European Journal of Plant Pathology **110:** 353–360, 2004.

Molecular identification of a new member of the clover proliferation Phytoplasma group (16SrVI) associated with Centaurea solstitialis virescence in Italy

Francesco Faggioli[1], Graziella Pasquini[1], Valentina Lumia[1], Gaetano Campobasso[2], Timothy L. Widmer[3,*] and Paul C. Quimby, Jr.[3]
[1]*Istituto Sperimentale per la Patologia Vegetale, Rome, Italy;* [2]*European Biological Control Laboratory, USDA-ARS, Via Colle Trugli No. 9, 00132 Rome, Italy;* [3]*European Biological Control Laboratory, USDA-ARS, Campus International de Baillarguet, CS 90013, Montferrier sur Lez, 34988 St. Gely du Fesc CEDEX, France;* [*]*Author for correspondence (Fax: +33499623049; E-mail: tlwidmer@ars-ebcl.org)*

Accepted 26 November 2003

Key words: 16S-23S rRNA, weeds, yellow starthistle

Abstract

In the United States, yellow starthistle (*Centaurea solstitialis*) is an annual invasive weed with Mediterranean origins. Malformed plants displaying witches' broom, fasciations, abortion of buds and flower virescence symptoms were observed in central Italy. Attempts to transmit the causal agent from the natural yellow starthistle host to periwinkle by grafting, resulted in typical symptoms of a phytoplasma, i.e. yellowing and shortening of internodes. The detection of phytoplasmas was obtained from both symptomatic yellow starthistle and periwinkle by the specific amplification of their 16S-23S rRNA genes. PCR amplification of extracted DNA from symptomatic plant samples gave a product of expected size. Asymptomatic plants did not give positive results. An amplicon obtained by direct PCR with universal primers P1/P7 was cloned and sequenced. The homology search using CLUSTALW program showed more than 99% similarity with Illinois elm yellows (ILEY) phytoplasma from Illinois (United States) and 97% with Brinjal little leaf (BLL) phytoplasma from India. Digestion of the nested-PCR products with restriction enzymes led to restriction fragment length polymorphism patterns referable to those described for phytoplasmas belonging to the clover proliferation (16S-VI) group. Since this is a previously undescribed disease, the name Centaurea solstitialis virescence has been tentatively assigned to it. This is a new phytoplasma with closest relationships to ILEY and BLL, but distinguishable from them on the basis of 16S rDNA homology, the different associated plant hosts and their geographical origin.

Introduction

Centaurea solstitialis, common name yellow starthistle, is a serious invasive weed in the United States belonging to the family Asteraceae. In the United States, yellow starthistle is a serious pest of pastures, rangelands, croplands, natural areas and recreational areas. It is an aggressive invader of disturbed environments and spreads quickly if left uncontrolled. Consumption of yellow starthistle by horses may be fatal resulting from 'chewing disease' (Cordy, 1978). The spiny flowerheads also reduce the use of recreational areas. Different strategies for its management have been assessed, including mowing, grazing, burning, reseeding with competitive grasses (Woo et al., 1999), biological control (Bruckart, 1989; Campobasso et al., 1998) and chemical control (Shinn and Thill, 1999).

Malformed yellow starthistle plants showing witches' broom, fasciations, abortion of buds and flower virescence symptoms were observed on a mountainous site in central Italy. The symptoms were consistent with those previously described by infection with a phytoplasma (Lee et al., 2000).

Phytoplasmas are unculturable, walless prokaryotes of the class *Mollicutes* (Gundersen et al., 1994). They are known to be associated with diseases in several hundred plants species, including weeds (Lee and Davis, 1992; Marcone et al., 1997). Until the last decade, differentiation and classification of unculturable phytoplasmas relied primarily on their biological properties such as plant and insect host specificity and symptomatology of infected plants. More recently, classification of phytoplasmas in a monophyletic clade within the class Mollicutes has been possible by using restriction fragment length polymorphism (RFLP) analysis and sequencing of the conserved 16S rRNA gene; the majority of phytoplasmas were divided and assigned into 15 (Lee et al., 1998; Montano et al., 2001) or 20 (Semüller et al., 1998) 16S rDNA groups, encompassing a large number of subgroups.

