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Macropsis mendax as a vector of elm yellows phytoplasma of *Ulmus* species

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A 3-year study was carried out in north-east Italy, the site of recent elm yellows epidemics, to identify vectors for the elm yellows phytoplasma. Using PCR analysis, *Ulmus minor* and *Ulmus pumila*, each with and without symptoms, were positive for the elm yellows phytoplasma. *Macropsis mendax*, a univoltine and monophagous leafhopper, was shown to be the vector of the elm yellows-associated disease agent. PCR analyses demonstrated that the insect was infected both in natural conditions and in the screenhouse after acquisition-feeding on infected elm plants. Groups of *M. mendax*, collected from naturally infected elm trees, transmitted elm yellows phytoplasma to elm test plants. In nature, *Alnus glutinosa* trees affected by alder yellows were found in the surroundings of yellows-affected elm trees; the associated disease agent of alder yellows was transmitted under controlled conditions from alder to elm test plants by grafting.

Keywords: alder yellows, epidemiology, leafhopper, transmission

Introduction

Elm yellows (EY) is a widespread, serious disease of elm. It is associated with a phytoplasma belonging to the elm yellows (= 16SrV) group (Lee *et al*., 1998; Seemüller *et al*., 1998). Elm yellows occurs in the eastern half of the USA, and contributed to a severe decline in native American elm, *Ulmus americana* and *Ulmus rubra* (Braun & Sinclair, 1979). Following the first description (Swingle, 1938), EY was reported to be restricted to north America (Griffiths *et al*., 1999). Since the 1950s the disease has been reported from Italy (Goidanich, 1951); the Czech Republic (Bojnansky, 1969); France (cited by Mäurer *et al*., 1993); and Germany (Seemüller, 1992). In Italy, after the first report EY was recorded in several areas including Emilia Romagna (Pisi *et al*., 1981; Lee *et al*., 1995); Tuscany (Conti *et al*., 1987); and the Po valley (Mittempergher *et al*., 1990). Natural infections were reported in *Ulmus minor* and *Ulmus pumila*.

In the USA the only confirmed vector of EY phytoplasma is the white-banded elm leafhopper *Scaphoideus luteolus* (Baker, 1949), which is not found in Europe (Della Giustina, 1989). The presence of additional active vectors was suspected in the USA because epidemics of EY are sometimes known to develop and persist where *S. luteolus* is scarce. Two possible vectors, for which single instances of EY phytoplasma transmission were recorded are the cercopid *Philaenus spumarius*, and the leafhopper *Allygidius atomarius* (Matteoni & Sinclair, 1988). Little information is available about the possible vector of EY phytoplasma in Europe, despite the fact that leafhoppers, vectors of phytoplasmas belonging to the EY group, are known. These are *Scaphoideus titanus*, *Macropsis fuscula* and *Oncopsis alni*, transmitting the associated disease agents of flavescence dorée (Schvester *et al*., 1963), rubus stunt (de Fluiter & van der Meer, 1953) and alder yellows (Maixner & Reinert, 1999), respectively.

Recently, in the Friuli Venezia Giulia (FVG) Region, north-east Italy, several elm trees showing symptoms of EY were observed. On the basis of symptomatology, hundreds of trees appeared to be infected in some restricted areas, indicating the presence of active vectors. Starting from this point, the objective of the present study was to identify the vectors of EY phytoplasma and to clarify the epidemiology of the disease in Italy and consequently in Europe, where *S. luteolus* is not present. An additional aim of the investigations was to verify the presence of possible alternative host plants of the EY-associated disease agent and of asymptomatically infected *Ulmus* spp. trees in nature.

