Genetic Relatedness of Mycoplasmalike Organisms Detected in *Ulmus* spp. in the United States and Italy by Means of DNA Probes and Polymerase Chain Reactions

I.-M. Lee, R. E. Davis, W. A. Sinclair, N. D. DeWitt, and M. Conti

First, second, and fourth authors: USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705; third author: Department of Plant Pathology, Cornell University, Ithaca, NY 14853; and fifth author, Istituto di Fitovirologia Applicata, CNR, Torino, Italy.

We thank Dawn E. Gundersen and T. Norene O'Sullivan for excellent technical assistance and thank J. P. Prince for assistance in preparing Figure 1. We thank L. N. Chiykowski, J. Dale, S. M. Douglas, C. Hiruki, and G. N. Oldfield for providing MLO-infected tissue.

Accepted for publication 12 April 1993.

ABSTRACT

Lee, I.-M., Davis, R. E., Sinclair, W. A., DeWitt, N. D., and Conti, M. 1993. Genetic relatedness of mycoplasmalike organisms detected in *Ulmus* spp. in the United States and Italy by means of DNA probes and polymerase chain reactions. Phytopathology 83:829-833.

DNA fragments of an elm yellows (EY) mycoplasmalike organism (MLO) from diseased periwinkle (*Catharanthus roseus*) were cloned in plasmid vector pSP6 and *Escherichia coli* strain JM83. DNA probes were prepared by nick translation of EY-specific recombinant plasmids with biotinylated nucleotides. None of the EY probes hybridized with DNA from four representative strains of the aster yellows MLO strain cluster. None of the probes tested at 50 C hybridized with DNA of the MLOs of ash yellows, potato witches'-broom, Canadian peach X, clover proliferation, and beet leafhopper-transmitted virescence diseases, but two tested at 42 C hybridized with DNA of one or another of these MLOs. All probes tested hybridized with MLOs detected in *Ulmus americana* and *U. parvifolia* in the United States and in *U. carpinifolia* in Italy, revealing a close relatedness among these MLOs. These data support the recognition of a unique strain cluster, the elm yellows MLO strain cluster, and identification of strains from the United States and Italy as members of this cluster. Polymerase chain reactions using oligonucleotide primer pairs, derived on the basis of the nucleotide sequence of probe pEY11, provided means for sensitive detection of EY MLOs in infected elm tissue and for differentiation among EY MLO variants. Preliminary results indicated the existence of various strains of EY MLOs in North America that were distinct from a strain of EY MLO present in Italy.

Additional keywords: elm phloem necrosis, elm witches'-broom, Mollicutes.

Elm yellows (EY) (=elm phloem necrosis) is believed to be caused by a mycoplasmalike organism (MLO) (1–3,30). The disease was first described in 1938 in the United States (29). Until 1970, EY seemed to be limited to midwestern states. However, in the next decade, EY killed American elms (*Ulmus americana* L.) and red elms (*U. rubra* Muhl.) in northeastern states, including New York, Pennsylvania, New Jersey, and Massachusetts (10,25–27). The disease now threatens the survival of elm shade trees on the Atlantic coastal plain (18). Recently, EY was found in the Niagara Peninsula in Canada (19). Several research groups have investigated the epidemiology and vector(s) of EY, but progress has been limited because of the lack of a rapid and specific pathogen-detection method (18).

Since 1981, MLO-associated diseases of elms (*U. carpinifolia* Gleditch, *U. villosa* Brandis, *U. pumila* L., and elm hybrids) have been reported in Italy (5,20,21). The syndrome includes epinasty, yellowing, dwarfing, and premature casting of leaves; witches'-brooms at the tips of twigs and branches; and precocious opening of vegetative buds. The name elm yellows was applied to the disease (5,20), although relatedness of MLOs affecting elms in Italy and North America was not demonstrated. In 1985, MLO

infection, detected by means of the DAPI (4′,6-diamidino-2-phenylindole-2HCl) procedure (24,28), was associated with witches'-brooms and dieback in Chinese elm (*U. parvifolia* Jacq.) at Ithaca, NY (W. A. Sinclair, *unpublished data*). The relationship of these MLOs to those causing lethal EY in American and red elms was unknown.

