

MOLECULAR IDENTIFICATION OF PHYTOPLASMAS FROM OLIVE TREES IN ITALY

G. Pasquini¹, C. Marzachi², C. Poggi Pollini³, F. Faggioli¹, A. Ragozzino⁴, R. Bissani³, A. Vischi²,
M. Barba¹, L. Giunchedi³ and G. Boccardo²

¹ *Istituto Sperimentale per la Patologia Vegetale, Via G.C. Bertero 22, I-00156 Rome, Italy*

² *Istituto di Fitovirologia Applicata del C.N.R., Strada delle Cacce 73, I-10135 Torino, Italy*

³ *Istituto di Patologia Vegetale, Via F. Re 8, I-40126 Bologna, Italy*

⁴ *Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Via Università 100, I-80055 Portici (Napoli), Italy*

SUMMARY

Olive trees (51 adult plants and 13 seedlings from different Italian regions) showing yellowing, shortening of internodes, witches' broom, bud abortion, little leaf, hypertrophied inflorescences, decline and fasciation symptoms were tested for the presence of phytoplasmas. DNAs extracted from leaf veins were amplified in reactions assisted by universal or group-specific primer pairs constructed on 16S rRNA phytoplasma sequences and analyzed by restriction with five different enzymes. Phytoplasmas, identified as members of 16Sr-IB, -V, and -XIIA groups (Lee *et al.*, 1998) on the basis of their RFLP patterns, were detected from 34 samples (53.0%). Of these, 24 (*ca* 68.0%) carried a single phytoplasma [21 members of subgroup IB (87.5 %), one (4.0%) XIIA, and two (*ca* 8.0%) V]. Seven samples (20.5%) carried two different organisms (IB and XIIA subgroups members) and 3 (*ca* 9.0%) carried phytoplasmas of all three subgroups here identified. In addition, a IB subgroup member was detected in one symptomless adult tree. It appears that these phytoplasmas are ubiquitous in the areas surveyed, but a clear correlation between a given syndrome and the presence of one or more phytoplasmas did not emerge.

Key words: olive tree, phytoplasmas, PCR detection, RFLP.

INTRODUCTION

Although descriptions of virus or virus-like diseases in olive (*Olea europea* L. var. *sativa*) go back about 60 years, virus and phytoplasma studies appear to be rather scarce and relatively recent in spite of the economic importance of the crop in the Mediterranean area (for a review, see Martelli, 2000).

Based on restriction fragment polymorphism (RFLP) analysis of conserved ribosomal genes (rDNA) specifi-

cally amplified by means of polymerase chain reaction (PCR) technology, the monophyletic clade of the phytoplasmas has been divided into 14 major 16S rDNA groups (I to XIV), encompassing 32 subgroups within the class *Mollicutes* (Lee *et al.*, 1998).

Following the first report of a group V phytoplasma infection of olive trees in Italy (Poggi Pollini *et al.*, 1996), phytoplasmas belonging to group I (B and C subgroups), and groups III, V and XII (A subgroup) have been sporadically identified in a few symptomatic olive trees (Danielli *et al.*, 1996; Del Serrone *et al.*, 1996; Ciampitti *et al.*, 1999; Marzachi *et al.*, 1999; Poggi Pollini *et al.*, 1999a, b). Affected trees exhibited a rather variable range of symptoms: shoot proliferation, leaf rolling, yellowing of leaves and branches, phyllody, fasciation, witches'-brooms, dwarfing, leaf bronzing, abortion of axillary buds, erect growth, appearance of small heads due to clustering of hypertrophoid inflorescences, decline and die-back.

None of these symptoms has however been firmly associated with a given phytoplasma and such disorders have at times been ascribed to other conditions (Camele *et al.*, 1999). The aim of this study was to assess by molecular analysis the association, if any, of specific organisms with phytoplasma-like disorders present throughout Italy, in order to improve our knowledge on the sanitary status of this economically important crop. We have examined symptomless as well as symptomatic plants and consequently reinvestigated some of the syndromes already described. Through 16S rDNA PCR amplifications and RFLP analysis we have identified the phytoplasmas present in olives exhibiting a wide array of symptomatology.