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Materials and methods

General conditions

The investigations were started in September 2000 and continued until 2002. Surveys were carried out in

Table 1 Nested PCR analyses to test for the presence of elm yellows phytoplasmas in trees with and without symptoms sampled in 2000, 2001 and 2002 in different localities of north-east Italy

Site	Species	No. plants with symptoms PCR-positive/tested	No. plants without symptoms PCR-positive/tested
Cornino	*Ulmus minor*	8/8	0/7
	Alnus glutinosa	nt[a]	0/2
Trieste	*Ulmus minor*	20/20	1/5
	Ulmus pumila	6/6	1/2
	Alnus glutinosa	nt	2/2
	Crataegus spp.	nt	0/8
	Rubus spp.	nt	0/10
	Vitis sp.	nt	0/2
Gorizia	*Ulmus minor*	3/3	nt
	Ulmus pumila	3/3	nt
Moruzzo	*Ulmus minor*	4/4	3/14
	Alnus glutinosa	1/1	1/2
	Corylus avellana	nt	0/2
	Fraxinus sp.	nt	0/2
	Rubus spp.	nt	0/2

[a]nt, Not tested.

four different areas of the FVG Region: Cornino, Trieste, Gorizia and Moruzzo. All the transmission experiments were carried out in an environmentally controlled glasshouse at 23–25°C with supplementary light and 16 h days.

Testing for the presence of EY phytoplasma in plants and insects, and RFLP analysis

PCR was used to test for the presence of EY phytoplasma in all the plants listed in Table 1, the sources of inoculum used for acquisition experiments, inoculated test plants, groups of collected insects and a representative number of negative elm controls. The EY 1 strain of the EY phytoplasma maintained in periwinkle was used as a reference (Griffiths *et al*., 1999). The DNA was extracted from plants and insects as described by Carraro *et al*. (2002). For DNA extraction from plants, randomly collected shoots were used; in the case of trees with symptoms referable to phytoplasma disease, affected parts were chosen. The presence of EY phytoplasma was determined by nested PCR using the universal phytoplasma primers P1/P7 (Schneider *et al*., 1995). After 1 : 50 dilution, 5 μL of PCR product obtained in the first amplification was used as a template for further amplification with the nested primers fB1/rULWS (Smart *et al*., 1996) or R16(V)F1/R1 (Prince *et al*., 1993). Both the nested primer pairs are specific for the EY group of phytoplasmas. Amplifications were performed with a DNA Thermal Cycler Gene Amp PCR System 2400 (Perkin-Elmer Cetus, Norwalk, CT, USA) in a 50 μL volume containing 200 μM of the four dNTPs, 0·5 μM each primer, 1·5 U of Red *Taq* DNA polymerase, 1 × Red *Taq* polymerase buffer (both Sigma, St Louis, MO, USA), 2 μL of template DNA, and water. The mixture was subjected to 35 cycles at the following incubations: 30 s denaturation at 95°C (90 s for the first cycle), 60 s annealing at 55°C (50°C with primer pair fB1/rULWS), and 90 s extension at 72°C (10 min for the last cycle). The final PCR products (5 μL) were analysed by electrophoresis in 1·5% agarose gel and visualized under UV illumination after staining with ethidium bromide. The DNA of samples that were positive using EY group-specific primers were also amplified by nested PCR using the primer pair P1/P7 followed by R16F2n/R2 (Gundersen & Lee, 1996) and used for RFLP analysis. Ten microlitres of the resulting PCR products were digested with *Bfa*I (Bio Laboratories, Beverly, MA, USA), which differentiates EY (16SrV-A) phytoplasma from alder yellows (16SrV-C) phytoplasma (Lee *et al*., 1998; Davis & Dally, 2001). Restriction fragments were resolved in a 5% polyacrylamide gel.

2000 survey

In September, some severely pruned *U. minor* trees, located along a road in the Cornino area, were observed. The plants showed symptoms of pronounced witches' broom and small yellow epinastic leaves. In that area, no symptoms of witches' broom or yellowing were observed in elm trees that had not been pruned. Elm trees with and without symptoms were sampled and tested for the presence of EY phytoplasma using PCR analysis.

2001 survey

Starting in April, surveys were carried out in several localities of the FVG Region. In the areas of Trieste, Cornino and Gorizia, yellows-affected *U. minor* and *U. pumila* trees were observed; some of them were sampled and analysed by PCR. Symptomless elm trees were included in the analyses. Other wild plants (*Alnus glutinosa*, *Vitis* sp., *Rubus* spp., *Crataegus* spp.) growing in the surroundings of affected elm trees were also collected and analysed by PCR for the presence of EY phytoplasma. Sprouts emerging from the base of yellows-infected elm trees were uprooted and transplanted into pots in the glasshouse.