Until now, studies on phytoplasma-infected weeds were mainly based on the role that the weeds could play as potential plant reservoirs for a given phytoplasma, and thus as alternative hosts for economically important phytoplasma diseases. In this case, the disease affecting yellow starthistle plants may be taken in consideration as a possible biological control agent of the weed, since the diseased plants do not produce achenes and thorns. Conventional control strategies have been inadequate due to the large infestations and the economical and environmental costs of chemical control. However, the first step would be to positively identify whether a phytoplasma is the causal agent and classify it in one of the identified groups.

Although described symptoms have been observed in the field since 1980, no study to identify the causal agent had been undertaken previously. This paper reports the detection and characterization of a phytoplasma associated with yellow starthistle plants in Italy.

Materials and methods

Origin of samples and disease transmission

Twenty-eight samples of yellow starthistle plants showing witches' broom, fasciations, abortion of buds and flower virescence symptoms were collected during 2000 and 2001 from a mountainous site in the Latium region of central Italy. Fifteen asymptomatic yellow starthistle plants were also collected as negative controls in the same area. Attempts were made to transmit the phytoplasma from the natural yellow starthistle host to periwinkle (*Catharanthus roseus*) by shoot grafting. Two shoots of symptomatic yellow starthistle material were grafted, by a cleft-graft, onto the main branches of periwinkle. Plants, in which the grafted material survived for at least one month, were used for confirmation of transmission. For use as positive control, eight phytoplasma DNAs extracted from periwinkle infected with phytoplasmas belonging to four groups (Table 1) were employed in PCR and RFLP analyses.

DNA extraction

Leaf midribs or whole small leaves (1.5 g) were excised from symptomatic and asymptomatic yellow starthistle plants. Total DNA was extracted according to Marzachì et al. (1999). Leaf midribs were taken also from healthy and phytoplasma-infected periwinkle plants. The total DNA was resuspended in 100 µl of sterile distilled water.

PCR conditions

Direct amplification by PCR was carried out using the universal phytoplasma primer pair P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1995). These oligonucleotides amplify a DNA fragment of ~1800 bp in length between the 5′ end of the 16S rRNA gene and the 5′ region of the 23S rRNA gene. PCR amplification was performed by adding 10 ng of template DNA to a solution consisting of 10 mM Tris–HCl, pH 9.0; 50 mM KCl; 0.1% Triton X-100; 1.5 mM $MgCl_2$; 200 µM dNTPs each; 0.4 µM P1 and P7 primers; 2 units of *Taq* polymerase (Promega, Madison, WI, USA). The amplification process was carried out for 35 cycles with a PTC-200 DNA Engine model thermocycler (MJ Research Co., Watertown, Massachusetts, USA) under the following condition: denaturation at 94 °C for 45 s (3 min first cycle), annealing at 55 °C for 1 min, and extension at 72 °C for 2 min (10 min last cycle). A nested-PCR was performed using the universal phytoplasma primers R16F2/R16R2 (Lee et al., 1995). These oligonucleotides amplify a DNA fragment of ~1250 bp in the 16S rRNA region. Direct PCR amplicons were diluted 1 : 40 and mixed in a solution containing 10 mM Tris–HCl, pH 9.0; 50 mM KCl; 0.1% Triton X-100; 2 mM $MgCl_2$; 250 µM dNTPs each; 0.4 µM R16F2 and R16R2 primers; 2 units of *Taq* polymerase (Promega, Madison, WI, USA). DNA amplification was achieved by denaturation at 95 °C for 3 min and 35 cycles under the following conditions: denaturation at 94 °C for 1 min, annealing at 55 °C for

