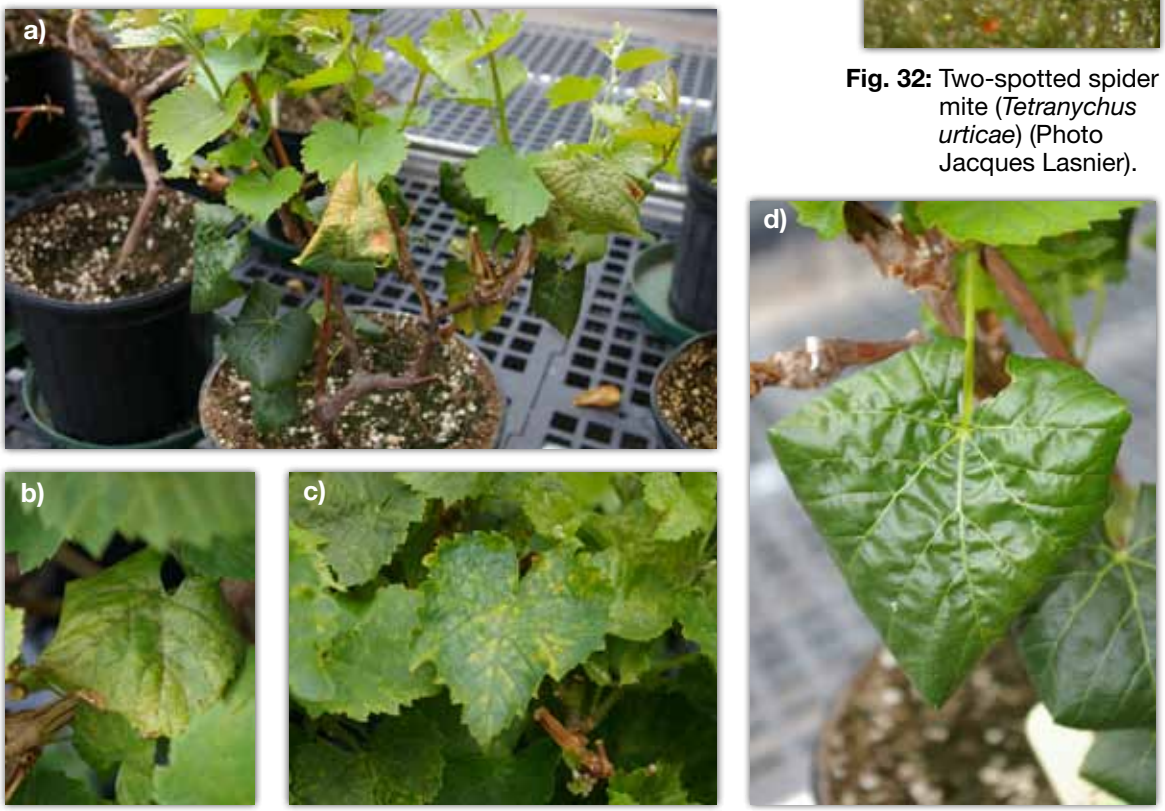

Fig. 33: Damage to grapevines by phytophagous mites: a) general view of a vine infected by mites; b) and c) yellowing of leaves; d) rolling of a leaf.

Other diseases

Other bacterial or fungal pathogens can also alter the physiology of the plant and result in leaf discoloration, yellowing, or both.

No matter what symptoms are observed, phytoplasma infection can be confirmed or ruled out only by molecular biology techniques.

5. Protection of vineyards from leafhoppers and phytoplasmas

Difficulties in identifying leafhopper species (notably at the nymphal stages), diagnosing diseases, and detecting phytoplasmas complicate the phytosanitary management of vineyards for phytoplasma diseases. However, various prevention and control strategies, based on preventive measures to avoid the spread of phytoplasmas, can be implemented. Prevention against phytoplasmas depends primarily on good management and maintenance of vineyards. It is also important to carry out increased monitoring in vineyards for leafhopper species, particularly those known to be vectors or potential vectors of phytoplasmas. Lastly, it is important to meet sanitary standards to prevent the introduction of contaminated plants and to ensure the removal of any contaminated plants as quickly as possible.

5.1. Integrated weed management and maintenance of vineyards and adjacent areas

Cultural practices

Cultural practices can have a major impact on the occurence of leafhoppers in vineyards. Leafhoppers can colonize different heights of the grapevine canopy, and the presence of some species of leafhoppers can be influenced by the density of the foliage. For example, the potato leafhopper (*Empoasca fabæ*) prefers to feed on the young parts of plants, whereas species of the genus *Erythroneura* prefer leaves near the ground and grape bunches. During the growing season, hedge pruning (Fig. 34) is carried out to keep each variety at the desired height. The purpose of hedging is to remove young shoots and canes, which are the parts of the plant preferred by some leafhopper species, such as *Empoasca fabæ*.

Leaf pulling carried out near grape clusters at the time of maturation reduces leafhopper populations that develop primarily on the lower leaves of the grapevine and reduces the risk of phytoplasma transmission.

Fig. 34: Two views of the mechanical hedging of grapevines to standardize vine size.

Fall and spring pruning can also be an important step in managing phytoplasma diseases favoring grapevine recovery, a poorly understood phenomenon. Recovery can be temporary or permanent depending on the cultivars, environmental conditions and the occurrence of re-infections. In Europe, a fall pruning, consisting of carefully removing the symptomatic canes, is known to reduce phytoplasma disease incidence the following year. It also reduces the survival of some insect pests that overwinter in the grapevine bark. Phytoplasma localized in grapevine roots may survive winter temperature. Spring pruning also allows the elimination of a part of phytoplasmas that recirculate in the plant during sap recirculation and bud burst, reducing the amount of inoculum available for the leafhoppers to be transmitted.