In this study, we cloned EY MLO-specific DNA fragments from a diseased periwinkle (*Catharanthus roseus* (L.) G. Don) artificially infected by dodder transmission of an EY MLO from a naturally infected American elm (3). Several cloned EY MLO DNA probes were used to investigate the relatedness of various elm-infecting MLOs to one another and to other types of MLOs. Oligonucleotide primers were designed on the basis of the nucleotide sequence of a cloned EY MLO (strain EY1) DNA probe and employed in polymerase chain reactions (PCR) for differentiation of these various elm MLOs. An abstract and a synoptic account of part of this research have been published (7,14).

MATERIALS AND METHODS

Source of mycoplasmalike organisms (MLOs). MLO strains EY1 and EY1A were previously dodder transmitted from a naturally infected American elm to a periwinkle (*C. roseus*) and a Chinese elm (*U. parvifolia*), respectively (3), and were maintained by propagating these plants by cuttings. The MLO strain EY2, detected in naturally infected Chinese elm at Ithaca, NY, was maintained by propagating the diseased elm by cuttings. European elm MLO strains (EYie1, EYie2, EYie3, and EYie4) propagated in European field elm (*U. carpinifolia*) originated in a naturally infected tree of this species in Italy (5). Aster yellows (AY)-affected periwinkle was collected in Maryland and maintained in a greenhouse at Beltsville, MD. Other strains were provided separately in tissue of periwinkle: ash yellows (AshY) by W. A. Sinclair; tomato big bud (BB) by J. Dale, University of Arkansas, Fayetteville; periwinkle little leaf, strain 0-1 (CN1) by S. M. Douglas, Connecticut Agricultural Experiment Station, New Haven; potato witches'-broom (PWB) and clover proliferation (CP) by C. Hiruki, University of Alberta, Edmonton, Canada; clover phyllody (CPh) and Canadian peach X-disease (CX) by L. N. Chiykowski, Agriculture Canada, Ottawa, Ontario; and vinca virescence (VR = beet leafhopper-transmitted virescence) by G. N. Oldfield, University of California, Riverside.

DNA cloning and preparation of probes. DNA extraction from EY MLO-infected periwinkle plants, cloning, and selection of MLO DNA fragments were performed as previously described (15). For cloning, the EY MLO (EY1) DNA plus plant DNA was partially digested with both *Eco*RI and *Hin*dIII restriction endonucleases, cloned in plasmid pSP6 (Promega Biotec, Madison, WI), and used to transform competent *Escherichia coli* strain JM83 according to the procedure described by Maniatis et al (17).

Colonies containing recombinant plasmids specific to EY were screened by dot hybridization with biotin-labeled nucleic acid preparations from EY-diseased plants as well as from healthy plants as described elsewhere (15). Colonies yielding DNA that hybridized with biotinylated nucleic acid from a periwinkle infected with EY1, but not with nucleic acid from healthy periwinkle, were considered as candidates for development of probes. The sizes of inserts in the recombinant plasmids were determined by restriction endonuclease digestion and agarose gel electrophoresis. Probes containing DNA of the EY MLO (EY probes) were prepared by nick translation of cloned recombinant plasmids with biotin-7-dATP (15).