MATERIALS AND METHODS

Plant samples, phytoplasma reference strains and DNA isolation. Samples of branches, buds when present and/or leaves were collected during February-mid April from 51 adult olive trees and 13 ten year-old seedlings showing various symptoms. Samples were collected in private gardens or industrial orchards from

Corresponding author: G. Boccardo

Fax: +39.011.343809

E-mail: g.boccardo@ifa.to.cnr.it

northeastern, central and southern Italy, along with corresponding parts of eight healthy-looking trees. As a rule, samples were divided and mailed to at least two of the laboratories partaking in the study.

For comparison, isolates [acronyms and taxonomic subgroup assignments (Lee *et al.*, 1998) in parenthesis] of European aster yellows (EAY; IB), clover phyllody (CPh; IC), Serbian stolbur strain from pepper (Stol; XIA) phytoplasmas were graft-propagated and maintained in periwinkle [*Catharanthus roseus* (L.) G. Don] (Marzachi *et al.*, 1999). American aster yellows (AAY; IA), faba bean phyllody (FBP; IIC), Italian clover phyllody (ICPh; IIIB), elm yellows and elm witches' broom (EY-1 and ULW; VA) phytoplasmas were obtained from Drs. M.F. Clark (Horticulture Research International, East Malling, U.K.; AAY, FBP), L. Carraro (Dipartimento di Biologia Applicata alla Difesa delle Piante, Udine, Italy; ICPh), and E. Seemüller (Institut für Pflanzenschutz im Obstbau, Dossenheim, Germany; EY-1, ULW) and likewise propagated and maintained. Apple proliferation, (AP; XA) and pear decline (PD; XC) phytoplasmas were propagated and maintained in periwinkle and pear cv. 'A20' or *Cydonia oblonga* Miller seedlings, respectively. Jujube (*Zizyphus jujuba* Miller) and blackberry (*Rubus fruticosus* L.), infected by jujube witches' broom (JWB; VB) and rubus stunt (RS; VC) phytoplasmas, were collected at the Institute of Pomology (courtesy of Dr. Zhong Zhe, Chinese Academy of Agricultural Sciences, Zhengzhou, Shanxi Province, China), and at the Istituto Sperimentale per la Patologia Vegetale in Rome, respectively. Grapevines infected by flavescence dorée (FD; VC; A. Vischi and G. Boccardo, unpublished) were collected in southeastern Piemonte.

DNA was extracted as detailed by Marzachi *et al.* (1999) from 1.5 g of midribs when possible or, in the case of severely affected olive samples, whole reduced leaves or inflorescences of freshly collected samples. Final products were resuspended in 100 µl sterile double distilled water and aliquots (typically 2 µl) used in PCR experiments.

PCR amplification and RFLP analysis. Two universal primer pairs derived from highly conserved ribosomal sequences, designed to amplify DNA fragments of different specific lengths were used in direct PCR assays: R16F2/R2 (Lee *et al.*, 1993; 1225 bp) and fU5/rU3 (Lorenz *et al.*, 1995; 880 bp). Products initially amplified with primer pair R16F2/R2 were diluted 1:40 and 2 µl aliquots used as templates in reactions primed by one of the following group-specific pairs: R16(I)F1/R1, R16(III)F2/R1 and R16(V)F1/R1 (Lee *et al.*, 1994; approximately 1100, 800 and 1100 bp, respectively) and

R16 (X) F1/R1 (Lee *et al.*, 1995; 1100 bp). Reactions and cyclings were programmed as detailed in the original papers. Dilutions of the DNA extracts were used in inhibition assays (Marzachi *et al.*, 1998).

Reaction products were analyzed by electrophoresis in 1% agarose gels buffered in 0.5x TBE (TBE buffer: 90 mM tris (hydroxymethyl) aminomethane, 90 mM boric acid, 3 mM ethylenediaminetetraacetate Na salt, pH 8.3) along with 1 Kb DNA size marker (Life Technologies-BRL, Gaithersburg, USA) and visualized by UV light after ethidium bromide staining.