Hilling consists of drawing the soil up to form a protective mound around the base of the grapevines. This practice is done late in the fall and kills many soil-borne pests and parasites. A second tillage operation is carried out in the spring to remove the mound and clean the grapevines. Hilling can probably reduce the spreading of leafhoppers and phytoplasmas and improve the protection of vineyards.

Management of soil cover and potential phytoplasma reservoirs

Some grapevine phytoplasma diseases, such as aster yellows, can affect other plants in and around vineyards. Working the soil between the rows (Fig. 35) eliminates a large majority of reservoir plants and weeds from the vineyard. Mechanical or hand weeding at the base of the grapevines can prevent the potential spread of phytoplasmas from weeds to grapevines by reducing the risk of disease transmission by leafhoppers and limiting reservoir plants close to the grapevines.

Fig. 35: Soil cultivation: a) harrowing; b) mechanical weeding; c) hand weeding.

In some vineyards, grass is planted between the rows of vines to prevent erosion, increase plant biodiversity, or drain the land. However, some grasses or clover species can also provide a refuge for many species of leafhoppers that can move into the grapevines. Weeds between the rows or along the edges of vineyards can act as phytoplasma reservoirs and can increase the risk of spread of phytoplasma diseases within vineyards. Plants on the periphery of vineyards can also provide a refuge for leafhoppers overwintering under dead leaves.

Fig. 36: Grass strips between grapevine rows: a) in Quebec; b) in Ontario (Photo Jacques Lasnier).

Grass strips between rows (Fig. 36, 37a) must therefore be properly maintained (e.g. shearing), and care must be taken in the choice of species planted between the rows and grown around the vineyard. However, some plant species are also hosts for predators and natural enemies that may provide positive defences against leafhoppers.

Weedy areas (Fig. 37b) around vineyards can be another source of disease transmission, because the plant and leafhopper species that such areas support can be phytoplasma vectors. A number of leafhopper species feed on weeds in or near vineyards and can inoculate grapevines with phytoplasmas (Weintraub and Beanland 2006). Such is the case of *Hyalesthes obsoletus* in Europe and *Scaphoideus titanus* and other vectors of phytoplasmas in the United States (Hopkins and Purcell 2002; Beanland et al. 2006).

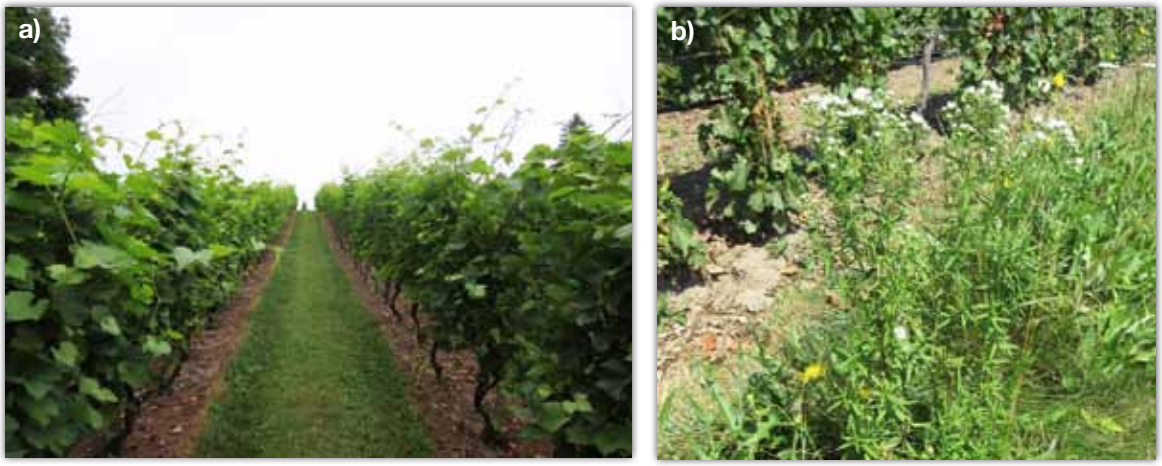

Fig. 37: a) Vineyard of Nova Scotia with grasses between rows; b) part of a vineyard with various weed species (Photos Jacques Lasnier).

Plants located on the periphery of vineyards can act as host or refuge plants for leafhoppers. Examples include Virginia creeper and wild grape (Fig. 38), which can act as host plants in the spring prior to grapevine budbreak and during the summer. Those plants can also act as refuge plants in the fall, when leafhoppers are looking for overwintering sites. These types of plants should therefore be removed in order to reduce leafhopper populations near vineyards.

Fig. 38: Virginia creeper and wild grape acting as refuge plants for leafhoppers.

Other plants are also found in or near vineyards, and several of those plants have been described as potential phytoplasma reservoirs. Below is a partial list of plant species that should be managed to prevent the spread of phytoplasma diseases (Fig. 39 to 56). The common names of the plants are consistent with Darbyshire (2003).

Fig. 39. Powell amaranth (*Amaranthus powellii*).

Fig. 40. Common ragweed (*Ambrosia artemisiifolia*): a) general view; b) isolated plant.

Fig. 41. Wild buckwheat (*Fallopia convolvulus*) (formerly *Polygonum convolvulus*).

Fig. 42. Large crab grass (*Digitaria sanguinalis*).

Fig. 43. Hairy galinsoga (*Galinsoga quadriradiata*) : a) general view; b) detail.

Fig. 44. Alfalfa (*Medicago sativa*).

Fig. 45. European wood-sorrel (*Oxalis stricta*).

Fig. 46. Lady's-thumb (*Persicaria vulgaris*) (formerly *Polygonum persicaria*).

Fig. 47. Timothy (*Phleum pratense*).

Fig. 48. Narrow-leaved plantain (*Plantago lanceolata*).

Fig. 49. Broad-leaved plantain (*Plantago major*).