Starting in May, in Cornino and areas of Trieste, the presence of possible insect vectors of the EY-associated disease agent was checked periodically by shaking them off elm trees onto underlying nets. The insects were collected from symptomless elm trees because the yellows-affected trees were very tall so insects could not be captured. The collected insects were selected and grouped using the following criteria: (i) preference for leafhoppers, preferably found when feeding on elm; (ii) insects available in sufficient number for experiments; (iii) insects common to the two localities considered. Some of the captured hoppers were analysed by PCR after forced acquisitions to verify their ability to acquire EY phytoplasma. Acquisition experiments involved caging the insects in small screenhouses (1·5 × 0·7 × 0·7 m) on the infected sprouts of elm trees previously transplanted into pots. The test insects, in groups of five individuals, were analysed by PCR for the presence of EY phytoplasma

1 month after being caged (if alive), or following their death. Other insects were used in transmission trials in order to determine their capacity to inoculate and infect plants. The inoculations were carried out by caging the collected insects, in groups of 10 individuals, on *U. minor* seedling test plants.

In September, a graft transmission trial was done, in which the sources of inoculum were both EY-infected elm trees and alder trees located in Trieste, that showed as positive for EY group phytoplasmas by PCR. The test plants were young seedlings of *U. minor*, *U. pumila* and *A. glutinosa*. Four chip buds and one approach graft were used for each test plant.

2002 survey

In 2002, the field surveys and sampling were continued to monitor the disease in elm trees with and without symptoms. Other trees (*Fraxinus* sp., *Corylus avellana*, *Rubus* spp., *A. glutinosa*) were also analysed by PCR. An area of Moruzzo was surveyed, where several young elm trees with yellows were present. Most of the EY-affected plants in Moruzzo were of a height that permitted the insects to be captured. In view of the results obtained during the acquisition experiments carried out the previous year, nymphs and adults of the leafhopper *Macropsis mendax* were collected from elm trees showing symptoms. The leafhoppers, in groups of 10 individuals, were used for transmission trials to elm seedling test plants. Four groups of five individuals were also analysed by PCR soon after capture.

Results

Insects found on elm trees

A few species of leafhoppers were regularly caught on elm trees (Table 2). These were *Iassus scutellaris*, *M. mendax* and *Metcalfa pruinosa*. The cercopids *Aphrophora alni* and *P. spumarius*, although both xylem-feeding, were included in the acquisition access experiments and in transmission trials because they are found in all the localities surveyed, and because *P. spumarius* is reported to be a possible secondary vector of EY phytoplasma. The other species of leafhoppers, *Issus* sp., *Aphrodes* sp. and *Hyalesthes luteipes*, although not regularly found on elm trees, were captured in sufficient number and so were included in the experiments.

Table 2 Faunistic data on the leafhoppers collected in 2001 and 2002 from elm trees in different areas of the Friuli Venezia Giulia Region (north-east Italy); insects were collected by shaking them off elm trees onto underlying nets

Species	Month of collection	Locality	Number of insects collected
Aphrodes sp.	June	Trieste	35
Aphrophora alni	June	Cornino, Trieste	20
Hyalestes luteipes	July, August	Trieste	30
Iassus scutellaris	May, June, July	Cornino, Trieste	140
Issus sp.	May, June	Trieste	25
Macropsis mendax	May, June, July	Cornino, Trieste	20
Metcalfa pruinosa	June, July, August	Cornino, Trieste	170
Philaenus spumarius	May, June, July	Cornino, Trieste	80
Macropsis mendax[a]	May, June, July	Moruzzo	220

[a]Leafhoppers collected in 2002; the remainder were collected in 2001.