Aliquots (8 µl) of group-specific nested-amplified 16S rDNA fragments were separately digested at 37°C for 2-16 h with 1.5-2 units of one of the following restriction endonucleases: *Bfa*I (New England Biolabs, Beverly, USA), *Alu*I, *Hha*I, *Mse*I, or at 65°C for 2 h with *Taq*I (Life Technologies). Restriction fragments were resolved in 5% polyacrylamide gels buffered in TBE and visualized as mentioned. The 1Kb plus DNA ladder (Life Technologies) was the size marker.

RESULTS

Field survey. Trees with suspected phytoplasma diseases exhibited a wide variety of symptoms, already described in Italy (Danielli *et al.*, 1996; Del Serrone *et al.*, 1996; Ciampitti *et al.*, 1999; Marzachi *et al.*, 1999; Poggi Pollini *et al.*, 1996, 1999b). Pronounced witches' brooming and yellowing (Fig. 1A and B), erect growth of lateral branches and appearance of small heads due to clustering of hypertrophied inflorescences (Fig. 1C) were some of the most spectacular symptoms encountered. Some regional variations in symptom expression were obvious: diffuse hypertrophied inflorescences were encountered only in the centre of Italy (Teramo), whereas decline and yellowing were almost exclusively observed in the northeast (Trento-Verona) (Table 1). Symptoms became obvious at the beginning of spring and did not worsen through the season, except that in the northeast size reduction and leaf yellowing became more obvious. The incidence of symptomatic trees was rather low (>10%), although over the years the disease appeared to spread slowly. A few trees, originally showing symptoms when first documented (Del Serrone *et al.*, 1996), were symptomless at the time of our survey, although the phytoplasma described was still detectable.

Seedlings, all coming from the same nursery (Table 1, samples U1-U15), showed remarkably uniform symptoms, which also appeared to be the commonest throughout the areas under examination.