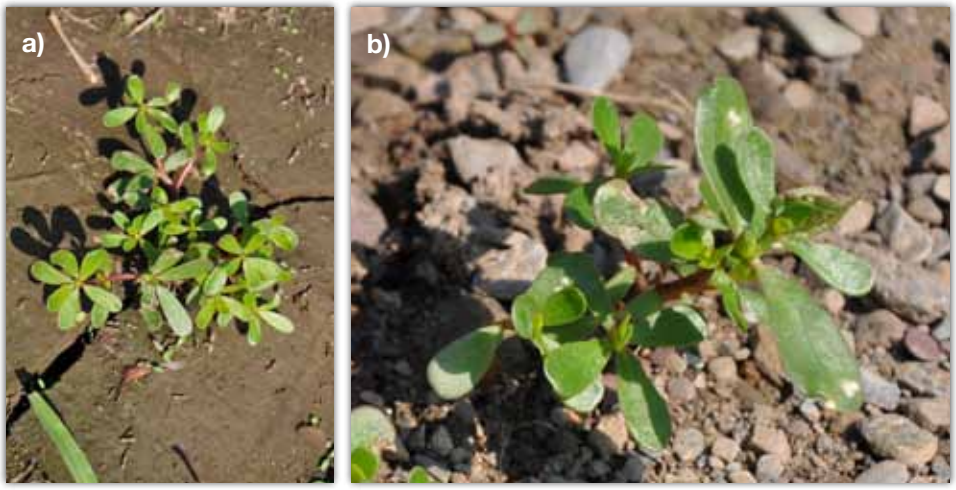

Fig. 50. Purslane (*Portulaca oleracea*): a) general view; b) detail.

Fig. 51. White cockle (*Silene pratensis*) (formerly *Lychnis alba*).

Fig. 52. Canada goldenrod (*Solidago canadensis*).

Fig. 53. New England aster (*Symphyotrichum novæ-angliæ*) (formerly *Aster novæ-angliæ*): a) general view; b) detail.

Fig. 54. Tall white aster (*Symphyotrichum lanceolatum)* (formerly *Aster simplex*): a) general view; b) detail.

Fig. 55. Red clover (*Trifolium pratense*).

Fig. 56. Tufted vetch (*Vicia cracca*).

5.2. Monitoring and management of leafhoppers

Although a large number of leafhopper species are associated strictly with grapevines, other species can be found in vineyards for short periods but do not necessarily feed on grapevines. Species feeding on broad-leaved plants (forbs and shrubs) may be vectors of disease, and hence the importance of the previously mentioned cultural practices for managing leafhopper populations in vineyards.

Visual monitoring

Leafhoppers can be detected visually. They are observed in flight in vineyards, specifically in the morning or following passes by agricultural equipment. Visual monitoring of plants also allows the detection of punctures on leaves, loss of pigmentation, or hopper burn caused by leafhoppers. Leafhopper eggs can be found by close inspection of the undersides of leaves.

Sweep netting

Using a net, 180 degree sweeps are made between the rows and on plants to capture flying insects (Fig. 57). This sampling technique can be used to collect leafhoppers at a specific time and to obtain an estimate of leafhopper populations. However, this technique is non-selective and collects many other insect species. Sweep netting can be used to collect live insects for rapid identification of leafhopper species present in the vineyard as well as for laboratory experiments that involve rearing or working with living individuals. Sweeping of grasses should be avoided, as some leafhopper species that do not threaten grapevine are very abundant on grasses.

Fig. 57: Leafhopper sampling with a sweep net (Photo Jacques Lasnier).

Trapping with yellow sticky traps

Yellow sticky traps can be used to monitor leafhoppers over a long period of time. Traps must be placed at various heights in the grapevine canopy, although placing traps near ground level should be avoided to minimize the trapping of grass-feeding insects. This non-selective monitoring method captures all flying insects attracted by the colour yellow (Fig. 58). The disadvantage of this method is that specimens caught in the traps are difficult to identify.

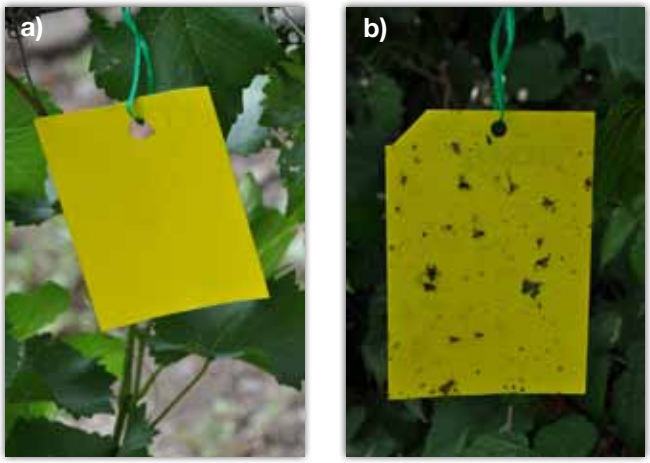

Fig. 58: Leafhopper monitoring with yellow sticky traps: a) freshly set trap; b) trap after 1 week.

Tapping

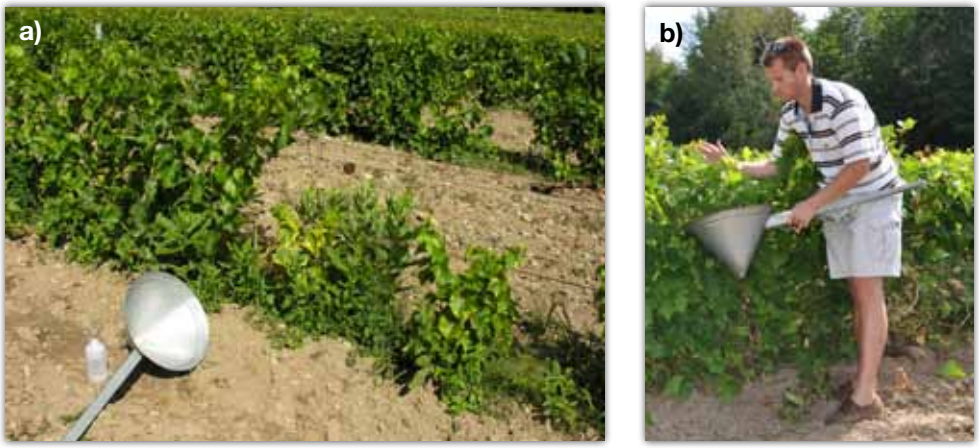

Fig. 59: Leafhopper sampling using the tapping method: a) equipment; b) technique.

