ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 101, No. 3 (2014), p. 313–320
DOI 10.13080/z-a.2014.101.040

Possible insect vectors of '*Candidatus* Phytoplasma asteris' and '*Ca.* Phytoplasma pruni'-related strains in Lithuania

Algirdas IVANAUSKAS[1], Deividas VALIŪNAS[1], Rasa JOMANTIENĖ[1], Luca PICCIAU[2], Robert Edward DAVIS[3]

[1]Institute of Botany, Nature Research Centre
Akademijos 2, Vilnius, Lithuania
E-mail: algirdasivanauskas@gmail.com

[2]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino
Via Leonardo Da Vinci, 44-10095 Grugliasco, Italy

[3]Molecular Plant Pathology Laboratory, Agricultural Research Service (USDA)
20705 Beltsville, USA

Abstract

Phytoplasma strains affiliated with groups 16SrI, 16SrIII, 16SrV and 16SrXII have been found in Lithuania, but their insect vectors in the country have not been determined. '*Candidatus* Phytoplasma asteris' and '*Ca*. Phytoplasma pruni'-related phytoplasma strains were identified in five leafhopper species and three spittlebug species occurring in Lithuania. The occurrence in Lithuania of *Anaceratagallia ribauti*, reported as a vector of stolbur phytoplasma ('*Ca*. Phytoplasma solani', subgroup 16SrXII-A) elsewhere in Europe, provokes the question of whether this phytoplasma may be present but yet undetected in Lithuania. The finding of subgroup 16SrI-C strains in *A. ribauti*, *Aphrodes* sp., *Macrosteles sexnotatus* and *Euscelis incisus*; 16SrI-B strains – in *Aphrophora alni*, *Aphrodes* sp. and *Cicadella viridis*; 16SrIII-B strains – in *A. ribauti*; and 16SrIII-P – in *Aphrodes* sp. and *E. incisus* is consistent with the hypothesis that these leafhopper species are vectors of the respective phytoplasmas in Lithuania. Transmission trials were initiated based on these results. Results thus far have revealed that Lithuanian biotype of *E. incisus* is capable of vectoring subgroup 16SrI-C phytoplasma strains that are found in Lithuania. The presence of diverse phytoplasmas in three spittlebug species, *Aphrophora alni*, *Lepyronia coleopterata* and *Philaenus spumarius*, indicates that these xylem feeders actually ingested phloem sieve cell contents during feeding on phytoplasma-infected plants, bringing into focus the question of whether some of such xylem feeders might act as occasional vectors of phytoplasmas.

Key words: *Cercopoidae*, *Cicadellidae*, leafhoppers, phytoplasma, vectors.

Introduction

Phytoplasmas are wall-less bacteria that inhabit plant phloem tissue and cause hundreds of plant diseases worldwide. Phytoplasmas are mainly transmitted from plant-to-plant by phloem-feeding insects, but also can be transmitted by grafting, vegetative propagation, and by the parasitic plant, dodder (*Cuscuta* spp.) (Weintraub, Beanland, 2006; Bertaccini, Duduk, 2009). Thus far, phytoplasmas classified in four 16Sr groups and twelve subgroups, affiliated with no less than four '*Candidatus* Phytoplasma' species, have been reported from various plant hosts in Lithuania (Valiunas et al., 2009), but data are lacking about insect species that could possibly serve as vectors of phytoplasmas in the country. The main objective of this study was to learn what phytoplasmas Auchenorrhyncha insect species may be transmitting in Lithuania. Our approach was to identify species harbouring phytoplasmas, then to identify those phytoplasmas and plant species serving as hosts for them in areas where the insects were collected, and to initiate tests of selected insect species to determine their ability to transmit phytoplasma. A preliminary synoptic report of part of this work was published in abstract form (Ivanauskas et al., 2011).

Materials and methods

Experiments were conducted in Nature Research Centre, Institute of Botany, Phytovirus Laboratory during 2011–2012 year period. Suspected insect vectors of phytoplasmas were collected by net-sweeping, in July–September, from a meadow and an orchard in Kaunas and Vilnius districts of Lithuania, where phytoplasma-infected plants had previously been found. Samples of plants, showing symptoms of possible phytoplasma infection, were also collected from the same locations to determine possible plant hosts, and deoxyribonucleic acid