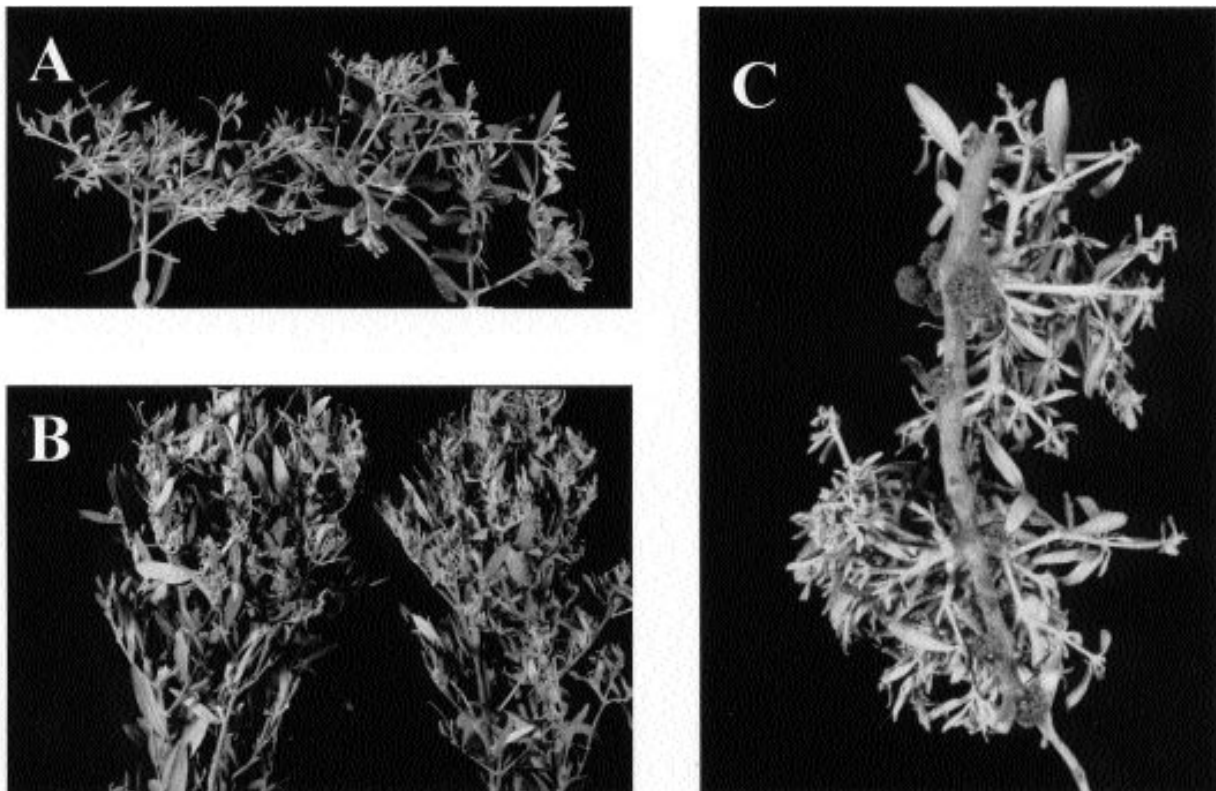


Fig. 1. Olive trees (for acronyms refer to Table 1) carrying various phytoplasmas (in parenthesis) showing some of the symptoms most commonly encountered. **A:** plant M3 (16Sr-IB) with witches' broom and shortening of internodes. **B:** plant 77N (16Sr-IB and -XIIA) with little leaves and witches' broom. **C:** plant A6 (16Sr-IB and -XIIA) with hypertrophied inflorescences, witches' broom and shortening of internodes.

Phytoplasma detection and identification. Table 2 summarizes PCR and RFLP results obtained with different primer pairs. While none of the DNAs possessed a phytoplasma titre high enough to yield an amplified fragment of the expected size with R16F2/R2 primer pair, about one third (13/35) did respond positively when amplifications were attempted with fU5/rU3 primer pair. Notwithstanding the negative response obtained with the former primer pair, all DNAs were tested with the adaptable and available group-specific primer pairs in nested assays. None of the extracts, except DNAs from reference isolates, yielded the expected phytoplasma-specific fragments using groups III- or X-specific primer pairs (not shown), whereas 33 and 5 DNA samples responded positively to groups I- and V-specific primer pairs, respectively. Three samples yielded a phytoplasma-specific fragment of the expected length with both these primer pairs (Table 2).

RFLP profiles of 16S rDNA sequences amplified by nested PCRs with the 16Sr-I group-specific primer pair further indicated that most of the positive samples were infected by phytoplasmas belonging to subgroup IB,

whereas sample A3 (Table 2) yielded a restriction pattern indistinguishable from that of the reference Stol (subgroup XIIA, Fig. 2). However, amplified fragments from ten samples gave complex RFLP patterns suggesting co-infection by phytoplasmas belonging to subgroups IB and XIIA (Fig. 2). In summary, 25 samples (71.4% of the positive ones) were found infected by a single phytoplasma [22 (88.0%) in IB and one (4.0%) in XIIA subgroups, and two (8.0%) in group V]. Seven samples (20.0%) were found carrying two different phytoplasmas (IB and XIIA members) and three (12.0%) phytoplasmas belonging to the three different identified groups (Table 2). Amplicons obtained by nested PCR driven by the 16S(V)F1/R1 primer pair digested with *Bfa*I appeared to be indistinguishable from those of VA or B subgroups reference isolates, but different from that of RS and FD (VC) (Fig. 3).

DNA from one of the healthy-looking samples (Table 1: sample L3) was amplified by nested PCR assisted by the group I-specific primer pair and found upon RFLP analysis to contain a IB subgroup member (Table 2).

Table 1. Summary of the full array of symptoms observed in the phytoplasma-positive samples. TN: Trento, VR: Verona, NA: Napoli, TE: Teramo, AP: Ascoli Piceno, PG: Perugia, RM: Roma, CS: Cosenza.