Table 1. Phytoplasma strains employed in molecular analyses

Phytoplasma strains	Acronym	Origin	16S rRNA group[a]	Researcher who provide organism or Gene Bank accession number
Centaurea solstitialis virescence	CSV	Italy	VI-D	This study
Beet leafhopper transmitted virescence	BLTVA	California	VI-A	Dr Lee, through Dr. Bertaccini
Catharanthus phyllody	CPS	Sudan	VI-C	Dr. Bertaccini
European aster yellows	EAY	Italy	I-B	This lab
Faba bean phyllody	FBP	Sudan	II-C	Dr. Bertaccini
Picris echioides phyllody	PEP	Italy	II-E	Dr. Marcone
Sesame phyllody	SEPT	Thailand	II-A	Dr. Bertaccini
Stolbur	STOL	Serbia	XII-A	Dr. Boccardo
Tomato big bud	TBB	Australia	II-D	Dr. Bertaccini
Almond witches' broom	AWB	Lebanon	IX	AF455038
Ash yellows	ASHY	USA	VII-A	AF092209
Bermuda grass white leaf	BGWL	Bermuda	XIV-A	AF248961
Black alder witches' broom	BaWB	Germany	X-E	X76431
Brinjal little leaf	BLL	India	VI	AF228052
Candidatus Phytoplasma brasiliense	Pbras	Brazil	XV	AF147708
Coconut letal yellowing	CLY	USA	IV-A	U18747
Elm yellows	EY	USA	V-A	AF189214
Epilobium phyllody	EpP	Estonia	I	AY101386
Erigeron witches' broom	EWB	Brazil	VII	AY034608
European stone fruit yellows	ESFY	Austria	X-B	AY029540
Faba bean phyllody	FBP	Sudan	II-C	X83432
Flavescence dorée	FD	France	V-C	AF176319
Fragaria multicipita	FM	Canada	VI-B	AF190224
Illinois elm yellows	ILEY	USA	VI-C	AF268895
Loofah witches' broom	LfWB	Taiwan	VIII-A	AF353090
Mexican periwinkle virescence	MPV	Mexico	XIII-A	AF248960
Peanut witches' broom	PWB	Asia	II-A	L33765
Picris echioides yellows	PiEP	Italy	IX	Y16389
Rice yellows dwarf	RYD	Japan	XI-A	D12581
Spartium witches' broom	SWB	Italy	X-D	X92869
Sugarcane white leaf	SCWL	Thailand	XI-B	X76432
Tomato big bud	TBB	Australia	II-E	Y08173
Walnut witches' broom	WWB	USA	III-G	AF190227

[a]Groups and subgroups are indicated following Lee et al., 1998; Lee et al., 2000; Montano et al., 2001; Jacobs et al., 2002.

2 min, extension at 72 °C for 3 min, followed by a final extension for 10 min at 72 °C. Amplified products were analyzed by electrophoresis in 1% agarose gel, stained with ethidium bromide, and visualized with a UV transilluminator.

RFLP analysis of nested-PCR products

Ten microlitres of each positive nested-PCR product was separately digested with *Alu*I, *Cfo*I, *Hpa*II, *Kpn*I, *Rsa*I and *Taq*I restriction endonucleases according to the manufacture's instructions (Roche Diagnostic, Mannheim, Germany). Digested products were loaded and resolved on vertical 5% polyacrylamide gels in TBE 1X buffer (45 mM Tris-borate, 1 mM EDTA, pH 8). Restriction fragments were visualized under UV light after staining with ethidium bromide. The size of digested products were estimated using the 100 bp GeneRuler™ DNA ladder (Fermentas, Vilnius, Lithuania).

Putative RFLP map

Nucleic acid from Brinjal little leaf (BLL), Illinois elm yellows (ILEY) and Fragaria multicipita (FM) was not available for comparison to the yellow