(DNA) was extracted from each plant as described by Lee et al. (2006). Insects were preserved in 90% ethanol until DNA extraction. Adult male specimens were identified to the species level using the keys of Ossiannilsson (1978; 1981; 1983) and Ribaut (1952) by morphological analysis under a stereomicroscope. DNA was extracted separately from each of six *Anaceratagallia ribauti* (Ossiannilsson, 1938), one *Aphrophora alni* (Fallen, 1805), six *Aphrodes* sp., three *Cicadella viridis* (Linnaeus, 1758), five *Lepyronia coleoptrata* (Linnaeus, 1758) and five *Philaenus spumarius* (Linnaeus, 1758) individuals using Genomic DNA purification kit ("Fermentas", Lithuania). Extracted DNA from insects and plant samples was used as template in three separate experiments, each involving nested polymerase chain reactions (PCRs) primed by universal primer pairs P1A/16S-Sr (Lee et al., 2003; 2006) and R16F2n/R16R2n (Lee et al., 2006). PCRs were carried out and products were analysed as previously described (Lee et al., 1998). Amplified DNA products of nested PCRs primed by primer pair R16F2n/R16R2n were analysed by single enzyme digestion, according to manufacturer's instructions, with *Alu*I, *Bfa*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Kpn*I, *Mse*I, *Taq*I and *Rsa*I ("Fermentas"). Restriction fragment length polymorphism (RFLP) patterns were analysed by electrophoresis through a 5% polyacrylamide gel. The DNA size marker was phiX174 DNA/*Bsu*RI (*Hae*III) ("Fermentas"). RFLP patterns were compared with patterns previously published (Lee et al., 1998; Jomantiene et al., 2002). Products of PCRs primed by R16F2n/R16R2n were cloned in *Escherichia coli* using the cloning kit InsTAclone™ PCR ("Fermentas") and were sequenced using automated DNA sequencing by Macrogen™ sequencing services. Sequence reads were assembled using SeqMan, and alignments of the sequences were accomplished with software *MegAlign*, *DNASTAR LaserGene* (DNASTAR, USA) and MEGA version 5 (Tamura et al., 2011). Nucleotide sequences of the phytoplasma 16S rDNA amplicons were analyzed through the use of BLAST searches at the National Centre for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov). The *i*PhyClassifier web tool (http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi) was used to obtain virtual RFLP gel images and to classify phytoplasma strains according to 16Sr groups and subgroups (Zhao et al., 2009). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011). Phylogenetic trees were constructed using the neighbour-joining method with default values and 1000 replications for bootstrap analysis.

For insect transmission tests, healthy plants of Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don) and white clover (*Trifolium repens* L.) were grown from seed in an isolated greenhouse section equipped with yellow sticky traps for surveillance for insects. Individuals of *Euscelis incisus* for artificial breeding were collected from healthy-appearing plants in Lithuanian meadows and were placed in insect-proof cages containing white clover plants. Insects in the artificially reared, caged colonies and plants on which they fed in the cages were analyzed for phytoplasma infection; the results were negative. To obtain source plants for insect transmission tests, plants of white clover were collected in a Lithuanian orchard and tested for infection by the '*Ca*. Phytoplasma asteris' strain, clover phyllody (CPh) phytoplasma, member of subgroup 16SrI-C. Phytoplasma-positive plants served as the original source of CPh phytoplasma for transmission of the phytoplasma to healthy plants of Madagascar periwinkle by the use of dodder (*Cuscuta* sp.). The CPh phytoplasma was then transmitted, using dodder, from the infected periwinkle to healthy white clover seedlings. This last set of clover plants was used as CPh source for insect acquisition feeding in the transmission tests. All plants were tested for phytoplasma-free or phytoplasma-infected status before and after the tests of CPh phytoplasma transmission from clover to clover.

The insect transmission tests were carried out in a greenhouse that was equipped with yellow sticky traps for insect surveillance. Groups of 20–30 artificially reared, phytoplasma-uninfected *E. incisus* 3rd to 4th stage nymphs were placed on caged plants (4 plants per cage) of CPh phytoplasma-infected white clover for an acquisition feeding period of 14 days. Surviving individuals (20–25–23, min-max-average) of these insects then were placed, for an inoculation feeding period of 2–3 weeks, on each of 20 white clover plants, and an equal number of healthy control white clover seedlings, without insects, were each held separately in insect-proof cages. After the inoculation feeding period, plants were treated with insecticide, and samples of plant tissue and insects were collected for phytoplasma detection and identification. The plants were held in the cages throughout the duration of the experiment until they died. All plants were tested for phytoplasma infection 2–3 weeks following the 2–3 week inoculation feeding period. Three identical trials were performed: one in 2011 (June–September) and two in 2012 (June–September).

Results and discussion

Two leafhopper species: *Anaceratagallia ribauti* and an unidentified *Aphrodes* species, three froghoppers (spittlebugs): *Aphrophore alni*, *Lepyronia coleoptrata* and *Philaenus spumarius*, and one sharpshooter *Cicadella viridis* (earlier annotated in Lithuania by Söderman et al. (2009) were chosen for phytoplasma testing among the insects collected from orchard and meadow. Phytoplasma-specific 1.2 kbp 16S rDNA fragments were obtained from four of six *A. ribauti*, from one *A. alni*, from all six *Aphrodes* sp., from all three *C. viridis*, from all five *L. coleoptrata*, and from four of five *P. spumarius* individual samples, indicating the presence of phytoplasmas in these insects (data not shown).