Extraction of nucleic acid from plants. Nucleic acid samples from healthy periwinkle and from periwinkle plants infected with EY1 and other strains of MLOs were prepared using procedures previously described (15). For extraction of nucleic acid from elm tissue, 2 g of leaf midribs stripped by a sharp forceps or of young, tender twigs near growing tips was pulverized in liquid nitrogen in a mortar. The pulverized sample was triturated in 14 ml of freshly prepared grinding buffer containing an equal volume of solution A (95 mM K_2HPO_4, $3H_2O$, 30 mM KH_2PO_4, 10% sucrose, 0.15% BSA [bovine serum albumin] fraction V, 2% PVP-10 [polyvinylpyrolidone 10], 0.53% ascorbic acid (pH 7.6) (9), and solution B (ethylene glycol monomethyl ether) and was filtered by squeezing the suspension in a Miracloth-lined (Johnson & Johnson, New Brunswick, NJ) funnel. The solids were triturated with 14 ml of grinding buffer and filtered again. The combined filtrates were centrifuged at 20,000 × *g* for 20 min. The pellet was resuspended in 4 ml of extraction buffer (100 mM Tris-HCl, pH 8.0; 100 mM EDTA; and 250 mM NaCl) containing proteinase K at 100 μg/ml, and 440 μl of 10% Sarkosyl was added to the suspension. The sample was first kept at room temperature for 10 min and then incubated for 1 h at 55 C and clarified by centrifugation at 7,700 × *g* for 10 min. Crude nucleic acid was precipitated from the supernatant by the addition of 0.6 volume of isopropanol at −20 C for 30 min or overnight and was pelleted by centrifugation at 7,700 × *g* for 15 min. The pellet was resuspended in 3 ml of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) containing proteinase K at 100 μg/ml and 0.5% sodium dodecyl sulfate (SDS) and was incubated for 40 min at 37 C. First, 525 μl of 5 M NaCl was mixed thoroughly with the sample, and then 420 μl of 10% cetyltri-methylammonium bromide (CTAB) in 0.7 M NaCl was added. After incubation for 10 min at 65 C, the sample was vigorously extracted at least once with an equal volume of chloroform-isoamyl alcohol (24:1) and was extracted once with an equal volume of TE-saturated phenol-chloroform-isoamyl alcohol (25:24:1), and the nucleic acid was precipitated and pelleted as described above. The pellet was washed with 3 ml of chilled 70% ethanol and resuspended in TE buffer. This crude nucleic acid, without further purification, was used as template for PCR. For use in dot hybridization, the nucleic acid was further purified using a procedure described by Ralph and Bellamy (21). In brief, the pellet was resuspended in 1 ml of deionized water and first mixed with 1 ml of 2.5 M K_2HPO_4 (pH 8.0) and then mixed with 1 ml of ethylene glycol monomethyl ether. The mixture was shaken vigorously for 2 min and was centrifuged at 4,300 × *g* for 5 min. The supernatant liquid was mixed with 0.25 volume of 1% CTAB, incubated for 30 min at −20 C, and centrifuged at 7,700 × *g* for 20 min. The pellets were washed three times (10 min each) with chilled 70% ethanol containing 0.1 M sodium acetate (pH 4.8) and were resuspended in TE buffer.

Dot hybridization. Eight EY MLO cloned DNA fragments from EY1 were used as probes in dot hybridization to study the genetic interrelatedness among elm MLOs from various sources and among these MLOs and those associated with nine other yellows diseases. Dot hybridization, posthybridization washes, and signal detection were performed as described previously (15). The presence of a detectable quantity of MLO DNA in each sample spot was verified by hybridization of a second set of samples to a homologous DNA probe. Hybridizations were performed under both moderately (42 C) and highly (50 C) stringent conditions in the presence of 45% formamide as described elsewhere (8,11,15). Wash conditions were the same in both cases (8,11,15).

Southern hybridization. In tests to determine whether cloned EY MLO DNA fragments might have derived from chromosomal or extrachromosomal (plasmid) DNA, each of the cloned EY MLO DNA probes was hybridized to Southern blotted DNA samples. Undigested DNA samples (2–4 μg of total nucleic acid per sample) from healthy or EY MLO-infected periwinkle plants were electrophoresed in a 0.7% agarose gel, alkali denatured, and transferred to nitrocellulose membrane by the method of Southern as described by Maniatis et al (17). The membranes were air-dried, baked, prehybridized, and hybridized with biotin-labeled DNA probes at 42 C as described previously (15).

Polymerase chain reaction. Three oligonucleotide primers (EY11-F1, EY11-R1, and EY11-R2) were designed on the basis of partial sequences of the EY MLO DNA insert in probe pEY11 (Fig. 1). Sequencing was performed using standard dideoxy nucleotide termination reactions (23). Two primer pairs (F1R1 and F1R2) were employed in PCR to detect the EY MLO in infected periwinkle and elm tissues. For the PCR, nucleic acid extracts from healthy or MLO-infected tissues were diluted in

TE buffer to give a final concentration of 20 ng/μl. The reactions were performed, as previously described (23), in total 50-μl reaction mixtures containing 20 ng of the dilute nucleic acid sample, PCR buffer (1× 10mM Tris HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin), 200 mM each dNTP, 1 μM each primer of a primer pair, and 1.25 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). The following parameters were used: 1 min (2 min for first cycle) denaturation at 94 C, annealing at 50 C for 2 min, and primer extension at 72 C for 3 min. Tubes with the reaction mixture devoid of DNA template were included in each experiment as negative controls. The presence of a sufficient amount of MLO DNA (as templates) in each of the reaction mixtures was verified by a separate PCR assay with a primer pair designed from an EY MLO 16S rRNA sequence that initiates amplification of MLO 16S rDNA sequences from all known members of the EY MLO strain cluster. (I.-M. Lee, R. E. Davis, W. A. Sinclair, N. D. DeWitt, and M. Conti, *unpublished data*). In a separate experiment, the reaction was performed under a higher annealing temperature at 60 C.