Sample	Location	Symptoms							
		Buds abortion	Decline	Fasciation	Hypertrophoyd inflorescence	Little leaves	Shortening of internodes	Witches broom	Yellowing
1TN1	Arco (TN)							•	
55TN1	Arco (TN)							•	•
56TN1	Arco (TN)		•					•	
57TN1	Arco (TN)	•		•		•	•	•	•
62TN1	Arco (TN)							•	
63TN1	Arco (TN)	•		•				•	•
69TN1	Arco (TN)								•
19VR	Garda (VR)		•					•	•
64VR	Garda (VR)		•						
1TN2	Riva (TN)							•	
2TN2	Riva (TN)							•	
3TN2	Riva (TN)		•					•	
4TN2	Riva (TN)							•	
77N	NA					•		•	
91N	NA					•			•
94N	NA							•	
A1	Morro D'oro (TE)				•	•	•	•	
A2	Morro D'oro (TE)				•	•	•	•	
A3	Morro D'oro (TE)				•	•	•	•	
A4	Morro D'oro (TE)				•	•	•	•	
A6	Giulianova (TE)				•	•	•	•	
M1	Ponzano di Fermo (AP)						•	•	
M3	Grottazzolina (AP)					•	•	•	
U1	PG					•	•	•	
U2	PG					•	•	•	
U3	PG					•	•	•	
U7	PG					•	•	•	
U10	PG					•	•	•	
U11	PG					•	•	•	
U12	PG					•	•	•	
U14	PG					•	•	•	
U15	PG					•	•	•	
L2	Montelibretti (RM)								• ¹
L3	RM								
C4	CS		•						•

¹ Leaf bronzing.

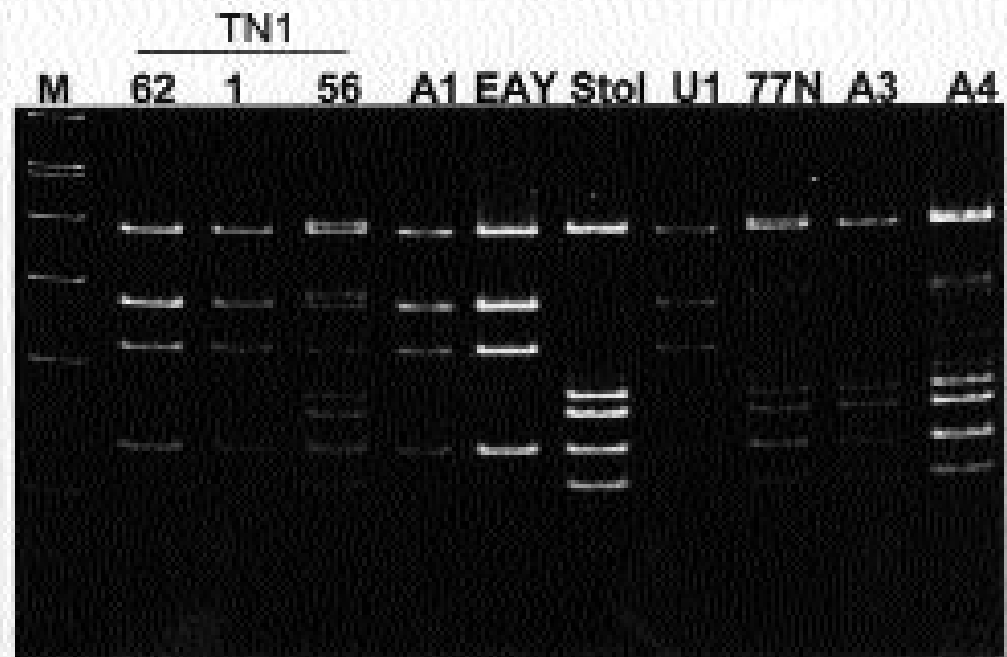


Fig. 2. Polyacrylamide gel electrophoresis of *Mse*I-restricted amplicons obtained in group-I-specific nested PCR. For acronyms see text and Table 1. M: size marker (1650, 1000, 850, 650, 500, 400, 300 bp).

DISCUSSION

Since we detected one or more phytoplasmas in about only 53.0% (25 adult trees and 9 seedlings) of the symptomatic samples analyzed, we can not correlate any established symptomatology with the presence of a particular phytoplasma. Furthermore, in one of the eight healthy-looking trees examined, a IB subgroup phytoplasma was detected. In Italy, members of this clade have been found in apparently symptomless fruit trees in northern regions (Lee *et al.*, 1995).