RFLP patterns of 16S rDNAs amplified from the detected phytoplasmas indicated that several phytoplasma subgroup taxa were present in the insects tested; RFLP patterns representing the subgroups detected are shown in Figures 1–8. Comparison of the RFLP patterns with patterns published for 16S rDNAs from other phytoplasmas revealed that the phytoplasmas detected in the various *Auchenorrhyncha* suborder species belonged to several different subgroup level taxa; subgroup 16SrI-B (Maryland aster yellows (AY1) phytoplasma,

'*Ca*. Phytoplasma asteris' subgroup), subgroup 16SrI-C (clover phyllody (CPh) phytoplasma subgroup), subgroup 16SrIII-B (clover yellow edge (CYE) phytoplasma) and subgroup 16SrIII-P ('*Ca*. Phytoplasma pruni'-related phytoplasma strains) (Table). The phytoplasmas found in *A. ribauti* specimens were placed in phytoplasma subgroups 16SrI-C and 16SrIII-B. The phytoplasma strains found in *A. alni* were affiliated with phytoplasma subgroup 16SrI-B. The strains found in *Aphrodes* sp. were assigned to phytoplasma subgroups 16SrI-B, 16SrI-C and 16SrIII-P. The phytoplasma infecting *C. viridis* was assigned to phytoplasma subgroup 16SrI-B; the strains detected in *L. coleoptrata* were assigned to phytoplasma subgroups 16SrI-B, 16SrI-C; and the strain found in *P. spumarius* was assigned to phytoplasma subgroup 16SrI-C. Thus, some insect species had fed upon naturally diseased plant(s) that had been infected by more than one phytoplasma taxon.

BLAST search analyses of 16S rDNA sequences from phytoplasmas found in the present work in *A. ribauti*, *Aphrodes* sp., *P. spumarius*, and from phytoplasmas found previously in *Euselis incisus* (Kirshbaum) and *Macrosteles sexnotatus* (Fallen, 1806) (Ivanauskas et al., 2011), revealed high similarities with sequences from phytoplasma subgroups 16SrI-B, 16SrI-C, 16SrIII-B and 16SrIII-P. The nucleotide sequences are deposited in GenBank database under accession numbers given in Table.

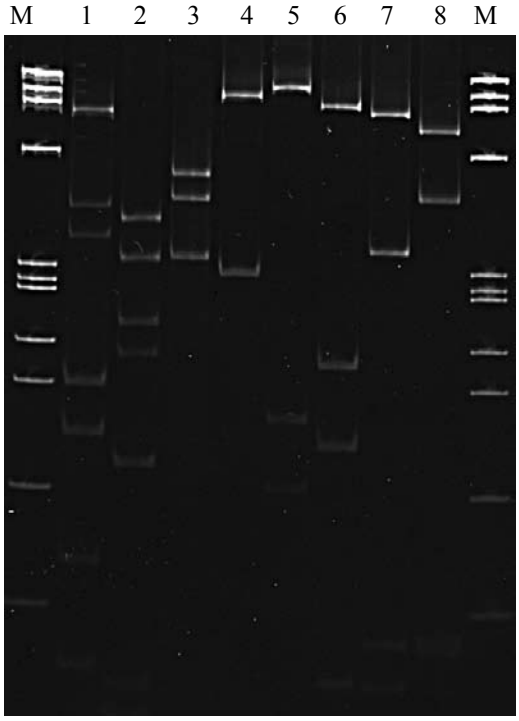
Table. Possible phytoplasma vectors and phytoplasmas detected in field-collected insects, and classified in 16S rDNA RFLP groups and subgroups

Insect species	Total/infected	16Sr subgroup classification of detected strain	GenBank No. of 16S rRNA gene sequences from detected phytoplasmas	Strain/GenBank number of reference strain
Anaceratagallia ribauti	6/4	16SrIII-B 16SrI-C	KC283217 NA	CYE /AF173558 CPh/L33762
Aphrophora alni	1/1	16SrI-B	NA	AY1/L33767
Aphrodes sp.	6/6	16SrIII-P 16SrI-B 16SrI-C	KC283216 KC283215 NA	DanVir/AF370119 AY1/L33767 CPh/L33762
Cicadella viridis	3/3	16SrI-B	KC283211	AY1/L33767
Euscelis incisus	10/10	16SrI-C 16SrIII-P	KC283213 KC283212	CPh/L33762 DanVir/AF370119
Lepyronia coleoptrata	5/5	16SrI-B 16SrI-C	NA NA	AY1/L33767 CPh/L33762
Macrosteles sexnotatus	10/3	16SrI-C	KC283214	CPh/L33762
Philaenus spumarius	5/4	16SrI-B 16SrI-C	NA KC283218	AY1/L33767 CPh/L33762

Note. Strain – reference strain of the indicated phytoplasma 16Sr subgroup, bolded – confirmed phytoplasma vector, default – possible phytoplasma host/vector; NA – not available; CYE – clover yellow edge, CPh – clover phyllody; AY1 – Maryland aster yellows, DanVir – dandelion virescence.