After amplification, a 5- to 15-μl aliquot from each sample was electrophoresed in 1.0% agarose gel and visualized by staining with ethidium bromide and UV illumination.

RESULTS

DNA cloning and preparation of probes. Ninety transformed colonies of *E. coli* were obtained in the cloning of DNA from periwinkle plants infected with the EY MLO. Nucleic acid preparations from 54 of these transformants hybridized with probes from diseased plants but not with probes from healthy plants. Eight clones selected from these 54 transformants were used for development of EY probes. The sizes of inserts in these eight recombinant plasmids ranged from 0.3 to 3.2 kb (Table 1). Inserts of pEY11, pEY24, pEY29, pEY39, and pEY42 have one to two internal restriction sites (*Eco*RI or *Hin*dIII; data not shown).

Fig 1. Partial sequence of elm yellows (EY) mycoplasmalike organism DNA segment in the probe pEY11. The positions of primers EY11-F1, EY11-R1, and EY11-R2 for polymerase chain reactions are indicated in boxes.

Dot hybridization. Six EY probes (pEY11, pEY14, pEY29, pEY39, pEY42, and pEY53) were used in hybridization assays at 50 C and two probes (pEY24 and pEY68) were used at 42 C (Table 1; Fig. 2). All six EY probes hybridized at 50 C with nucleic acid from the New York sample of Chinese elm naturally infected with unidentified MLO (EY2) and the Italian sample of MLO-infected European field elm (EYie1), as well as Chinese elm (EY1A) and periwinkle (EY1) (Table 1; Fig. 2). None of these probes hybridized with nucleic acid preparations from healthy plants or from plants infected with AY (a strain from Maryland), BB, CN1, CPh, or other MLOs, i.e., AshY, PWB, CX, CP, or VR. These results indicated that the unidentified MLOs in Chinese and European field elms were closely related to strain EY1 but did not reveal relatedness to other MLOs investigated. Under hybridization conditions of moderate stringency (42 C), probe pEY24 hybridized with nucleic acid from

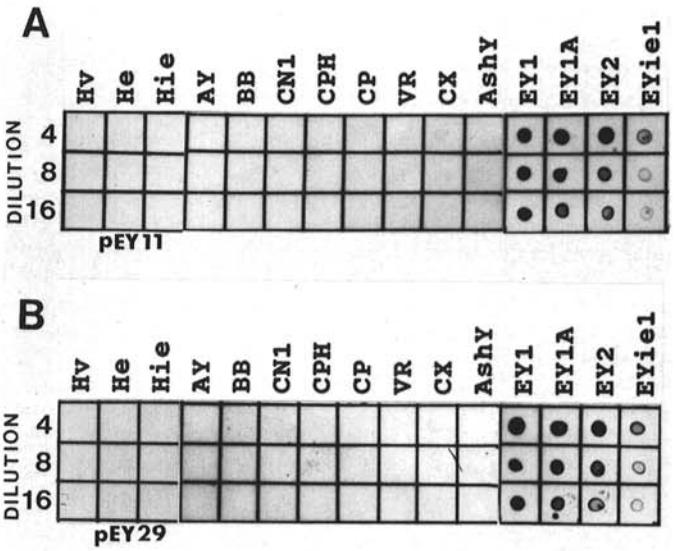

Fig 2. Dot hybridization of biotinylated cloned elm yellows (EY) mycoplasmalike organism (MLO) DNA probes, **A,** pEY11 and **B,** pEY29, to nucleic acid preparations from MLO-infected and healthy plants. Strain descriptions are the same as those in Table 1. The numbers 4, 8, and 16 indicate reciprocals of dilutions. The first fourfold dilution contained 1.1 μg of total nucleic acid.

TABLE 1. Summary of results from dot hybridizations of cloned DNA probes derived from elm yellows (EY) MLO strain EY1 to nucleic acid preparations from healthy or MLO-infected plants

Probe[a]	Insert size (kb)	Hybridization with nucleic acid from indicated source plant[b]														
		Hv	He	Hie	EY1	EY1A	EY2	EYie1	AshY	AY	BB	CN1	CPh	PWB	CP	CX	VR
pEY11	1.6	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY14	0.3	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY24	2.0	−	−	−	+	ND	ND	ND	+	−	−	−	ND	+	+	+	+
pEY29	3.2	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY39	3.1	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY42	3.1	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY53	0.5	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−
pEY68	0.4	−	−	−	+	ND	ND	ND	−	−	−	−	ND	−	−	−	w

[a] Dot hybridization was carried out at 50 C; pEY24 and pEY68 were at 42 C as described.

