

SHORT COMMUNICATION

SUBGROUP 16SrIII-F PHYTOPLASMA STRAINS IN AN INVASIVE PLANT, *HERACLEUM SOSNOWSKYI*, AND AN ORNAMENTAL, *DICTAMNUS ALBUS*D. Valiunas^{1,3}, M. Samuitiene¹, V. Rasomavicius², M. Navalinskiene¹, J. Staniulis¹, and R.E. Davis³¹Laboratory of Phytoviruses, Institute of Botany, Zaliuju ezeru 49, Vilnius LT-08406, Lithuania²Laboratory of Flora and Geobotany, Institute of Botany, Zaliuju ezeru 49, Vilnius LT-08406, Lithuania³Molecular Plant Pathology Laboratory, USDA-ARS, Beltsville, MD 20705, USA

SUMMARY

Phytoplasma strains were detected in an aggressive and widespread, invasive plant species, *Heracleum sosnowskyi* (hogweed), and in *Dictamnus albus* (gas-plant) exhibiting yellows disease symptoms in northern Lithuania. Analysis of PCR-amplified 16S rDNAs revealed that the strains, designated heracleum yellows (HerY) and dictamnus stunt (DicSt), respectively, belong to subgroup 16SrIII-F (milkweed yellows phytoplasma subgroup). The results established that phytoplasma is a natural enemy of hogweed and *D. albus* in the Baltic region. While subgroup 16SrIII-F strains occur in four plant species (*Vaccinium corymbosum*, *V. myrtillus*, *H. sosnowskyi* and *D. albus*) in Europe, *Asclepias syriaca* (milkweed) in North America is the only known host elsewhere.

Key words: Mollicutes, RFLP, identification, phylogeny, hogweed, gas-plant.

Hogweed (*Heracleum sosnowskyi* Manden.; family *Apiaceae*) is a perennial or short-lived perennial monocarpic plant native to the Caucasus Mountains, where it grows in open sites of beech and mixed forests, and in subalpine forb meadows (Satsyperova, 1984). During the 1960s and 1970s, *H. sosnowskyi* was introduced into Baltic countries as a prospective silage plant, but for reasons including health problems during harvesting, its cultivation was ceased about twenty years ago. Now hogweed is the most aggressive alien plant in the Baltic region, very rapidly occupying both natural and disturbed habitats (Gudžinskas, 1998). In northern Lithuania, it is wide spread in open areas of forest habitats – glades, forest edges, fire breaks, roadsides, banks of drainage ditches. Here we report a phytoplasma belonging to group 16SrIII, subgroup F (III-F, milkweed yellows phytoplasma subgroup), in diseased hogweed and also in diseased

plants of *Dictamnus albus* L. (gas-plant, family *Rutaceae*), an ornamental shrub containing a volatile oil that causes dermatitis on contact (Evans and Schmidt, 1980).

Leaves were collected from naturally infected hogweed exhibiting twisting and recumbent growth of stems, stunting, phyllody, leaf yellowing, and leaf crinkle (Fig. 1) in Suvainiškis, Rokiškis district, and from ornamental gas-plant exhibiting yellowing and stunting in Kursenai region, northern Lithuania. DNA was extracted from excised veins using Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania). 16S rDNA products from nested PCRs, primed by P1/P7 and R16F2n/R16R2 (Lee *et al.*, 1993), were analyzed by single enzyme digestion with *AluI*, *MseI*, *HbaI*, *HaeIII*, *HpaII*, *RsaI*, and *TbaI* (New England Biolabs, Beverly, MA, USA). Restriction fragment length polymorphism (RFLP) patterns were compared with those previously published (Lee *et al.*, 1998). Phloem tissue for examination of ultrathin sections was fixed in glutaraldehyde, postfixated in osmium tetroxide, and embedded in Epon resin by standard methods.

Amplification of phytoplasma-characteristic DNA products in nested PCRs and electron microscopy of phloem tissue (data not shown) suggested phytoplasma infections in both plant species. The hogweed and gas-plant phytoplasmas were designated heracleum yellows (HerY) and dictamnus stunt (DicSt) phytoplasmas, respectively. The collective RFLP patterns of 1.2 kbp segments of 16S rDNA (products of PCRs primed by R16F2n/R16R2) (Fig. 2) were indistinguishable from those published by Lee *et al.* (1998) for strain MW1 (milkweed yellows phytoplasma from North America), a member of group 16SrIII (X-disease phytoplasma group), subgroup III-F (milkweed yellows phytoplasma subgroup).