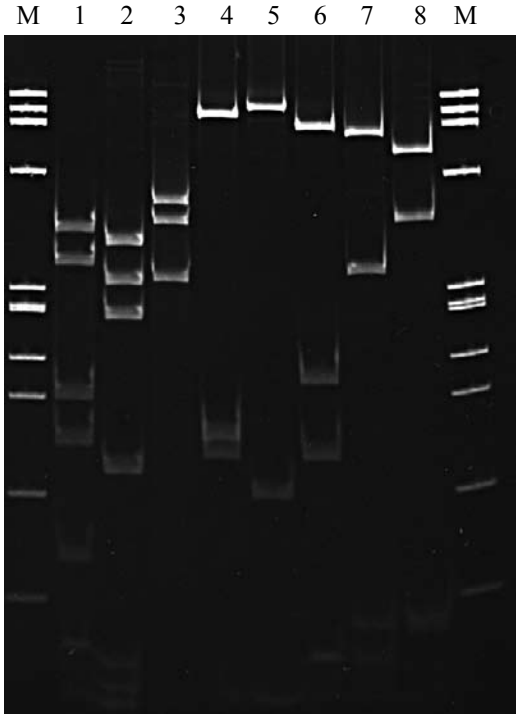
The newly determined phytoplasma gene sequences, and previously published phytoplasma gene sequences from various phytoplasma rDNA RFLP groups, were aligned and subjected to phylogenetic analysis using *Acholeplasma laidlawii* as the outgroup. The phytoplasmas found in insects in the present work clustered with phytoplasmas previously classified in subgroups 16SrI-C, 16SrI-B, 16SrIII-B and 16SrIII-P subgroups (data not shown), supporting the current work's classification of the insect-harboured strains. Following the transmission trials, DNA extracted from plants and insects was used as template in PCRs with phytoplasma specific primers. The PCRs yielded products of 1.2 kbp 16S rDNA fragments, confirming phytoplasmal infection in all inoculated plants. RFLP analysis of the products clearly indicated that the plants and insects had been infected by a strain assigned to phytoplasma subgroup 16SrI-C (data not shown), indicating that *E. incisus* had vectored the CPh phytoplasma from white clover to white clover. The infected white clover plants that had been inoculated by *E. incisus* in the transmission trials developed symptoms including stunting, leaf chlorosis and leaf reddening.

Data obtained from experiments revealed five known *Auchenorryhcha* species and one unidentified species from the genus *Aphrodes* as possible candidate vectors/hosts of three different phytoplasmas in Lithuania. Some phytoplasmas may be transmitted by more than one insect species, and a single insect species possibly is capable of transmitting any of several different phytoplasmas. For example, it is possible that the subgroup 16SrI-C phytoplasma, CPh phytoplasma, is transmitted/harboured in Lithuania by six different *Auchenorrhyncha* species: *A. ribauti*, *L. coleoptrata*, *P. spumarius*, *E. incisus*, *M. sexnotatus* and *Aphrodes* sp. (Ivanauskas et al., 2011). Phytoplasmas belonging to subgroup 16SrI-B are possibly transmitted/harboured in Lithuania by five different insect species: *A. alni*,



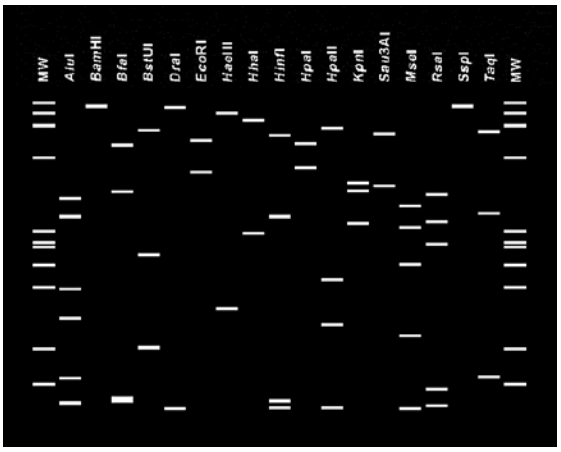
Notes. For phytoplasmas that have rRNA interoperon sequence heterogeneity, the actual RFLPs show DNA bands from both of two sequence heterogeneous rRNA operons. M – marker (phiX174 DNA/*Bsu*RI (*Hae*III)); 1 – *Alu*I, 2 – *Mse*I, 3 – *Kpn*I, 4 – *Hha*I, 5 – *Hae*III, 6 – *Hpa*II, 7 – *Hinf*I, 8 – *Bfa*I.

Figure 1. RFLP analysis of 16S rRNA gene sequence from strain CPhM15-7, GenBank No. KC283214, classified in subgroup 16SrI-C



Explanation of numbers under Figure 1

Figure 3. RFLP analysis of 16S rRNA gene sequence from strain AY1A112, GenBank No. KC283215, classified in subgroup 16SrI-B



Note. The *i*PhyClassifier figure shows results from analysis of the nucleotide sequence from just one of two rRNA operons of a given phytoplasma.

Figure 2. RFLP analysis, made by *i*PhyClassifier (Zhao et al., 2009), of 16S rRNA gene sequence from strain CPhM15-7, operon *rrnA*, GenBank No. KC283214, classified in subgroup 16SrI-C