PCR detection and characterization of phytoplasmas in plants and insects

DNA was amplified in nested PCR from both elm and alder trees, and also from groups of *M. mendax* (Tables 1, 3–5). No differences in detection were observed when the EY group-specific primers R16(V)F1/R1 and fB1/rULWS were used to detect phytoplasmas from the same samples. Fragments of the expected size were also amplified from reference strain EY 1 from periwinkle plants (Fig. 1). No DNA amplification was obtained from the negative controls. After digestion with *Bfa*I, the PCR products obtained from samples of periwinkle EY 1, elm trees and groups of *M. mendax* showed the restriction profile belonging to the subgroup 16SrV-A. The PCR

Table 3 Results of graft transmission experiments using elm and alder trees both as sources of inoculum and test plants; 8 months after grafting the plants were analysed by nested PCR for the presence of elm yellows phytoplasmas

	Test plants	
Source of inoculum	Species	No. PCR positive/ grafted
Ulmus minor	*Ulmus minor*	4/4
	Ulmus pumila	4/4
	Alnus glutinosa	0/3
Alnus glutinosa	*Ulmus minor*	1/3
	Alnus glutinosa	3/3

Table 4 Nested PCR analyses carried out in 2001 to test for the presence of elm yellows phytoplasmas in groups of five insects after acquisition access on infected elm plants; insects were analysed 1 month after the beginning of acquisition, if still alive, or after their death

Species	Groups of insects positive/tested (no.)
Aphrodes sp.	0/1
Aphrophora alni	0/2
Hyalesthes luteipes	0/2
Iassus scutellaris	0/6
Issus sp.	0/1
Macropsis mendax	2/2
Metcalfa pruinosa	0/10
Philaenus spumarius	0/8

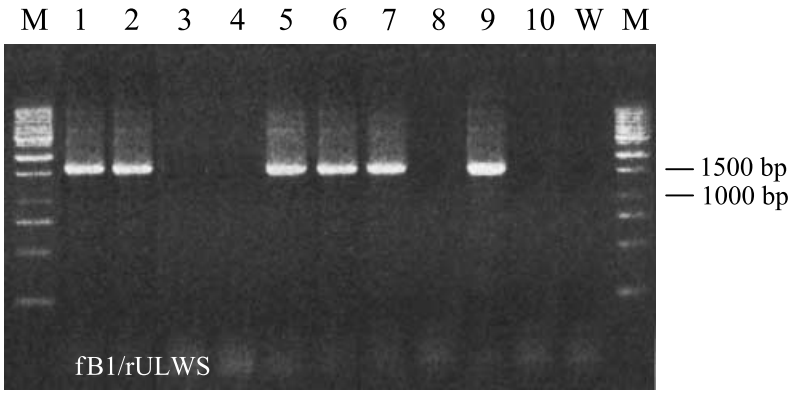

Figure 1 Agarose gel electrophoresis of PCR products from *Ulmus* spp., *Alnus glutinosa*, and groups of *Macropsis mendax* amplified in nested PCR with the primer pair fB1/rULWS. Lanes 1–3: *M. mendax*; 4–7: *Ulmus* spp.; 8–10: *A. glutinosa*; W, water control; M, DNA marker (1 kb DNA ladder, Fermentas, Lithuania).

Table 5 Results of transmission trials carried out in 2001 and 2002 by feeding insects collected from elm trees. Ten individuals per plant were caged on seedlings of *Ulmus minor*. Test plants were tested by nested PCR for the presence of elm yellows phytoplasmas 8 months after exposure to insects

Inoculation by	Number of plants PCR-positive/fed with insects
Aphrodes sp.	0/3
Aphrophora alni	0/1
Hyalesthes luteipes	0/2
Iassus scutellaris	0/11
Issus sp.	0/2
Macropsis mendax	0/1
Metcalfa pruinosa	0/12
Philaenus spumarius	0/4
Macropsis mendax[a]	5/22

[a]Trials carried out in 2002; remainder in 2001.