Failure to amplify phytoplasma DNA from ~47% of the symptomatic samples was not because of presence of enzyme inhibitors in the extracts, but could have been due either to titres below detection limits (even using very sensitive techniques) or because the symptoms were caused by other agents (Barba, 1993). For example, DNAs from adult trees showing crown decline or leaf rolling only, never yielded positive results with any of the primer pairs used in this study. It is also worth noting that from DNA extracts from trees with symptoms very similar to those in Fig. 1C, the same group-specific primer sets that we used did not amplify phytoplasma DNA (Camele *et al.*, 1999). Conversely, Stol-type phytoplasmas were identified from olive trees with witches' broom and buds with cauliflower-like appearance in Spain (Font *et al.*, 1998).

As expected from previous reports on a wide range of hosts (Bertaccini *et al.*, 1990; Lee *et al.*, 1995; Vibio

et al., 1996; Marcone *et al.*, 1997a, b; Marzachi *et al.*, 1999), subgroup IB phytoplasmas were the most frequently found. Members of the 16Sr-V group were detected in extracts from five samples only, all coming from the northeast (Table 2).

In Italy, mixed infections by phytoplasmas of the same or different phylogenetic groups have been reported from dicot and monocot hosts, and most frequently described from perennial fruit trees, shrubs or grapevines (Bertaccini *et al.*, 1995; Lee *et al.*, 1995; Bianco *et al.*, 1996; Danielli *et al.*, 1996; Marcone *et al.*, 1996a, b; Vibio *et al.*, 1996; Marcone *et al.*, 1997b; Bertaccini *et al.*, 1999; Marzachi *et al.*, 1999; Poggi Pollini *et al.*, 1999a, b). We did not detect phytoplasmas belonging to groups III, previously reported in symptomatic olive trees from central Italy (Danielli *et al.*, 1996), or group X, rather common and readily detectable in fruit trees or shrubs growing near the sampled trees (Lee *et al.*, 1995; Poggi Pollini *et al.*, 1995; Marcone *et al.*, 1996a, b; Del Serrone *et al.*, 1998).

PCR detection in olive trees of phytoplasmas in single, double or multiple infections confirms that they may be widely present in this crop, but does not identify any of them as causal agents of any of the long time known disorders and stresses the need of further biological assays.

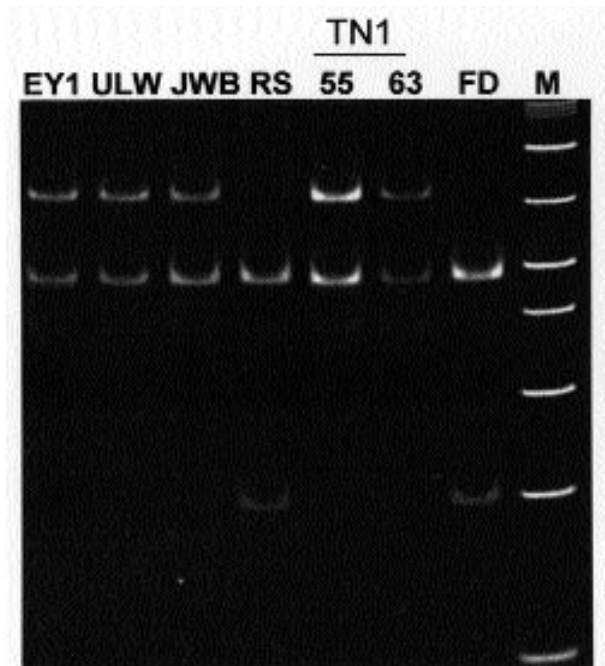
Table 2. Direct and nested PCR detection of phytoplasmas in olive trees. Assignment to phylogenetic subgroups has been attained by RFLP analysis of nested amplicons.