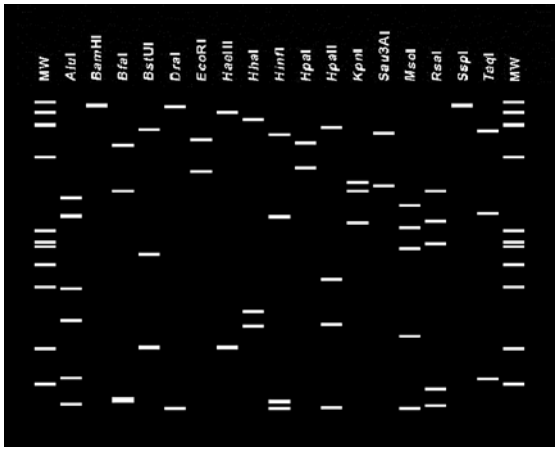
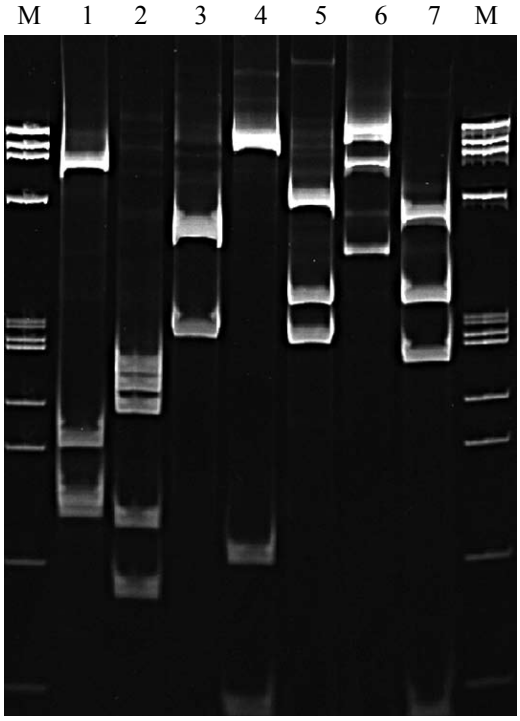


Figure 4. RFLP analysis, made by *i*PhyClassifier (Zhao et al., 2009), of 16S rRNA gene sequence from strain AY1A112, GenBank No. KC283215, classified in subgroup 16SrI-B

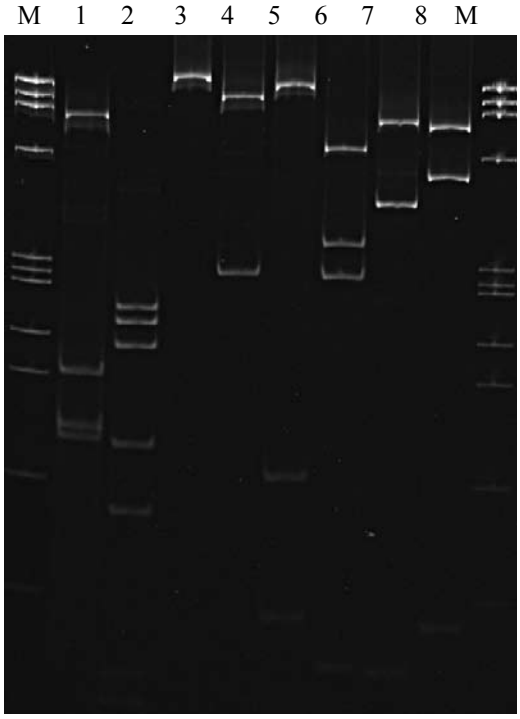
C. viridis, *L. coleoptrata*, *P. spumarius* and *Aphrodes* sp. *A. ribauti* is a possible candidate as vector of phytoplasma subgroup 16SrIII-B. Two insect species, *Aphrodes* sp. and *E. incisus* were identified as possible vectors of dandelion virescence phytoplasma (subgroup 16SrIII-P) (Ivanauskas et al., 2011).

Results from analyses of plants growing in the area where the insects were collected for this work revealed that phytoplasma subgroup 16SrI-B was present in cocksfoot (*Dactylis glomerata* L.), hawkweed oxtongue (*Picris hieracioides* L.), common centaury (*Centaurium erythraea* Rafn.), red clover (*Trifolium pratense* L.). On the other hand, phytoplasma strains belonging to subgroup 16SrI-C were found in cherry plum (*Prunus cerasifera* Ehrh.), white clover (*T. repens* L.) and creeping thistle (*Cirsium arvense* (L.) Scop.). Phytoplasma strains belonging to subgroup 16SrIII-P were found only in dandelion (*Taraxacum officinale* F.H. Wigg). If these and other findings are representative of the natural habitat of



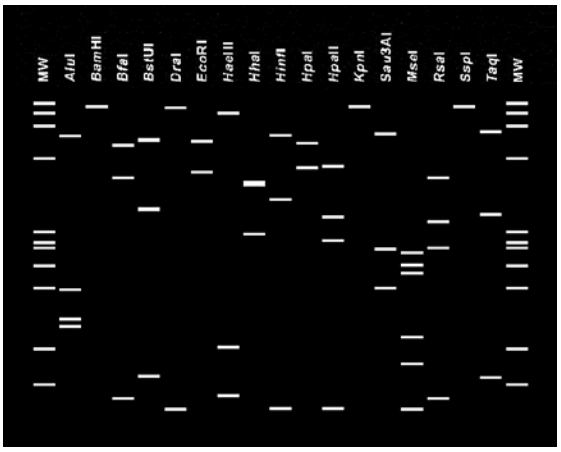
Notes. For phytoplasmas that have rRNA interoperon sequence heterogeneity, the actual RFLPs show DNA bands from both of two sequence heterogeneous rRNA operons. 1 – *Alu*I, 2 – *Mse*I, 3 – *Hha*I, 4 – *Hae*III, 5 – *Hpa*II, 6 – *Hinf*I, 7 – *Rsa*I; M – marker (phiX174 DNA/*Bsu*RI (*Hae*III)).