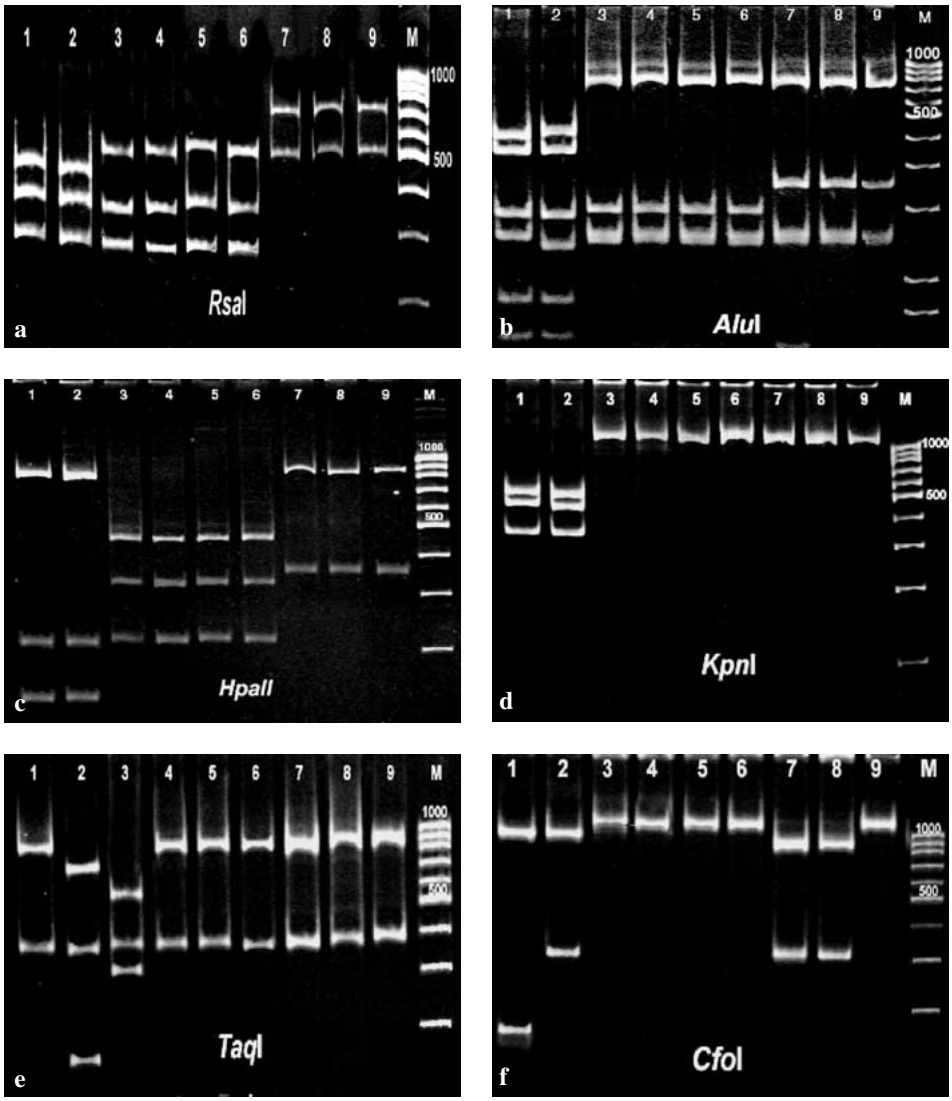

Figure 1. Restriction profiles of ribosomal phytoplasma DNA amplified by PCR assay using *Rsa*I (a), *Alu*I (b), *Hpa*II (c), *Kpn*I (d), *Taq*I (e) and *Cfo*I (f) endonucleases. Lane 1, European aster yellows (EAY); lane 2, Stolbur (STOL); lane 3, Seasame phyllody (SEPT); lane 4, Faba bean phyllody (FBP); lane 5, Tomato big bud (TBB); lane 6 Pichis echioides phyllody (PEP); lane 7, Beet leafhopper transmitted virescence (BLTVA); lane 8, Catharanthus phyllody (CPS); lane 9, CSV; lane M, 100 bp GeneRuler marker (Fermentas), fragment sizes (bp) from top to bottom: 1000, 900, 800, 700, 600, 500, 400, 300, 200.

starthistle-infecting phytoplasma so a virtual RFLP was conducted on the three sequences from GenBank. Endonucleases *Alu*I, *Cfo*I, *Hpa*II, *Kpn*I, *RsaI, Sau*3AI and *Taq*I were employed on the sequences from nucleotide 1–1250. The origin and GenBank accession number of these strains are reported in Table 1. The putative RFLP map was generated on each sequence separately using the Max Heiman's Webcutter 2.0 program (copyright 1997).

Cloning, sequencing and phylogenetic analyses

A DNA fragment amplified from the yellow starthistle-infecting phytoplasma isolate by P1/P7 primer pair was purified using a PCR DNA purification system (Microcon PCR Kit – Amicon, Bedford, MD, USA) and cloned using Topo TA cloning kit according the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). A P1/P7 clone was sequenced on both

strands by fluorescent automated sequencing on a ABI Prism Model 377 (PE Applied Biosystems, CA, USA). The obtained sequence was deposited in the GenBank database (AY270156) and compared with other sequences by CLUSTALW program (Table 1). Genetic distances among nucleotide sequences were calculated for multiple substitutions by the Kimura's two-parameters method (Kimura, 1983). A phylogenetic tree was constructed by the neighbour-joining method (Saitou and Nei, 1987). *Acholeplasma palmae*, a culturable Mollicutes that is phylogenetically related to the phytoplasmas, was designated as the out group to root the tree. The reliability of the tree was assessed by Bootstrap analysis with 1000 times replications (Efron, 1982).