Samples	fU5/rU3	Nested I	Nested V	16S rDNA ubgroups
1TN1	-	+	-	IB
55TN1	+	+	+	IB, V, XIII A
56TN1	+	+	-	IB, XIII A
57TN1	+	+	+	IB, V, XIII A
62TN1	+	+	-	IB
63TN1	-	+	+	IB, V, XIII A
69TN1	+	-	+	V
19VR	-	-	+	V
64VR	-	+	-	IB, XIII A
1TN2	-	+	-	IB
2TN2	-	+	-	IB
3TN2	-	+	-	IB
4TN2	-	+	-	IB
77N	+	+	-	IB, XIII A
91n	-	+	-	IB
94N	-	+	-	IB, XIII A
A1	-	+	-	IB
A2	-	+	-	IB, XIII A
A3	-	+	-	XIII A
A4	-	+	-	IB, XIII A
A6	-	+	-	IB, XIII A
M1	+	+	-	IB
M3	+	+	-	IB
U1	-	+	-	IB
U2	+	+	-	IB
U3	+	+	-	IB
U7	-	+	-	IB
U10	+	+	-	IB
U11	-	+	-	IB
U12	+	+	-	IB
U14	-	+	-	IB
U15	-	+	-	IB
L2	+	+	-	IB
L3	-	+	-	IB
C4	-	+	-	IB

ACKNOWLEDGEMENTS

We thank all colleagues mentioned in the text for gifts of various materials, Elisabetta Vindimian (Stazione Sperimentale di San Michele all'Adige, Trento), Paolo Miorelli (Ufficio Fitosanitario Provinciale, Riva, Trento), Severino Belleggia (Agricoop, Grottazzolina, Ascoli Piceno), Angelo Tarquini (ERSA,

Teramo), Luciana Baldoni (Istituto di ricerche sulla Olivicoltura CNR, Perugia) for their help in collecting samples, and Elso Piccolini for computer graphics. We thank Prof. G.P. Martelli (Università degli Studi di Bari, Italy) for sending preprints. This work was funded in part by MiPAF [Progetto Nazionale Biotecnologie Vegetali (Area 4.2.10 - Diagnostici)].

**Fig. 3.** Polyacrylamide gel electrophoresis of *Bfa*I-restricted amplicons obtained in group V-specific nested PCR. For acronyms see text and Table 1. M: size marker (850, 650, 500, 400, 300, 200, 100 bp).**REFERENCES**

- Barba M., 1993. Viruses and virus-like diseases of olive. *Bulletin OEPP/EPPO Bulletin* **23**: 493-497.
- Bertaccini A., Davis R.E., Lee I.M., 1990. Distinctions among mycoplasma-like organisms (MLOs) in *Gladiolus*, *Ranunculus*, *Brassica* and *Hydrangea* through detection with non-radioactive cloned DNA probes. *Phytopathologia Mediterranea* **29**: 107-113.
- Bertaccini A., Fránová J., Paltrinieri S., Martini M., Navrátil M., Lugaresi C., Nebesárová J., Simkova M., 1999. Leek proliferation: a new phytoplasma disease in the Czech Republic and Italy. *European Journal of Plant Pathology* **105**: 487-493.
- Bertaccini A., Vibio M., Stefani E., 1995. Detection and molecular characterization of phytoplasmas infecting grapevine in Liguria (Italy). *Phytopathologia Mediterranea* **34**: 137-141.