[b] Source plant of periwinkle: Hv, healthy; He, healthy Chinese elm; Hie, healthy European field elm; EY1, EY MLO strain maintained in periwinkle; EY1A, EY MLO strain maintained in Chinese elm; EY2, EY MLO strain EY-2 naturally in infected Chinese elm; EYie1, an Italian strain of EY MLO in European field elm; AshY, ash yellows MLO; AY, Maryland strain of aster yellows; BB, tomato big bud; CN1, periwinkle little leaf MLO strain Orch 1; CPh, clover phyllody; PWB, potato witches'-broom; CP, clover proliferation; CX, Canada peach X; VR, vinca virescence (=beet leafhopper-transmitted virescence agent). ND, not determined; +, strong to moderate hybridization signal; w, weak hybridization signal; and −, no hybridization signal.

periwinkle infected with EY1, as well as with nucleic acid from PWB, AshY, CX, and CP MLO-infected plants, and probe pEY68 hybridized weakly with nucleic acid from a VR MLO-infected plant (Table 1), indicating sequence homology with these MLOs.

Polymerase chain reaction. Two primer pairs (EY11-F1R1 and EY11-F1R2) were employed in PCR for amplification of EY MLO-specific DNA fragments. Under moderate stringency with an annealing temperature at 50 C, no specific PCR products were obtained in the control reaction mixture containing water or with nucleic acid samples prepared from healthy plants of periwinkle, Chinese, American, and European field elms (Fig. 3A and B). With the primer pair EY11-F1R1, a specific DNA fragment (about 1.1 kb) was amplified in each of the reaction mixtures containing nucleic acid prepared from infected periwinkle (EY1), Chinese elm (EY1A and EY2), and one symptomatic American elm (EYae2). Nonspecific DNA fragments (about 0.6 and 1.3 kb) were amplified from each of the nucleic acid samples prepared from one apparently healthy and two symptomatic European field elms (Hie2, EYie1, and EYie2) (Fig. 3A). The two nonspecific DNA fragments were not amplified when the annealing temperature was 60 C (Fig. 3C). No PCR products were obtained with samples of two symptomatic European elms (EYie3 and EYie4) and one symptomatic American elm (EYae1). With the primer pair EY11-F1R2, a specific DNA fragment (about 0.9 kbp) was amplified from each nucleic acid sample prepared from infected periwinkle (EY1) and Chinese elm (EY1A and EY2) (Fig. 3B). No PCR products were obtained when template consisted of DNA from any asymptomatic European and American elm trees.

The same nucleic acid samples prepared from periwinkle and various elm plants were used in a separate PCR assay. When a primer pair derived from an EY MLO 16S rRNA sequence was employed in PCR, an EY MLO-specific 16S rDNA sequence was amplified in each reaction mixture containing template DNA prepared from each symptomatic periwinkle or elm (EY1, EY1A, EY2, EYae2, EYie1, EYie2, EYie3, or EYie4) with one exception, diseased American elm (EYae1). No PCR products were amplified with samples of healthy or asymptomatic periwinkle and elm plants (data not shown).

Southern hybridization. In Southern hybridization analyses, (data not shown) all eight EY probes hybridized with a DNA band corresponding in position to chromosomal MLO DNA as seen in previous work (11). No evidence of extrachromosomal MLO DNA was noted.

DISCUSSION

Nucleic acid dot hybridization has been applied to detect various MLOs and to study genetic relatedness among various strains of MLOs (reviewed by Lee and Davis [12]). Based on dot hybridization analyses using DNA probes derived from AY, BB, CN1, and other MLOs, we have previously distinguished groups of MLOs referred to as strain clusters (6,8,11–16). MLO strain EY1 represented one cluster (12). The present results indicate that previously unidentified MLOs in Chinese elm in New York and European field elm in Italy also are members of the EY MLO strain cluster. Analyses using the EY probes support the previous suggestion that EY MLOs are only distantly related, if at all, to members of the AY MLO strain cluster, because none of the eight EY probes hybridized with nucleic acid preparations from any members (AY, BB, CN1, and CPh MLOs) of the AY cluster tested. The EY MLOs appeared to be related to, but distinct from, AshY, PWB, CX, CPh, and VR MLOs under moderately stringent (42 C) hybridization conditions, but relatedness was not revealed under highly stringent conditions (50 C). At 42 C, two probes did hybridize weakly to moderately with nucleic acid preparations from plants infected by one or another of these MLOs.