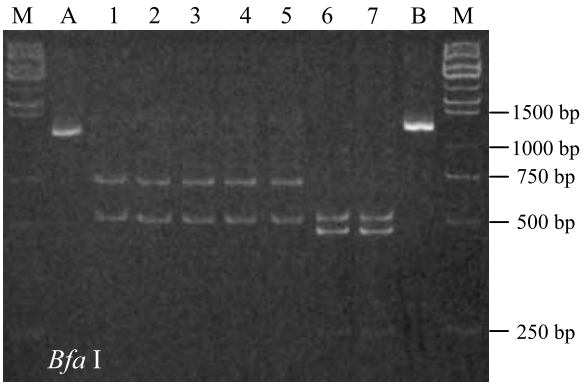

Figure 2 *Bfa*I restriction profiles of phytoplasma 16S rDNA amplified in nested PCR with the primer pair R16F2n/R2. The template DNA was from elm yellows phytoplasma-infected *Macropsis mendax* (lane 1) and *Ulmus minor* (lanes 2–5); and from alder yellows phytoplasma-infected *Alnus glutinosa* (lanes 6–7). Lanes A and B: undigested amplified elm yellows phytoplasma 16S rDNA (controls); M, DNA marker.

products from samples of alder trees, including the elm test plant graft inoculated using alder as source of inoculum, showed the profile of the subgroup 16SrV-C (Fig. 2).

Presence of EY phytoplasmas in trees with and without symptoms

Using PCR, the EY phytoplasma was detected in *U. minor* and *U. pumila* trees growing in four localities of the FVG Region (Table 1). All 44 yellows-affected elm trees were phytoplasma positive. No differences in symptom expression between the two species of elm were observed. Both species showed clear to faint symptoms of witches' broom, yellowing and small epinastic leaves. Five of the 28 symptomless elm trees were positive for EY phytoplasma. In the localities of Trieste and Moruzzo, four out of the five *A. glutinosa* trees tested positive for the presence of phytoplasmas belonging to EY group. Only one of these infected trees showed faint symptoms of phytoplasma infection such as yellowing and decline. Phytoplasmas were not detected in other species of plants using PCR.

Graft transmission experiment using elm and alder trees as sources of inoculum and test plants

The associated disease agent was transmitted from infected elm to healthy elm test plants by grafting (Table 3). The test plants showed symptoms of yellowing. Using elm as inoculum, the transmission to alder was negative. When infected alder was used as the donor plant, the agent was transmitted to all the alder test plants and also to one of the three elm seedlings. In this case the infected test plants, both alder and elm, also showed symptoms associated with phytoplasma infection, such as yellowing.

Transmission trials by insects

All the insect transmission trials carried out in 2001 were negative (Table 5). Phytoplasmas were detected in *M. mendax* which had fed on EY phytoplasma-infected plants (Table 4). In 2002 research concentrated exclusively on this species of leafhopper. In the Moruzzo area it was possible to collect several individuals, nymphs and adults, of *M. mendax* from infected elm trees with yellows. Some of these insects were found to be naturally infected (one group out of the four tested by PCR), and five of the 22 groups of *M. mendax* transmitted EY phytoplasma to elm seedling test plants (Table 5). The test

plants showed yellowing symptoms typical of phytoplasma-associated disease.

Discussion

The main object of the present work was to investigate the European vector of EY phytoplasma. In various localities of the FVG Region, EY appeared to be widespread, indicating the presence of active vectors. During 2001, individuals from eight species of hoppers were collected from symptomless elm trees in two EY-infected areas and used for transmission trials. None of these insects transmitted the associated agent of the disease to test plants. Individuals of the same species were forced to feed on infected elm trees in the screenhouse and then tested, using PCR, for the presence of EY phytoplasma. Only two groups of *M. mendax* were positive according to PCR analysis, indicating their ability to acquire the EY agent, but not to transmit it. *Macropsis mendax* used in acquisition experiments may have been infected with EY phytoplasma before collection; they were collected from symptomless *Ulmus* spp. trees that can, however, be infected by EY phytoplasma. But there is the possibility that *M. mendax* acquired EY phytoplasma from sources of inoculum in the glasshouse. The two groups of insects tested PCR positive after acquisition feeding; the other group, used in transmission trials without forced acquisition, did not transmit EY phytoplasma to test plants. It should be noted that *P. spumarius*, reported to be a secondary vector of EY phytoplasma in the USA, although feeding on xylem, did not test positive for the associated disease agent.