Tapping (Fig. 59) consists of striking the leaves of grapevines several times over a metallic funnel that is filled with 70% ethanol. This method is non-selective, since all arthropod species located on the foliage may fall into the funnel. Grapevines are struck five times per meter of row at different heights in the canopy. Nymphs and adults fall into the funnel and are immediately killed. They remain on the surface of the funnel owing to the ethanol. Individuals can then be removed from the funnel using tweezers and transferred to a vial containing 70% ethanol until identification is performed. This method is used most commonly for field monitoring and taxonomic studies (identification with morphological or molecular biology techniques).

Insecticides

Fig. 60: Pesticide spraying in a vineyard: a) airblast sprayer in Quebec; b) recycling sprayer in Ontario (Photo Queen's Printer for Ontario 2015. Reproduced with permission; c) airblast sprayer in Ontario (Photo Wendy McFadden-Smith).

Several insecticides are registered for the control of leafhoppers in Canada. However, action thresholds are rarely reached, and the use of insecticides (Fig. 60) is seldom needed to control leafhopper populations. Recommended rates of pesticides vary considerably. Depending on the timing of applications, some broad-spectrum pesticides used to control other pests may be sufficient to reduce leafhopper populations. It is therefore important to verify the recommendations made by each province. Information regarding recommended products is generally published by provincial ministries of agriculture.

The Ontario Ministry of Agriculture, Food and Rural Affairs (2014) publishes recommendations for the production of fruit crops every year. The Centre de référence en agriculture et agroalimentaire du Québec (2014) also provides a plant protection guide for Quebec. A list of pesticides used in Nova Scotia vineyards is provided by Perennia (2013). These documents should be used to determine the appropriate products and concentrations and the optimal spraying conditions for the province in question. Another source of information is the Pest Management Regulatory Agency of Health Canada (PMRA), which publishes on its website the labels of all pest control products registered in Canada (PMRA 2015).

As pesticides can be toxic to bees, predators, and beneficial insects, such products must be used in a cautious manner, in order to reduce adverse effects on beneficial arthropod populations in vineyards. Through rational management of pesticide use in Quebec vineyards over the past 20 years, it has been possible to maintain the presence of certain leafhopper predators, such as the predatory mite *Anystis baccarum* (Bostanian et al. 2005, 2006; Laurin and Bostanian 2007; Lasnier, unpublished data). The judicious use of insecticides will also help prevent the development of insecticide resistance in leafhoppers.

Natural enemies

Although able to quickly flee from enemies (by walking, hopping, or flying), leafhoppers are prey or host species for many natural enemies in vineyards. Various generalist predators (e.g., beetles, ants, and plant bugs) are very good predators of leafhoppers and feed on large numbers of nymphs. This is also true of many of the species of spiders that have been reported in vineyards (Bolduc et al. 2005). One example is *Anystis baccarum* (Fig. 61), a small red predatory mite that is visible to the naked eye. It moves quickly on grapevine leaves and can capture a large number of preys every day.

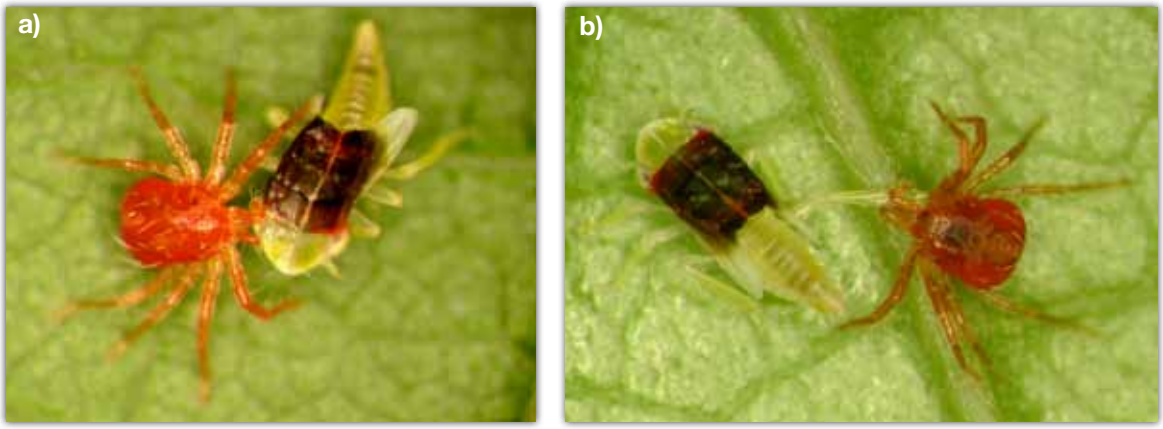

Fig. 61: *Anystis baccarum* attacking a leafhopper nymph: a) by an eye; b) by a leg.

Several insect species parasitize leafhoppers. For example, the females of some mymarid species (at least four genera) lay their eggs in leafhopper eggs. Larvae of Big-headed flies (family Pipunculidae) develop in leafhoppers, and an inflated abdomen of the host is a sign of parasitism. Larvae of Dryinidae (parasites) live subcutaneously in leafhopper hosts. Some Strepsiptera parasites extrude themselves from abdominal sclerites of leafhoppers.