Results

Disease transmission and detection of phytoplasma by PCR assay

Three months after inoculation, the grafted periwinkle plants showed typical phytoplasma symptoms, including yellowing and shortened internodes. Primer pair P1/P7 amplified the target DNA from all samples of diseased yellow starthistle plants and from symptomatic grafted periwinkle leaves. A 1.25-kbp product was amplified in nested-PCR using the R16F2/R16R2 primer pair when amplicons of P1/P7 diluted 1 : 40 were used as template. At the same time, all reference phytoplasmas yielded an amplification product of the expected size. No PCR products were amplified from asymptomatic yellow starthistle and periwinkle samples (data not shown).

RFLP analyses, actual and putative

All 28 phytoplasma-positive samples from yellow starthistle showed the same restriction profiles when their PCR products were separately digested with all restriction endonucleases, indicating that all the yellow starthistle-infecting phytoplasmas from Italy were molecularly indistinguishable from each others (data not shown). Amplified products obtained from grafted periwinkle samples were indistinguishable from those obtained from yellow starthistle-infected samples, showing the same pattern with each restriction endonucleases (data not shown). The profiles obtained from yellow starthistle-positive samples

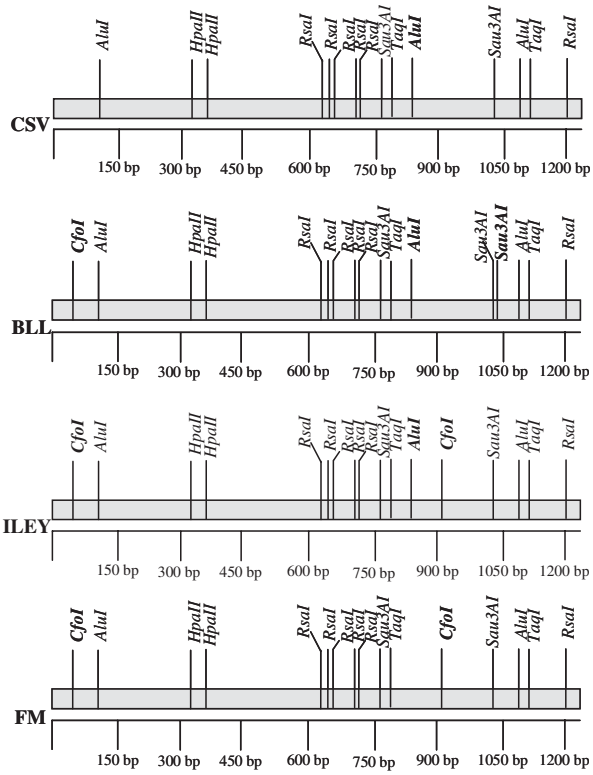

Figure 2. Putative restriction fragment length polymorphism maps, based on 16S rRNA sequences extracted from GeneBank (from nucleotide 1 to 1250), of three phytoplasma strains determined to have an high homology to Centaurea solstitialis virescence phytoplasma (CSV): Brinjal little leaf (BLL); Illinois elm yellow phytoplasma (ILEY); and Fragaria multicipita (FM). The endonuclease restriction sites not in common among the four strains are indicated in bold.

with *Rsa*I, *Alu*I and *Hpa*II restriction endonucleases, showed the same pattern as CPS and BLTVA, but were different to EAY, STOL, SEPT, FBP, TBB, and PEP (Figure 1a–c). Digestion with *Cfo*I showed a different pattern to CPS and BLTVA but was similar to SEPT, FBP, TBB and PEP. In fact, with this enzyme phytoplasmas belonging to the 16Sr-II group and the unknown phytoplasma were uncut while the profiles of digested DNA from EAY, STOL, CPS and BLTVA had several different bands for each (Figure 1f). *Kpn*I endonuclease cleaved PCR fragments only in the EAY and STOL reference strains (Figure 1d). *Taq*I endonuclease (Figure 1e) showed a singular pattern identical for the yellow starthistle-infecting phytoplasma, CPS, BLTVA, PEP, FBP, TBB and EAY; whereas, STOL and SEPT showed unique patterns.