Native American elms infected by EY MLOs exhibit chlorosis, epinasty and premature casting of leaves; witches'-broom (in *U. rubra*); root mortality; and degeneration of phloem in roots and at the base of the tree (2,26). The symptoms induced by EY MLOs in naturally or artificially (3) infected Asiatic or European elms are different, with greater frequency of witches'-brooms and less necrosis of phloem and roots (2,3,21,26). Based on symptoms in European and Chinese elms to which EY MLOs were transmitted by grafting (3), we suspected that naturally occurring witches'-broom and declines in Chinese elm at Ithaca, NY, and in various elm species and hybrids in Italy were caused by EY MLOs. Before the availability of EY-specific DNA probes, however, no reliable method existed for specific diagnosis of EY mycoplasmal infections. The present dot hybridization analyses provided these diagnoses and expanded to three the number of MLO

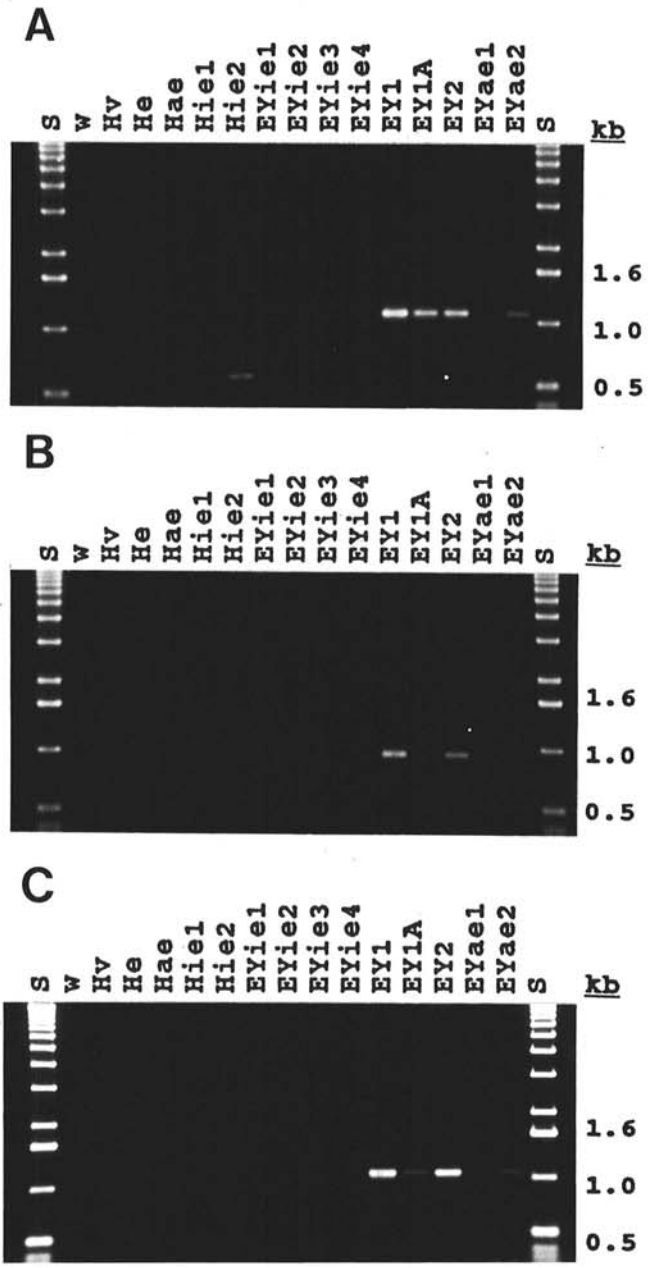

Fig 3. Polymerase chain reaction (PCR) analysis of DNA extracted from healthy plants and plants infected with mycoplasmalike organisms (MLOs). Reaction products are the result of PCR amplification with primer pairs **A and C,** EY11-F1R1 and **B,** EY11-F1R2. Annealing temperature in PCR was **A and B,** 50 C or **C,** 60 C. Figure shows results from electrophoresis of reaction products in a 1.2% agarose gel after staining with ethidium bromide. Lane S, DNA ladder; W, water control; Hv, healthy periwinkle; He, healthy Chinese elm; Hae, healthy American elm; Hie1 and Hie2, two apparently healthy (or asymptomatic) European field elm samples; EYie1, EYie2, EYie3, and EYie4, four symptomatic European field elm samples; EYae1 and EYae2, two symptomatic American elm samples; EY1, EY1A, and EY2, strain descriptions are the same as those in Table 1.

strains assigned to the EY MLO strain cluster.