Physical methods

Recent years have seen the development of other methods, such as the installation of nets several metres high around parts of vineyards to create a physical barrier that prevents insects from reaching their host plants. Ultraviolet-absorbing screening has also been tested in tunnels to assess the ability of this method to keep leafhoppers out of greenhouses (Weintraub et al. 2008; Olivier et al. 2012).

Resistant plants

Grapevine cultivars differ in leaf structure and physiology, and these differences can have an impact on the plants that leafhoppers choose. Research programs are also underway to identify varieties and rootstocks that are resistant to leafhoppers, phytoplasmas, or both (Olivier et al. 2012).

5.3. Prevention

There is no treatment available for the direct control of phytoplasmas in infected plants. Strict sanitary measures and control methods therefore must be implemented if certain diseases are detected.

Treatment of imported plants: hot water treatment

In the 2000s, Canada imported about 1.5 to 2 million grapevines from Europe every year. Given the presence of Flavescence Dorée and Bois Noir in Europe, the risk of importing these phytoplasmas through infected plants was significant (Foissac and Wilson 2010). To prevent the introduction of Flavescence Dorée or the reintroduction of Bois Noir, which are regulated in Canada, the Canadian Food Inspection Agency (CFIA) implemented strict regulations concerning imports of plants and the reporting of infected vines or part of vineyards. Growers have been asked to implement increased monitoring and report all grapevines that show symptoms of phytoplasma diseases, to help determine the origin of infected, imported grapevines. Another directive sets out the import requirements for grapevine propagative material and provides a list of approved countries, clones, and nurseries for the importation of disease-free rootstock and varieties to Canada (CFIA 2014).

A permit is required for grapevine importation. The material must come from a certified establishment, and the source of the plant material must be approved by the CFIA (CFIA 2014). The CFIA inspects plants at their point of entry into Canada, and an inspector may take samples for analysis.

Grapevines must meet phytosanitary requirements for importation to Canada. One treatment is hot water treatment, a technique that involves submersing canes, root cuttings, and young plants in hot (50 °C) water for at least 35 minutes in order to disinfect grapevine cuttings intended for grafting or import. The CFIA has introduced measures similar to those implemented in European countries (CFIA 2014).

Removal of contaminated plants and reporting of infected vines

In Europe, Flavescence Dorée and Bois Noir are quarantine diseases. Ministerial orders set out mandatory control measures for Flavescence Dorée and its vector. When these diseases are detected, the following control measures must be taken:

- Report infected grapevines.
- Uproot and burn infected plants.
- Define a control perimeter.
- Perform insecticide treatments against the vector, in vineyards and in nurseries.
- Destroy infected grafts or rootstock in nurseries.

When a municipality is infected by Flavescence Dorée, the municipality must inform the plant protection authorities and is classified as a “contaminated zone” for a minimum of two years. Only once the area is free of plants infected with Flavescence Dorée is it no longer considered a “contaminated zone.”

For growers, when infection levels reach 20%, all infested plants in the vineyards must be uprooted and burned. To harvest wood, a 1km safety perimeter is established around the infected block. The wood harvested must be treated with hot water, and planting within a 300m radius of the infested area is prohibited.

In high-risk regions where Flavescence Dorée is reported, insecticide treatments of infested vineyards are enforced every year by the phytosanitary authorities, which set the number of treatments (maximum of three) on the basis of the emergence and size of the vector leafhopper populations.

In Europe, there are no mandatory control measures for Bois Noir. However, if the presence of this disease is confirmed, infected plants must be removed.

Flavescence Dorée has not been detected in Canada to date, although its vector, *Scaphoideus titanus* is indigenous in Eastern Canada. However, when grapevines infected by Bois Noir were discovered in 2007, plants had to be destroyed, and the infested area of vineyards were subjected to strict monitoring over the following years (Rott et al. 2007). Although Aster yellow is widespread in vineyards and in other crops, no specific measures have been developed so far to control it. The same is true of other phytoplasma diseases in vineyards, but programs to control leafhopper populations are available to growers, as discussed above (see § 5.2. *Monitoring and management of leafhopper species*).

6. Techniques of study

6.1. Leafhopper rearing

As the identification of some leafhopper nymphs may be difficult, it may also be helpful to rear them to adulthood. Some leafhopper species associated with grapevines can be reared under laboratory conditions for purposes such as studying their development, behaviour, and resistance or susceptibility to pesticides. For example, Saguez and Vincent (2011) developed a method of rearing *Erythroneura elegantula*, *Erythroneura vitis*, and *Erythroneura ziczac* on grapevine leaves in Petri dishes (Fig. 62). This method requires relatively fewer grapevine plants in comparison with mass rearing on entire plants. A grapevine leaf is placed in a Petri dish containing an agar-agar solution in the process of forming a gel. Agar-agar is used to maintain moisture in the Petri dishes, preventing the overly rapid dehydration of isolated leaves exposed to feeding by roughly 30 nymphs, which remove the contents of the leaf cells. The lid of the Petri dish is covered with filter paper to collect the droplets of honeydew excreted by the leafhoppers as well as excess moisture. The entire set-up is inverted so that the leaf is positioned over the filter paper.