Figure 5. RFLP analysis of 16S rRNA gene sequence from strain DanVirA213, GenBank No. KC283216, classified in subgroup 16SrIII-P



Explanation of numbers under Figure 1

Figure 7. RFLP analysis of 16S rRNA gene sequence from strain CYEA1, GenBank No. KC283217, classified in subgroup 16SrIII-B



Explanation under Figure 2

Figure 6. RFLP analysis, made by *i*PhyClassifier (Zhao et al., 2009), of 16S rRNA gene sequence from strain DanVirA213, operon *rrnA*, GenBank No. KC283216, classified in subgroup 16SrIII-P

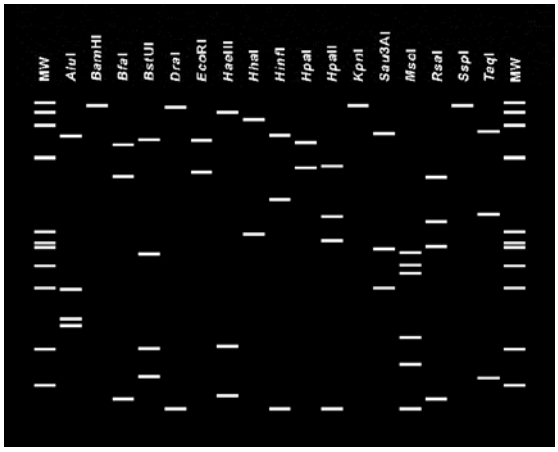


Figure 8. RFLP analysis, made by *i*PhyClassifier (Zhao et al., 2009), of 16S rRNA gene sequence from strain CYEA1, GenBank No. KC283217, classified in subgroup 16SrIII-B

the studied insect species and associated phytoplasmas (Staniulis et al., 2000; Jomantiene et al., 2002), the data not only could reveal plant hosts of the phytoplasmas, but also may be providing insights into feeding preferences of these insects in their natural habitats.

The most common phytoplasma vectors are leafhoppersandplanthoppers(suborder*Auchenorrhyncha*) and psyllids (suborder *Sternorrhyncha*) in the order Hemiptera. Xylem feeding *Auchenorrhyncha* insects from superfamily *Cercopoidae* belonging to subfamily *Cicadellinae* are considered as not capable of transmitting phytoplasmas (Bosco, D'Amelio, 2010), as phytoplasmas are phloem restricted organisms. Insects such as *A. alni*, *L. coleoptrata*, *P. spumarius* and *C. viridis* are described as strict xylem-feeders (Purcell, 2008; Weintraub, Wilson, 2010). However, phytoplasmas have been reported in xylem-feeding *Cercopoidea* insects, e.g., *P. spumarius* (Matteoni, Sinclair, 1988; Seemüller, 1990; Sinclair, 2000; Landi et al., 2007; Országová et al., 2011), and studies on xylem feeding by spittlebugs showed that by

positioning their stylets they can puncture phloem and ingest its sap (Crews et al., 1998).

The finding of several different phytoplasma lineages in diverse *Auchenorrhyncha* species in Lithuania assumes increased significance in light of published reports that these insect species could be active vectors in the spread of phytoplasmas in other geographic areas. For example, research carried out in other regions has shown that *A. ribauti* is a vector of 16SrXII-A phytoplasmas (Riedle-Bauer et al., 2008), and *A. alni* is regarded as a possible vector (to date unconfirmed) of apple proliferation phytoplasma (phytoplasma group 16SrX) (Seemüller, 1990). *Aphrodes bicincta* (Shrank) is a confirmed vector of phytoplasma subgroups 16SrI-A, 16SrI-C, 16SrIII-B, 16SrIV and 16SrXII-A, and possibly other phytoplasmas in other regions (Nielson, 1968; Brčák, 1979; Lee et al., 1998; Weintraub, Beanland, 2006). *C. viridis* has been reported as a suspected vector of yellows disease (Mazzoni et al., 2001). *L. coleoptrata* is described as a possible vector of apple proliferation phytoplasma ('*Ca.* Phytoplasma mali', phytoplasma group 16SrX) (Seemüller, 1990) (to date unconfirmed); we found that this insect species also may be a host/vector of phytoplasmas belonging to subgroups 16SrI-B and 16SrI-C in our region. *P. spumarius* is an unconfirmed vector of phytoplasmas classified in group 16SrV (Matteoni, Sinclair, 1988), subgroup 16SrIII-A (Landi et al., 2007) and possible vector of group 16SrX (apple proliferation phytoplasma group) (to date unconfirmed) (Seemüller, 1990). This species is possibly a host/vector, not only for subgroup 16SrI-C strains, but also for 16SrIII and 16SrV group phytoplasmas in Lithuania. From earlier studies of suspected viruses, *E. incisus* is known as a vector of the clover phyllody pathogen (now classified as a phytoplasma belonging to subgroup16SrI-C), clover witches' broom, clover stolbur, clover stunt and parastolbur diseases in regions outside of Lithuania (Nielson, 1968). *E. incisus* is also now known elsewhere as a vector of phytoplasma strains classified in subgroups 16SrXII-A, 16SrVI, 16SrI-C and 16SrI-B (Brčák 1979; Weintraub, Beanland, 2006). We have confirmed that this species is capable of transmitting a subgroup 16SrI-C phytoplasma, and possibly transmit subgroup 16SrIII-P phytoplasmas, which thus far have been found only in Lithuania and only in a single host plant species, *T. officinale* (Jomantiene et al., 2002; Ivanauskas et al., 2011). Perhaps, it is more important that *E. incisus* may pose a threat as a vector of stolbur phytoplasma in Lithuania.