During the year 2002, work concentrated on *M. mendax*. In Moruzzo it was possible to collect several individuals of this species from elm trees with symptoms associated with yellows disease. One group out of the four tested by PCR was naturally infected. Five groups were able to transmit the associated disease agent to elm test plants, clearly indicating that *M. mendax* is the vector or one of the European vectors of EY phytoplasma. *Macropsis mendax* is monophagous and has one generation per year on elm, where it overwinters as eggs (Pavan, 2000). It is interesting to note that *M. mendax* belongs to the subfamily *Macropsinae*, as do vectors of other phytoplasmas of the EY group in Europe, i.e. the associated disease agents of rubus stunt and alder yellows.

In the present work, EY was found to be widespread in north-east Italy on *U. minor* and *U. pumila* trees. Symptoms such as witches' broom, yellowing and small leaves are characteristic of associated phytoplasma infection. Symptomless but infected trees were also found. A phytoplasma belonging to the EY group was also detected in alder trees with and without symptoms. The alder yellows phytoplasma was easily transmitted to alder test plants by graft transmission and, in one case, also to an elm test plant. Both alder yellows and EY appear to be closely related diseases: (i) the relative associated disease agents belong to the same group of phytoplasmas; (ii) both are present in the same localities; (iii) they have a common experimental host (elm). The molecular relationships between the two phytoplasmas associated with these diseases were studied by Marcone *et al.* (1997) and Maixner & Reinert (1999). Using RFLP analyses of ribosomal and nonribosomal DNA fragments, they found geographic variation of alder yellows phytoplasma. Davis & Dally (2001) recently revised the subgroup classification of the EY group of phytoplasmas, placing the associated disease agents of EY and alder yellows in two different subgroups, A and C, respectively. The results obtained in the present work confirm this subgroup classification. Further investigations, involving the host range and the vectors, are necessary to clarify the relationships between the two diseases. The indication of a common host plant (*U. minor*) for the two diseases is a promising starting point.

The natural spread of EY in Europe is clarified now it is known that an insect as common as *M. mendax* (Ribaut, 1952) is a vector of the associated disease agent. No information is yet available on the efficiency of *M. mendax* as a vector. This is an interesting subject for future research.

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References

Baker WL, 1949. Notes on the transmission of the virus causing phloem necrosis of American elm, with notes on the biology of its insect vector. *Journal of Economic Entomology* **42**, 729–32.

Bojnansky V, 1969. Elm witches'-broom, a new disease in Czechoslovakia. *6th Proceedings of the Conference of the Czechoslovak Plant Virologists, Olomuc, Czechoslovakia.* High Tatras, Czechoslovakia, 211–3.

Braun EJ, Sinclair WA, 1979. Phloem necrosis of elm: symptoms and histopathological observations in tolerant hosts. *Phytopathology* **69**, 354–8.

Carraro L, Ferrini F, Ermacora P, Loi N, 2002. Role of wild *Prunus* species in the epidemiology of European stone fruit yellows. *Plant Pathology* **51**, 513–7.

Conti M, D'Agostino G, Mittempergher L, 1987. A recent epiphytotic of elm yellows in Italy. *7th Proceedings of the Mediterranean Phytopathological Union Congress*. Granada, Spain: Spanish Phytopathological Society, 208–9.

Davis RE, Dally EL, 2001. Revised subgroup classification of group 16SrV phytoplasmas and placement of flavescence dorée-associated phytoplasmas in two distinct subgroups. *Plant Disease* **85**, 790–7.

Della Giustina W, 1989. Homoptères Cicadellidae III. *Faune de France 73*. Paris, France: FFSSN/INRA.

de Fluiter HJ, van der Meer FA, 1953. Rubus stunt, a leafhopper borne virus disease. *Tijdschrift over Plantenziekten* **59**, 195–7.