Analyses by PCR with two oligonucleotide primer pairs designed on the basis of the sequence of probe pEY11 (Fig. 1) indicated that American EY MLO strains were more closely related to one another than to a strain of EY MLO from Italy. Using these two primer pairs, no EY MLO DNA fragments with specific sizes (1.1 and/or 0.9 kb) were amplified when reaction mixtures contained nucleic acid samples from EY-infected European elm trees collected in the field in Italy. In contrast, EY MLO-specific DNA fragments were readily amplified when the reaction mixture contained EY1 MLO or other American EY MLO DNA as template (Fig. 3).

Although a lack of specific PCR products from nucleic samples prepared from the symptomatic European elm trees could be due to factors such as insufficient MLO DNA templates or to the presence of *Taq* polymerase inhibitors in the reactions mixtures, these alternative explanations are not applicable, because we readily obtained EY MLO-specific PCR products with the same amounts of these nucleic acid samples as templates in PCR with another primer pair designed from an EY MLO 16S rRNA sequence. However, such factors probably account for the failure of specific sequence amplification when the reaction mixture containing EYae1 MLO DNA as templates was used for PCR with both primer pairs derived from pEY11 and from an EY MLO 16S rRNA sequence. The failure of these two primer pairs (EY11-F1R1 and EY11-F1R2) to detect all strains in the EY MLO strain cluster was not totally unexpected, because genetic diversity is probable among EY MLO strains from different geographical regions. The discrepancy could simply be due to sequence variation among these EY MLO strains in a variable region of the genome from which the primer pairs were derived.

The PCR analyses also suggested genomic dissimilarities among the EY MLO strains present in North America; EYae2 MLO could be differentiated from EY1 and EY2 MLOs by analysis with the two primer pairs derived from probe pEY11. Based on sizes of amplified DNAs (Fig. 3A and B), EY MLOs in Chinese elm, EY1A may represent a mixed population of at least two strains, one resembling EYae2 MLO and the other resembling EY1 MLO.

EY is spreading and is characterized by explosive epiphytotics in North America (4,10,18,26). In Italy, it has become a serious disease of elm (5,20). The sensitive and reliable MLO detection afforded by the EY DNA probes and by EY MLO-specific PCR should contribute to progress in epiphytological studies, such as investigation of insect vectors and possible alternative host plants and could facilitate selection of elms tolerant of, or resistant to, the EY MLO.