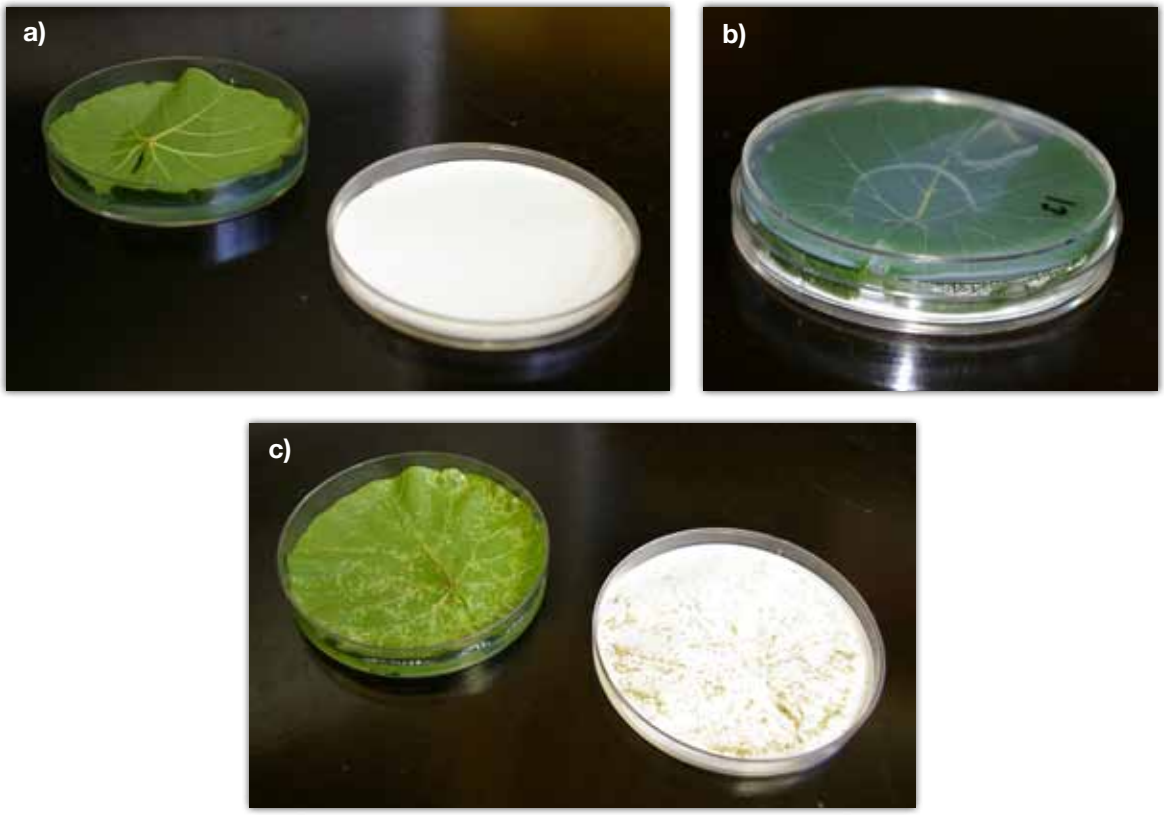

Fig. 62: Rearing of leafhoppers in Petri dishes: a) and b) set-up; c) view of the set-up after 1 week.

This set-up has a number of advantages. It makes it possible to control and synchronize leafhopper development and to test the effectiveness of certain insecticides or biopesticides at specific stages of leafhopper development. This technique can be used to initiate a new rearing, for instance in the event of infection by a pathogen or another insect.

6.2. Maintenance of phytoplasma strains

Until now, *in vitro* culture of phytoplasmas in laboratory conditions was considered impossible. However, Contaldo et al. (2012) succeeded in culturing a few phytoplasmas *in vitro*, including 16SrXII-A phytoplasmas associated with a grapevine yellow. The only way to ensure the conservation of strains is by rearing leafhoppers on host plants infected by phytoplasmas. This requires the availability of leafhopper species that are phytoplasma vectors or of plants infected by the pathogen in order to facilitate the epidemiological cycle. It is also important to have a large number of insects and plants to ensure the infection of new plants. Species such as *Macrosteles quadrilineatus* can be vectors of aster yellows and can be easily reared on barley plants. However, to ensure the sustainability of the strain of phytoplasma, the plants must be regularly renewed. This is tedious when perennials such as grapevine are involved. Projects requiring the use of phytoplasmas, particularly regulated quarantine diseases such as Flavescence Dorée and Bois Noir, should be carried out in containment greenhouses.

6.3. Histological studies

Histological techniques can be used to study damage caused by leafhoppers to grapevines. When they penetrate a plant, leafhoppers insert their stylets into its tissues. At the beginning of penetration and sometimes during feeding, leafhoppers secrete saliva that forms a sheath around their stylets. Salivary sheaths limit the production of plant defences and protect the insect against plant compounds. Microscopic observation techniques involving staining are used to locate leafhopper punctures in various plant tissues (Figs. 63, 64) and to determine the risk of disease transmission. The staining of salivary sheaths with Trypan blue makes it possible to locate them in leaves. Tissue staining with TBO (toluidine blue O) followed by the preparation of ultra-fine cross-sections by means of a microtome is used to observe leaf structure and the organization of the various tissues. The combination of the two techniques makes it possible to observe the stylet pathway within the plant.

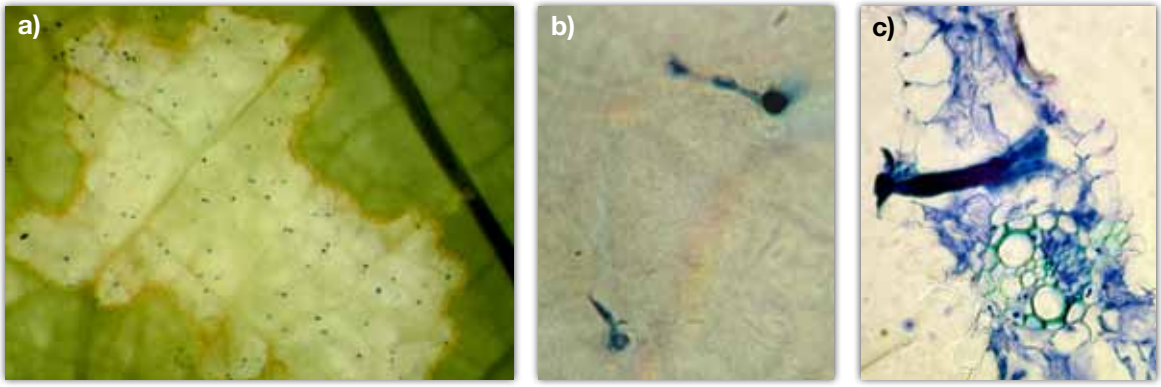

Fig. 63: Trypan blue method for showing leafhopper punctures: a) leafhopper punctures in a grapevine leaf; b) salivary sheath of an *Erythroneura* sp. (Source: Saguez et al. 2015); c) barley leaf punctured by *Macrosteles quadrilineatus*.