Goidanich G, 1951. Gli scopazzi dell'olmo. *Informatore Fitopatologico* **14**, 8.

Griffiths HM, Sinclair WA, Boudon-Padieu E, Daire X, Lee IM, Sfalanga A, Bertaccini A, 1999. Phytoplasmas associated with elm yellows: molecular variability and differentiation from related organisms. *Plant Disease* **83**, 1101–4.

Gundersen DE, Lee IM, 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea* **35**, 144–51.

Lee IM, Bertaccini A, Vibio M, Gundersen DE, Davis RE, Mittempergher L, Conti M, Gennari F, 1995. Detection and characterization of phytoplasmas associated with disease in *Ulmus* and *Rubus* in northern and central Italy. *Phytopathologia Mediterranea* **34**, 174–83.

Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk IM, 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48**, 1153–69.

Maixner M, Reinert W, 1999. *Oncopsis alni* (Schrank) (Auchenorrhyncha: Cicadellidae) as a vector of the alder yellows phytoplasma of *Alnus glutinosa* (L.) Gaertn. *European Journal of Plant Pathology* **105**, 87–94.

Marcone C, Ragozzino A, Seemüller E, 1997. Identification and characterization of the phytoplasma associated with elm yellows in southern Italy and its relatedness to other phytoplasmas of the elm yellows group. *European Journal of Forest Pathology* **27**, 45–54.

Matteoni JA, Sinclair WA, 1988. Elm yellows and ash yellows. In: Hiruki C, ed. *Tree Mycoplasmas and Mycoplasma Diseases*. Alberta, Canada: University of Alberta Press, 19–31.

Mäurer R, Seemüller E, Sinclair WA, 1993. Genetic relatedness of mycoplasma-like organisms affecting elm, alder, and ash in Europe and north America. *Phytopathology* **83**, 971–6.

Mittempergher L, Fagnani A, Ferrini F, D'Agostino G, 1990. Elm yellows a disease to be taken into consideration when breeding elm for disease resistance. *8th Proceedings of the Mediterranean Phytopathological Union Congress*. Agadir, Morocco: Actes, 433–5.

Pavan F, 2000. Occurrence on elm and phenology of Auchenorrhyncha potential vectors of the phytoplasma associated with elm yellows disease. *Bollettino di Zoologia agraria e di Bachicoltura* **32** (II), 59–68.

Pisi A, Marani F, Bertaccini A, 1981. Mycoplasma-like organisms associated with elm witches' broom symptoms. *Phytopathologia Mediterranea* **20**, 189–91.

Prince JP, Davis RE, Wolf TK, Lee IM, Mogen BD, Dally EL, Bertaccini A, Credi R, Barba M, 1993. Molecular detection of diverse mycoplasma-like organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. *Phytopathology* **83**, 1130–7.

Ribaut H, 1952. Homoptères Auchénorhynques II (Jassidae). *Faune de France 57*. Paris, France: Lechevalier.

Schneider B, Seemüller E, Smart CD, Kirkpatrick B, 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG, eds. *Molecular and Diagnostic Procedures in Mycoplasmology*. San Diego, CA, USA: Academic Press, 369–80.

Schvester D, Carle P, Moutous G, 1963. Transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. (*Homopt. Jassidae*). *Annales des Epiphyties* **14**, 167–74.

Seemüller E, 1992. Laubgehölzmycoplasmosen in Europa. *Nachrichtenblatt des Deutschen Pflanzenschutzdientes* **44**, 145–8.

Seemüller E, Marcone C, Lauer U, Ragozzino A, Göschl M, 1998. Current status of molecular classification of the phytoplasmas. *Journal of Plant Pathology* **80**, 3–26.

Smart CD, Schneider B, Blomquist CL, Guerra LJ, Harrison NA, Ahrens U, Lorenz KH, Seemüller E, Kirkpatrick B, 1996. Phytoplasma specific primers based on sequences of the 16S–23S rRNA spacer region. *Applied and Environmental Microbiology* **62**, 2988–93.

Swingle RU, 1938. A phloem necrosis of elm. *Phytopathology* **28**, 757–9.