Products (1.8 kbp) of PCRs primed by P1/P7 were cloned in *E. coli* using TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA USA) and sequenced using automated DNA sequencing. The nucleotide sequences were deposited in the GenBank database under DQ164213 (strain HerY) and DQ164212 (strain DicSt).

Putative restriction maps of the 16S rDNAs, constructed by using the MapDraw program of sequence analysis software suite Lasergene (DNASTAR, Madison,



Fig. 1. Naturally infected *Heracleum sosnowskyi* (hogweed) exhibiting symptoms of stunting, twisting and recumbent growth of stems, leaf yellowing, and little crinkle leaf.

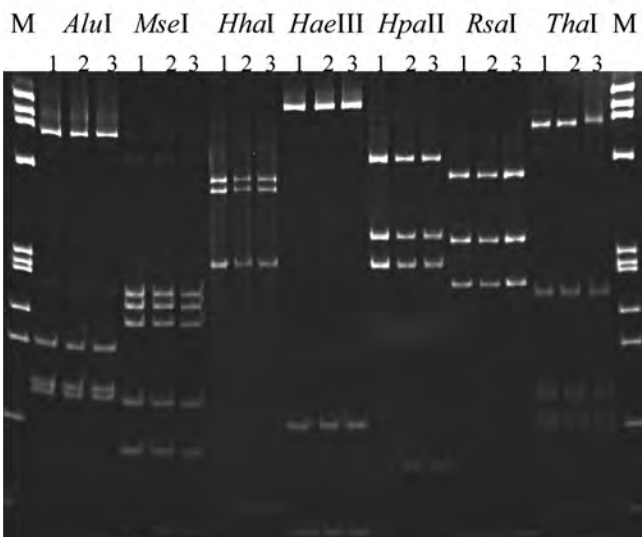


Fig. 2. RFLP patterns of 16S rDNA amplified in nested PCR primed by oligonucleotide pair R16F2n/R16R2 from heracleum yellows (HerY), dictamnus stunt (DicSt), and reference milkweed yellows (MW1) phytoplasmas. Results are from single enzyme digests using *AluI*, *MseI*, *HhaI*, *HaeIII*, *HpaII*, *RsaI*, and *ThaI*, respectively. 1, HerY; 2, DicSt; 3, MW1. M, ØX174 RFI DNA *HaeIII* digest size standard (Life Technologies, Gaithersburg, MD, USA), fragment sizes: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp.

WI), were in excellent agreement with results from enzymatic RFLP analysis (data not shown). Multiple alignments, using Lasergene's MegAlign program, revealed that strains HerY and DicSt shared high rDNA sequence similarity with strains VAC-L (GenBank no. AY034090, *Vaccinium myrtillus* phytoplasma from Lithuania) and MW1. The HerY and DicSt 1.8 kbp rDNA sequences shared 99.9% similarity and differed at only two base positions in the 16S rDNA region. The 1.8 kbp rDNAs from strains VAC-L, HerY, and DicSt phytoplasmas shared 99.5%, 99.9% and 99.8% sequence similarity, respectively, with that of strain MW1. European subgroup III-F strains VAC-L, HerY, DicSt, and VAC (GenBank no. X76430) differed from strain MW1 in two base positions, corresponding to bases 1237 and 1393 in GenBank sequences DQ164213 and DQ164212, respectively. Branching order in a phylogenetic tree of 16S rRNA gene sequences indicated that strains HerY, DicSt, VAC-L, VAC, and MW1 descended from a common ancestor (Fig. 3).

This study revealed that the noxious weed, *H. sosnowskyi* (hogweed), has a natural enemy in the Baltic Region. Considered among the worst invasive species, *H. sosnowskyi* forms monospecific stands, threatening the biodiversity of local flora and fauna (Sampson,

1994). The root and outer rind of hogweed contain an acrid sap that can inflame and ulcerate skin and cause dermatitis; contact results in blistering of the affected area and allergic reaction (Nielsen, 1970). Consequently, an intensive study of control methods to reduce/prevent hogweed dispersal is underway (Andersen, 1994; Tiley and Philp, 1997).

While subgroup III-F phytoplasma strains have been

found in four plant species (*Vaccinium corymbosum*, *V. myrtillus*, *H. sosnowskyi* and *D. albus*) in Europe (Valiunas *et al.*, 2004), *Asclepias syriaca* (milkweed) in North America is the only known host elsewhere. It is not yet known whether phytoplasmal infection plays any role in limiting spread of invasive *H. sosnowskyi*, but hogweed could possibly contribute to spread of subgroup III-F phytoplasmas.