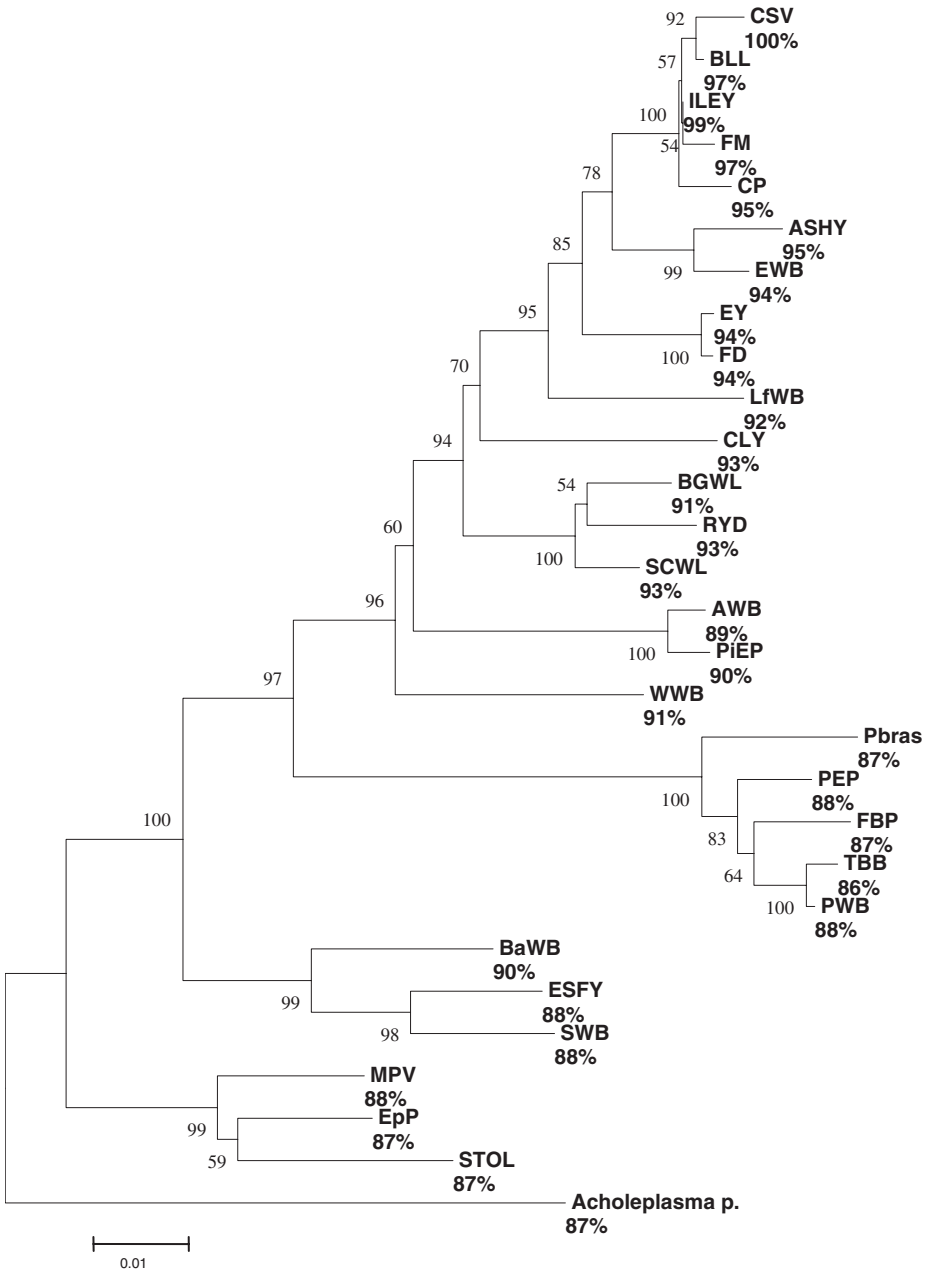

Figure 3. Phylogenetic tree constructed by the neighbour-joining method of 16S rRNA gene sequences from 28 phytoplasma taxa and employing culturable *Acholeplasma palmae* as the out group. Bootstrap values are shown on the branch. Ash yellows (ASHY); Almond witches' broom (AWB); Black alder witches' broom (BaWB); Bermuda grass white leaf (BGWL); Brinjal little leaf (BLL); Coconut lethal yellowing (CLY); '*Candidatus* Phytoplasma brasiliense' (Pbras); Centaurea solstitialis virescence (CSV); Clover proliferation (CP); Epilobium phyllody phytoplasma (EpP); Elm yellows (EY); European stone fruit yellows (ESFY); Erigeron witches' broom (EWB); Faba bean phyllody (FBP); Flavescence dorée (FD); Fragaria multicipita (FM); Illinois elm yellows (ILEY); Loofah witches' broom (LfWB); Mexican periwinkle virescence (MPV); Picris echioides phyllody (PEP); Picris echioides yellows (PiEP); Peanut witches' broom (PWB); Rice yellows dwarf (RYD); Sugarcane white leaf (SCWL); Stolbur (STOL); Spartium witches' broom (SWB); Tomato big bud (TBB); Walnut witches' broom (WWB). The homology (%), obtained using CLUSTALW program by comparison to CSV 16S and all considered phytoplasma sequences, is reported at the extremities of the corresponding branch.

Putative RFLP maps showed that the *Cfo*I RFLP pattern of the unknown phytoplasma differed from those of its close relatives BLL, ILEY, and FM. In fact, this endonuclease cleaved the PCR fragment in BLL, ILEY and FM 16S rDNAs (one, two and two restriction sites, respectively), but not in the yellow starthistle-infecting phytoplasma. *Sau3*AI showed two restriction sites in the unknown phytoplasma, EP, and FM 16S rDNAs and three restriction sites in BLL. A second *Alu*I restriction site is absent in the FM sequence. No differences were observed with the other endonucleases (Figure 2).

Sequence and phylogenetic analyses

The sequence of the cloned yellow starthistle-infecting phytoplasma DNA fragment consisted of 1805 nucleotides of the 16S rRNA gene, the spacer region between the 16S and 23S rRNA gene, and the start of the 23S rRNA gene regions of the phytoplasma genome. Comparison by the CLUSTALW program showed a 99.4% similarity with ILEY and a 97.8% similarity with BLL, both belonging to the clover proliferation (CP) group. Figure 3 lists the sequence similarities obtained with phytoplasma of different groups.