Further studies are needed to determine whether the five identified *Auchenorrhyncha* species and the unidentified *Aphrodes* sp., found to carry phytoplasmas in Lithuania, are also capable of transmitting the Lithuanian phytoplasma strains to plants. Continued study should also reveal additional insect species as candidate vectors of phytoplasmas in this region. In providing base data for further experimentation, this work opens a new pathway toward understanding the spread of phytoplasmal diseases in Lithuania, and toward eventual design of effective disease management strategies.

Conclusions

1. *Euscelis incisus* was confirmed to transmit 16SrI-C subgroup phytoplasmas in Lithuania.

2. *Anaceratagallia ribauti*, *Aphrophora alni*, *Cicadella viridis*, *Lepyronia coleoptrata*, *Philaenus spumarius*, *E. incisus*, *Macrosteles sexnotatus* and *Aphrodes* sp. were found to harbour phytoplasmas from 16Sr I-C, 16SrI-B and 16Sr III-B, 16SrIII-P subgroups.

3. *A. ribauti*, *L. coleoptrata*, *P. spumarius*, *E. incises* and *Aphrodes* sp. were found to harbour phytoplasmas that belong to more than one 16Sr subgroup.

4. Although *A. alni*, *L. coleopterata* and *P. spumarius* are described as xylem feeders, they ingested phloem sieve cell contents with phytoplasmas during experimental feeding on infected plants. Their role in phytoplasma transmission is under question.

Acknowledgments

We thank A. Bertaccini for organizing A. Ivanauskas' Short-Term Scientific Mission (STSM) to Italy, and A. Alma and R. Tedeschi for hosting A. Ivanauskas and providing resources and guidance during the STSM. We acknowledge COST action FA0807 Integrated management of phytoplasma epidemics in different crop systems for funding the STSM.

Received 07 10 2013
Accepted 19 02 2014

References

Bertaccini A., Duduk B. 2009. Phytoplasma and phytoplasma diseases: a review of recent research. Phytopatologia Mediterranea, 48: 355–378

Bosco D., D'Amelio R. 2010. Transmission specificity and competition of multiple phytoplasmas in the insect vector. Weintraub P. G., Jones P. (eds). Phytoplasmas: genomes, plant hosts and vectors, p. 293–308

Brčák J. 1979. Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. Harris K. F., Maramorsh K. (eds). Leafhopper vectors and plant disease agents, p. 97–153

Crews L. J., Mccully E. M., Canny J. M., Huang X. C., Ling E. C. L. 1998. Xylem feeding by spittlebug nymphs: some observations by optical and cryo-scanning electron microscopy. American Journal of Botany, 85 (4): 449–460 http://dx.doi.org/10.2307/2446427

Ivanauskas A., Valiunas D., Jomantiene R., Staniulis J., Alma A., Picciau L., Davis R. E. 2011. First report of potential phytoplasma vectors *Euscelis incisus* and *Macrosteles sexnotatus* in Lithuania. Bulletin of Insectology, 64: S131–S132

Jomantiene R., Davis R. E., Valiunas D., Alminaite A. 2002. New group 16SrIII phytoplasma lineages in Lithuania exhibit rRNA interoperon sequence heterogeneity. European Journal of Plant Pathology, 108 (6): 507–517 http://dx.doi.org/10.1023/A:1019982418063

Landi F., Prandini A., Paltrinieri S., Mori N., Bertaccini A. 2007. Detection of different types of phytoplasmas in stone fruit orchards in northern Italy. Bulletin of Insectology, 60 (2): 163–164

Lee I.-M., Dawn E., Gundersen-Rindal D., Bertaccini A. 1998. Phytoplasma: ecology and genomic diversity. Phytopathology, 88 (12): 1359–1366 http://dx.doi.org/10.1094/PHYTO.1998.88.12.1359

Lee I.-M., Martini M., Bottner K. D., Dane R. A., Black M. C., Troxclair N. 2003. Ecological implications from a molecular analysis of phytoplasmas involved in an aster yellows epidemic in various crops in texas. Phytopathology, 93: 1368–1377 http://dx.doi.org/10.1094/PHYTO.2003.93.11.1368

Lee I.-M., Bottner K. D., Munyaneza J. E., Davis R. E., Crosslin J. M., du Toit L. J., Crosby T. 2006. Carrot purple leaf: a new spiroplasmal disease associated with carrots in Washington State. Plant Disease, 90 (8): 989–993 http://dx.doi.org/10.1094/PD-90-0989

Matteoni J. A., Sinclair W. A. 1988. Elm yellows and ash yellows. Hiruki C. (ed.). Tree mycoplasmas and mycoplasma diseases, p. 19–31