- Bianco P.A., Davis R.E., Casati P., Fortusini A., 1996. Prevalence of aster yellows (AY) and elm yellows (EY) group phytoplasmas in symptomatic grapevines in three areas of northern Italy. *Vitis* **35**: 195-199.
- Camele I., Rana G.L., Murari E., Bertaccini A., 1999. Indagini preliminari su alcune alterazioni morfologiche e cromatiche dell'olivo. *L'Informatore Agrario* **22**: 79-81.
- Ciampitti M., Perucca M., Pinotti A., 1999. Fitoplasmi associati all'olivo in Lombardia. *Atti Incontro Nazionale sulle Malattie da Fitoplasmi, Udine* 1999, 32-33.
- Danielli A., Bertaccini A., Vibio M., Rapetti S., 1996. Identificazione di fitoplasmi associati allo scopazzo dell'ulivo. *Atti Convegno Annuale Società Italiana di Patologia Vegetale, Udine* 1996, C28-C29.
- Del Serrone P., Bianchi E., Liberatore A., 1998. Outbreak of apricot chlorotic leaf roll in apricot orchards of Latium, Italy. *Phytopathologia Mediterranea* **37**: 133-139.
- Del Serrone P., Faggioli F., Arzone A., Tarquini A., Barba M., 1996. Fitoplasmi associati allo scopazzo dell'ulivo. *Petria* **6**: 83-88.
- Font I., Abad P., Dally E.L., Davis R.E., Jorda C., 1998. Nueva enfermedad en le olivar español. *Phytoma España* **102**: 211-212.
- Lee I.M., Bertaccini A., Vibio M., Gundersen D.E., 1995. Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. *Phytopathology* **85**: 728-735.
- Lee I.M., Gundersen D.E., Hammond R.W., Davis R.E., 1994. Use of mycoplasma like organism (MLOs) group specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. *Phytopathology* **84**: 449-566.
- Lee I.M., Gundersen D.E., Hammond R.W., Davis R.E., Bartoszyk I.M., 1998. Revised classification scheme of phytoplasmas based on RFLP analysis of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48**: 1153-1169.
- Lee I.M., Hammond R.D., Davis R.E., Gundersen D.E., 1993. Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma like organisms. *Phytopathology* **83**: 834-842.
- Lorenz K.H., Schneider B., Ahrens U., Seemüller E., 1995. Detection of apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non ribosomal DNA. *Phytopathology* **85**: 771-776.
- Marccone C., Ragozzino A., Schneider B., Lauer U., Smart C.D., Seemüller E., 1996b. Genetic characterization and classification of two phytoplasmas associated with *Spartium junceum* witches'-broom disease. *Plant Disease* **80**: 365-371.
- Marccone C., Ragozzino A., Seemüller E., 1996a. European stone fruit yellows phytoplasma as the cause of peach vein enlargement and other yellows and decline diseases of stone fruits in southern Italy. *Journal of Phytopathology* **144**: 559-564.
- Marccone C., Ragozzino A., Seemüller E., 1997a. Detection and identification of phytoplasmas in yellows-diseased weeds in Italy. *Plant Pathology* **46**: 530-537.
- Marccone C., Ragozzino A., Seemüller E., 1997b. Detection and identification of phytoplasmas infecting vegetable, ornamental and forage crops in southern Italy. *Journal of Plant Pathology* **79**: 211-217.
- Martelli G.P., 2000. Infectious diseases and certification of olive: an overview. *Bulletin OEPP/EPPO Bulletin* **29** (in press.)
- Marzachi C., Alma A., d'Aquilio M., Minuto G., Boccardo G., 1999. Detection and identification of phytoplasmas infecting cultivated and wild plants in Liguria (Italian Riviera). *Journal of Phytopathology* **81**: 127-136.
- Marzachi C., Veratti F., Bosco D., 1998. Direct PCR detection of phytoplasmas in experimentally infected insects. *Annals of Applied Biology* **133**: 45-54.
- Poggi Pollini C., Bissani R., Giunchedi L., Vindimian E., 1995. Occurrence of phytoplasma infection in European plums (*Prunus domestica*). *Journal of Phytopathology* **143**: 701-703.
- Poggi Pollini C., Bissani R., Giunchedi L., Vindimian E., 1996. First report of phytoplasma infection in olive trees (*Olea Europea* L.). *Journal of Phytopathology* **144**: 109-111.
- Poggi Pollini C., Bissani R., Vindimian E., Giunchedi L., 1999a. Utilizzo della tecnica PCR-ELISA nella diagnosi di fitoplasmi associati all'olivo. *Atti Incontro Nazionale sulle Malattie da Fitoplasmi, Udine* 1999, 27-29.
- Poggi Pollini C., Ragozzino A., Faggioli F., Marzachi C., Pasquini G., Bissani R., Boccardo G., Barba M., 1999b. Identificazione e caratterizzazione molecolare di fitoplasmi in olivo. *Atti Incontro Nazionale sulle malattie da Fitoplasmi, Udine* 1999, 16-17.
- Vibio M., Bertaccini A., Lee I.M., Davis R.E., Clark M.F., 1996. Differentiation and classification of aster yellows and related European phytoplasmas. *Phytopathologia Mediterranea* **35**: 33-42.