Results of phylogenetic analyses on the 16S rRNA (Figure 3) revealed that the yellow starthistle-infecting phytoplasma is new having closest relationship to ILEY, BLL, CL and FM phytoplasmas. These results support the conclusion that the yellow starthistle-infecting phytoplasma is closely related to phytoplasmas belonging to the 16SrVI group, but that it represents a distinct phytoplasma, for which the name of Centaurea soltitialis virescence (CSV) phytoplasma is suggested.

Discussion

Molecular analyses were used to identify and classify the phytoplasma causing a vegetative disorder in yellow starthistle. The phytoplasma was detected only in diseased plants and never in asymptomatic ones, and moreover, it was transmitted to and then detected in healthy periwinkle plants. To our knowledge the examined disease has not been previously reported in the literature.

After confirmation that a phytoplasma was present in the symptomatic tissue and most likely the causal agent of the vegetative disorder, the phylogenetic position was determined for comparison with known reference strains. Sequence, phylogenetic and RFLP analyses show that the CSV phytoplasma could be the same as BLL from India and ILEY phytoplasma from the United States because the homology is higher than 97.5%. But on the basis of the molecular data (the *Cfo*I RFLP pattern of CSV differed from that of rDNA from all phytoplasmas previously described in group VI), the different associated plant hosts, and the geographical isolation, which is also in accord with the Phytoplasma Working Team (Phytoplasma Working Team, 13th IOM, Fukuoka, Japan, 14–19 July 2000) for similarity of phytoplasmas with more than 97.5% homology, we would consider the CSV phytoplasma a new subgroup, 16Sr-VI-D, of the CP group. Moreover, in our knowledge, this is the first identification of a 16Sr-VI group phytoplasma in Italy.

In Italy, as in most parts of Mediterranean Europe, yellow starthistle is not considered problematic. One hypothesis for this is that natural enemies have coevolved with the plant in its native habitat keeping populations at a balanced level. It is estimated that plants invasive to the United States have 77% fewer fungi and viruses than those plants in their native habitats (Mitchell and Power, 2003). The original intention of this study was to determine if the identified causal agent that appeared to help limit the spread and colonization of yellow starthistle in central Italy could be used as a biological control agent. However, the high homology with the ILEY phytoplasma (Jacobs et al., 2003), a pathogen of elm trees in the United States, makes the CSV phytoplasma not practical to pursue further as a biological control agent against yellow starthistle.