LITERATURE CITED

1. Braun, E. J. 1977. A freeze-etch and thin-section study of mycoplasmas in *Vinca rosea* phloem. J. Ultrastruct. Res. 60:44-51.
2. Braun, E. J., and Sinclair, W. A. 1976. Histopathology of phloem necrosis in *Ulmus americana*. Phytopathology 66:598-607.
3. Braun, E. J., and Sinclair, W. A. 1979. Phloem necrosis of elms: Symptoms and histopathological observations in tolerant hosts. Phytopathology 69:354-358.
4. Carter, J. C., and Carter, L. R. 1974. An urban epiphytotic of phloem necrosis and Dutch elm disease: 1944–1972. III. Nat. Hist. Surv. Bull. 31:113-143.
5. Conti, M., D'Agostino, G., and Mittempergher, L. 1987. A recent epiphytotic of elm yellows in Italy. Proc. 7th Cong. Mediterr. Phytopathol. Union:208-209.
6. Davis, R. E., Lee, I.-M., Douglas, S. M., and Dally, E. L. 1990. Molecular cloning and detection of chromosomal and extrachromosomal DNA of the mycoplasmalike organism associated with little leaf disease in periwinkle (*Catharanthus roseus*). Phytopathology 80:789-793.
7. Davis, R. E., Lee, I.-M., Douglas, S. M., Dally, E. L., and DeWitt, N. D. 1988. Cloned nucleic acid hybridization probes in detection and classification of mycoplasmalike organisms (MLOs). Acta Hortic. 234:115-122.
8. Davis, R. E., Sinclair, W. A., Lee, I.-M., and Dally, E. L. 1992. Cloned DNA probes specific for detection of a mycoplasmalike organism associated with ash yellows. Mol. Plant-Microbe Interact. 5:163-169.
9. Dellaporta, S. L., Wood, J., and Hicks, J. B. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19-21.
10. Lanier, G. N., Schubert, D. C., and Manion, P. D. 1988. Dutch elm disease and elm yellows in central New York: Out of the frying pan into the fire. Plant Dis. 72:189-194.
11. Lee, I.-M., and Davis, R. E. 1988. Detection and investigation of genetic relatedness among aster yellows and other mycoplasmalike organisms by using cloned DNA and RNA probes. Mol. Plant-Microbe Interact. 1:303-310.
12. Lee, I.-M., and Davis, R. E. 1992. Mycoplasmas which infect plants and insects. Pages 379-390 in: Mycoplasmas: Molecular Biology and Pathogenesis. J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman, eds. American Society for Microbiology, Washington, D.C.
13. Lee, I.-M., Davis, R. E., Chen, T. A., Chiykowski, L. N., Fletcher, J., Hiruki, C., and Schaff, D. A. 1992. A genotype-based system for identification and classification of mycoplasmalike organisms (MLOs) in the aster yellows MLO strain cluster. Phytopathology 82:977-986.
14. Lee, I.-M., Davis, R. E., and DeWitt, N. D. 1988. Molecular cloning of and screening by a new method for DNA fragments from elm yellows (EY) and tomato big bud (BB) mycoplasmalike organisms (MLOs). (Abstr.) Phytopathology 78:1602.
15. Lee, I.-M., Davis, R. E., and Hiruki, C. 1991. Genetic interrelatedness among clover proliferation mycoplasmalike organisms (MLOs) and other MLOs investigated by nucleic acid hybridization and restriction fragment length polymorphism analyses. Appl. Environ. Microbiol. 57:3565-3569.
16. Lee, I.-M., Gundersen, D. E., Davis, R. E., and Chiykowski, L. N. 1992. Identification and analysis of a genomic strain cluster of mycoplasmalike organisms associated with Canadian peach (eastern) X disease, western X disease, and clover yellow edge. J. Bacteriol 174:6694-6698.
17. Maniatis, T. A., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
18. Matteoni, J. A., and Sinclair, W. A. 1988. Elm yellows and ash yellows. Pages 19-31 in: Tree Mycoplasmas and Mycoplasma Diseases. C. Hiruki, ed. The University of Alberta Press, Edmonton, Alberta, Canada.
19. Matteoni, J. A., and Sinclair, W. A. 1989. A note on the presence of elm yellows in the Niagara Peninsula. Phytoprotection 70:137-139.
20. Mittempergher, L., Fagnani, A., Ferrini, F., and D'Agostino, G. 1990. Elm yellows a disease to be taken into consideration when breeding elm for disease resistance. Proc. 8th Cong. Mediterr. Phytopathol. Union. Pages 433-435.
21. Pisi, A., Marani, F., and Bertaccini, A. 1981. Mycoplasma-like organisms associated with elm witches' broom symptoms. Phytopathol. Mediterr. 20:189-191.
22. Ralph, R. K., and Bellamy, A. R. 1963. Isolation and purification of undegraded ribonucleic acids. Biochem. Biophys. Acta 87:9-16.
23. Schaff, D. A., Lee, I.-M., and Davis, R. E. 1992. Sensitive detection and identification of mycoplasmalike organisms by polymerase chain reactions. Biochem. Biophys. Res. Commun. 186:1503-1509.
24. Seemuller, E. 1976. Investigations to demonstrate mycoplasmalike organisms in diseased plants by fluorescence microscopy. Acta Hortic. 67:109-112.
25. Sinclair, W. A. 1972. Phloem necrosis of American and slippery elms in New York. Plant Dis. Rep. 56:159-161.
26. Sinclair, W. A. 1981. Elm yellows. Pages 25-31 in: Compendium of Elm Diseases. R. J. Stipes and R. J. Campana, eds. American Phytopathological Society, St. Paul, MN.
27. Sinclair, W. A., Braun, E. J., and Larsen, A. O. 1976. Update on phloem necrosis of elms. J. Arboric. 2:106-113.
28. Sinclair, W. A., Iuli, R. J., Dyer, A. T., and Larsen, A. O. 1989. Sampling and histological procedures for diagnosis of ash yellows. Plant Dis. 73:432-435.
29. Swingle, R. U. 1938. A phloem necrosis of elm. Phytopathology 28:757-759.
30. Wilson, C. L., Seliskar, C. E., and Krause, C. R. 1972. Mycoplasmalike bodies associated with elm phloem necrosis. Phytopathology 62:140-143.