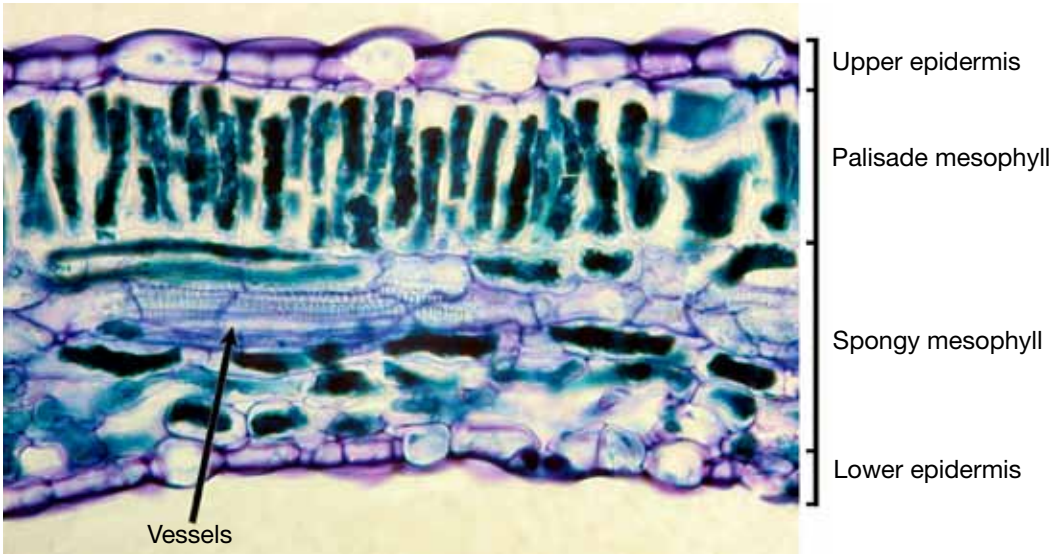

Fig. 64: Cross-section of a grapevine leaf showing the different leaf tissues (Source: Saguez et al. 2015).

6.4. Analysis of feeding behaviour using the electrical penetration graph technique

To understand the interactions between leafhoppers, grapevines, and phytoplasmas, histological studies can be complemented by behavioural studies. The feeding behaviour of leafhoppers can be studied using the electrical penetration graph (EPG) technique. In EPG, a leafhopper is made part of an electrical circuit (Fig. 65), where the leafhopper is connected to the positive electrode and the plant is connected to the negative electrode (ground). Briefly, a brass pin is soldered to a 2cm copper wire (Fig. 65a). Then, a 5cm gold wire (20µm in diameter) is glued to the copper wire with silver glue, and a gold wire is glued to the leafhopper's pronotum (Fig. 65a, b). Because plant cells have an electrical charge, each time the insect's stylet penetrates the plant, the circuit is completed, resulting in a fluctuating voltage. The waveform varies depending on the plant tissue penetrated and is characteristic of the type of food ingested (i.e., cell contents or fluids). The EPG technique makes it possible to differentiate between the penetration, saliva excretion, and ingestion phases (Fig. 66).

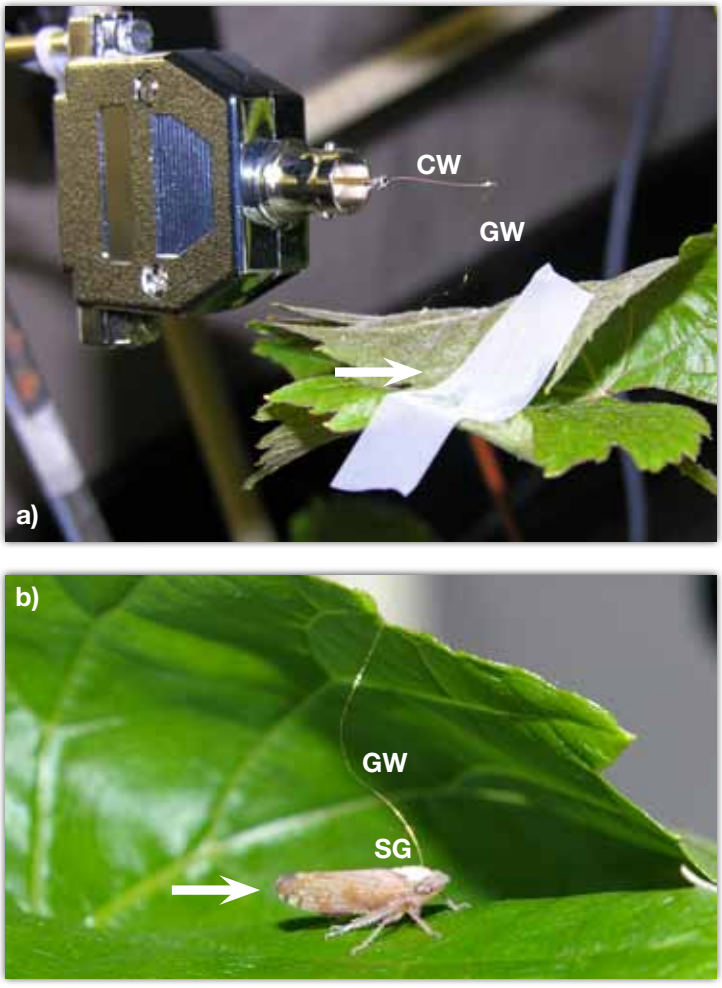

Fig. 65: EPG technique: a) positive electrode; b) leafhopper (white arrow) connected to the positive electrode and in contact with the leaf (CW: copper wire; GW: gold wire; SG: silver glue).