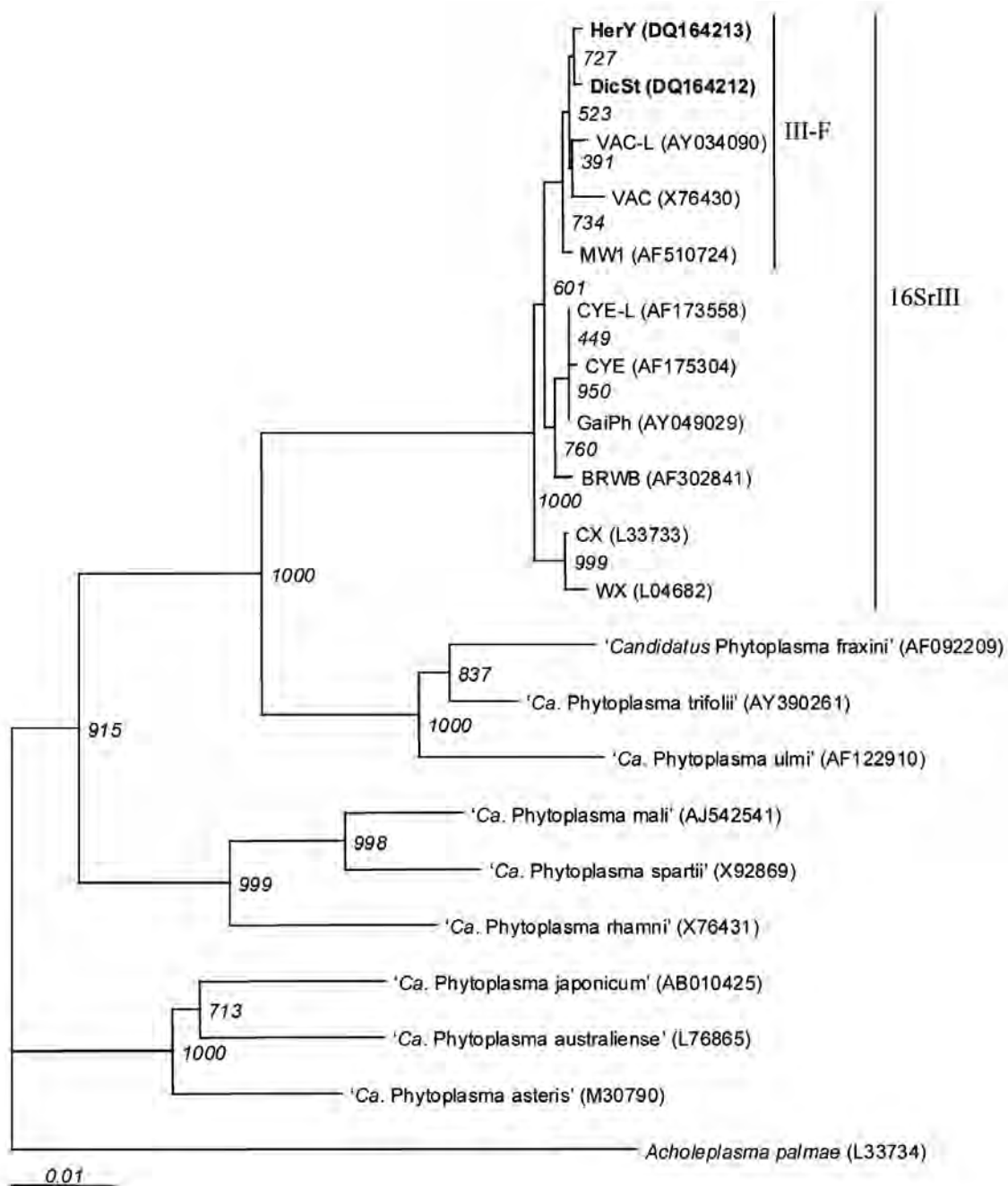


Fig. 3. Phylogenetic tree constructed of 1.2 kbp 16S rDNAs from 20 phytoplasmas and *Acholeplasma palmae*. GenBank numbers are in parentheses. Sequences were aligned using Clustal X 1.63b (Thompson *et al.*, 1997) and viewed using TreeViewPPC (Page, 1996). Group 16SrIII and subgroup III-F are indicated. Numbers at nodes are bootstrap (confidence) values. Phytoplasmas are: HerY, heracleum yellows; DicSt, dictamnus stunt; VAC-L, vaccinium witches' broom from Lithuania; VAC, vaccinium witches' broom; MW1, milkweed yellows; CYE-L, clover yellow edge from Lithuania; CYE, clover yellow edge; GaiPh, gaillardia phylloidy; BRWB, black raspberry witches' broom; CX, Canada X-disease; WX, western X-disease.

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