Acknowledgements

The authors thank Dr. Marcone, Dr. Bertaccini, and the late Dr. Boccardo for kindly providing some phytoplasma isolates employed in this study and the Bureau of Land Management, U.S. Department of Interior for partial funding of this project.

References

Bruckart WL (1989) Host range determination of *Puccinia jaceae* from yellow starthistle. Plant Disease 73: 155–160.

Campobasso G, Sobhian R, Knutson L and Terragitti G (1998) Host specificity of *Bangastemus orientalis* Capiomont (Coleoptera: Curculionidae) introduced into the United States

for Biological control of yellow starthistle (*Centaurea solstitialis* L., Asteraceae: Carduae). Environmental Entomology 27: 1525–1530.

Cordy DR (1978) *Centaurea* species in equine nigropallidal encephalomalacia. In: Keller RF, Van Kampen KR and James LF (eds) Proceedings of the IX International Symposium on Biological Control of Weeds. University of Cape Town, South Africa, pp. 327–336.

Deng SJ and Hiruki C (1991) Genetic relatedness between two non-culturable mycoplasmalike organism revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathology 81: 1475–1479.

Efron B (1982) The Jackknife, the Bootstrap and Other Resembling Plans. SIAM, Philadelphia, PA.

Gundersen DE, Lee IM, Rehner SA, Davis RE and Kingsbury DT (1994) Phylogeny of mycoplasmalike organisms (phytoplasmas): A basis for their classification. Journal of Bacteriology 176: 5244–5254.

Jacobs KA, Lee IM, Griffiths HM, Miller FD Jr. and Bottner KD (2003) A new member of the clover proliferation phytoplasma group (16SrVI) associated with elm yellows in Illinois. Plant Disease 87: 241–246.

Kimura M (1983) The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, UK.

Lee IM, Bertaccini A, Vibio M, and Gundersen DE (1995) Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. Phytopathology 85: 728–735.

Lee IM and Davis RE (1992) Mycoplasmas which infect plants and insects. In: Maniloff J, McElhaney RN, Finch LR and Baseman JB (eds), Mycoplasmas: Molecular Biology and Pathogenesis. American Society for Microbiology, Washington, D.C., pp. 379–390.

Lee IM, Davis RE, Dawn E and Gundersen-Rindal DE (2000) Phytoplasma: Phytopathogenic Mollicutes. Annual Review of Microbiology 54: 221–255.

Lee IM, Dawn E, Gundersen-Rindal DE, Davis RE and Bartoszyk M (1998) Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. International Journal of Systematic Bacteriology 48: 1153–1169.

Marcone C, Ragozzino A and Seemüller E (1997) Detection and identification of phytoplasmas in yellows-diseased weeds in Italy. Plant Pathology 46: 530–537.

Marzachì C, Alma A, d'Aquilio M, Minuto G and Boccardo G (1999) Detection and identification of phytoplasmas infecting cultivated and wild plants in Liguria (Italian Riviera). Journal of Plant Pathology 81: 127–136.

Mitchell GE and Power AG (2003) Release of invasive plants from fungal and viral pathogens. Nature 421: 625–627.

Montano HG, Davis RE, Dally EL, Hogenhout S, Pimental JP and Brioso Paulo ST (2001) '*Candidatus* phytoplasma brasiliense', a new phytoplasma taxon associated with hibiscus witches' broom disease. International Journal of Systematic and Evolutionary Microbiology 51: 1109–1118.

Saitou N and Nei M (1987) The neighbour-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Schneider B, Cousin MT, Klinkong S and Seemüller E (1995) Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunnhemp, sesame, soybean and eggplant. Pflanzenkrankheiten und Pflanzenschutz 102: 225–232.

Seemüller E, Marcone C, Lauer U, Ragozzino A and Göschl M (1998) Current status of molecular classification of the Phytoplasmas. Journal of Plant Pathology 80: 3–26.

Shinn S and Thill DC (1999) Yellow starthistle control with imazapic. Research Progress Report of the Western Society of Weed Science, p. 31.

Woo I, Swiadon L, Drilk T and Quarles W (1999) Integrated management of yellow starthistle. IPM Practioner 21: 1–10.