The EPG technique is used to identify whether leafhoppers are xylem, phlœm, or mesophyll feeders and to determine the time spent in each plant tissue. This technique provides information on the resistance of the plant to attack by a pest and makes it possible to determine whether a leafhopper can be considered a potential vector of disease.

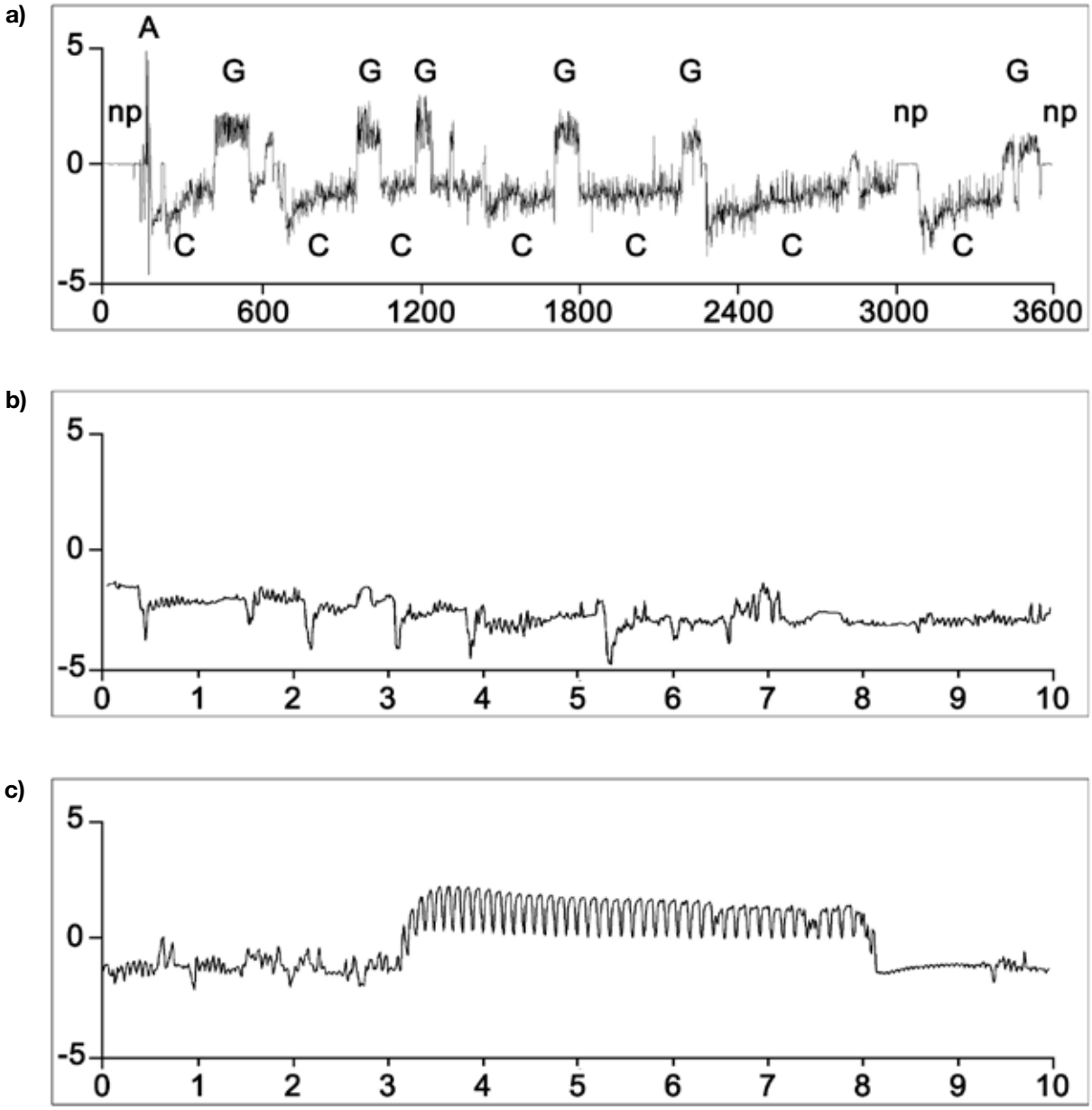

Fig. 66: EPG waveforms: a) general waveform recorded for 1 hour (np: non-probing; A: attack; C: mesophyll feeding; G: xylem feeding); b) signal characteristic of feeding in mesophyll (10 minutes); c) signal characteristic of feeding in xylem (10 minutes) (Source: Saguez et al. 2015).

Conclusion

The identification of leafhoppers, either nymphs or adults, is difficult. Many species are similar in appearance and can be confused with one another. If the presence of leafhoppers is suspected and a species of high risk to vineyards is identified, it is recommended to have the identification of the leafhoppers verified by a specialist in taxonomy.

Similarly, the identification of plants infected by phytoplasmas can be difficult, because plants can be asymptomatic and because some symptoms can be confused with those of other grapevine diseases. The presence of disease in samples suspected of being infected by phytoplasmas can be confirmed only by molecular biology techniques, which can take several days or even weeks to provide reliable results. It is therefore recommended to call on the services of researchers or specialists to avoid any diagnostic errors.

Growers can improve the protection of their grapevines by applying careful crop management practices and implementing effective methods to control leafhoppers and host plants that may be phytoplasma reservoirs. Growers should always select healthy and certified material for planting.

To limit the risk of spread of phytoplasma diseases in vineyards, the following best practices should be adopted:

- Monitor leafhopper populations and implement controls if large outbreaks of certain leafhopper populations are detected.
- Identify leafhopper species.
- Identify symptoms on grapevines.
- Contact specialists if a disease is suspected.
- Eliminate as many host plants of known and potential vectors as possible.
- Adopt sustainable control methods based on the use and protection of the natural enemies of leafhoppers.
- Remove infected plants.

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