Mazzoni V., Cosci F., Lucchi A., Santini L. 2001. Leafhoppers and planthoppers vectors in Ligurian and Tuscan vineyards. Integrated Control in Viticulture IOBC wprs Bulletin, 24 (7): 263–266

Nielson M. W. 1968. The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae) taxonomy, biology and virus transmission. USDA Technical Bulletin 1382, Agricultural Research Service, US Department of Agriculture. Washington D.C., USA

Orságová H., Březíková M., Schlesingerová G. 2011. Presence of phytoplasmas in hemipterans in Czech vineyards. Bulletin of Insectology, 64: S119–S120

Ossiannilsson F. 1978. The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. Part 1. Introduction, infraorder Fulgoromorpha. Klampenborg, Denmark

Ossiannilsson F. 1981. The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. Part 2. The Families Cicadidae, Cercopidae, Membracidae, and Cicadellidae (excl. Deltocephalinae). Klampenborg, Denmark

Ossiannilsson F. 1983. The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. Part 3. The Family Cicadellidae. Copenhagen, Denmark

Purcell A. H. 2008. Transmission of *Xylella fastidiosa* bacteria by xylem-feeding insects. Capinera L. J. (ed.). Encyclopaedia of entomology (2nd ed.), p. 3885–3886

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

Riedle-Bauer M., Sára A., Regner F. 2008. Transmission of a stolbur phytoplasma by the Agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae). Journal of Phytopathology, 156: 687–690 http://dx.doi.org/10.1111/j.1439-0434.2008.01416.x

Seemüller E. 1990. Apple proliferation. Jones A. L., Aldwinckle H. S. (eds). Compendium of apple and pear diseases, p. 67–68

Sinclair W. A. 2000. Elm yellows in North America. Dunn C. P. (ed.). The elms breeding, conservation, and disease management, p. 121–136

Söderman G., Gillerfors G., Endrestöl A. 2009. An annotated catalogue of the *Auchenorrhyncha* of Northern Europe (Insecta, Hemiptera: Fulgoromorpha et Cicadomorpha). Cicadina, 10: 33–69

Staniulis J., Davis R. E., Jomantiene R., Kalvelyte A., Dally E. L. 2000. Single and mixed phytoplasma infections in phyllody and dwarf-diseased clover plants in Lithuania. Plant Disease, 84: 1061–1066 http://dx.doi.org/10.1094/PDIS.2000.84.10.1061

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28: 2731–2739 http://dx.doi.org/10.1093/molbev/msr121

Valiunas D., Jomantiene R., Davis R. E. 2009. Establishment of a new phytoplasma subgroup, 16SrI-Q, to accommodate a previously undescribed phytoplasma found in diseased cherry in Lithuania. Journal of Plant Pathology, 91 (1): 71–75

Weintraub P. G., Beanland L. 2006. Insect vectors of phytoplasma. Annual Review of Entomology, 51: 91–111 http://dx.doi.org/10.1146/annurev.ento.51.110104.151039

Weintraub P. G., Wilson R. M. 2010. Control of phytoplasma diseases and vectors. Weintraub P. G., Jones P. (eds). Phytoplasmas: genomes, plant hosts and vectors, p. 233–249

Zhao Y., Wei W., Lee I-M., Shao J., Suo X., Davis R. E. 2009. Construction of an interactive online phytoplasma classification tool, *i*PhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). International Journal of Systematic and Evolutionary Microbiology, 59 (10): 2582–2593 http://dx.doi.org/10.1099/ijs.0.010249-0

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 101, No. 3 (2014), p. 313–320
DOI 10.13080/z-a.2014.101.040

Lietuvoje aptikti vabzdžiai, galimi fitoplazmų '*Candidatus* Phytoplasma asteris' ir '*Ca.* Phytoplasma pruni' gimininių kamienų pernešėjai

A. Ivanauskas[1], D. Valiūnas[1], R. Jomantienė[1], L. Picciau[2], R. E. Davis[3]

[1]Gamtos tyrimų centro Botanikos institutas
[2]Turino universiteto Žemės ūkio, miškininkystės ir maisto fakultetas, Italija
[3]Jungtinių Amerikos Valstijų žemės ūkio departamento Molekulinės augalų patologijos laboratorija

Santrauka

Iki šiol Lietuvoje aptikti fitoplazmų kamienai, priklausantys 16SrI, 16SrIII, 16SrV ir 16SrXII fitoplazmų grupėms, tačiau jas pernešantys vabzdžiai vis dar nenustatyti. Tyrimo metu '*Candidatus* Phytoplasma asteris' bei '*Ca*. Phytoplasma pruni' gimininги fitoplazmų kamienai buvo aptikti penkiose cikadėlėse ir trijose putinėse cikadose. Lietuvoje aptinkama *Anaceratagallia ribauti* kitose Europos šalyse žinoma kaip stolburo vektorius ('*Ca*. Phytoplasma solani', 16SrXII-A pogrupis), todėl yra tikimybė, kad šio pogrupio fitoplazmų yra ir Lietuvoje. Fitoplazmų kamienai, priklausantys 16SrI-C pogrupiui, buvo aptikti *A. ribauti*, *Aphrodes* sp., *Macrosteles sexnotatus* ir *Euscelis incisus*, 16SrI-B – *Aphrophora alni*, *Aphrodes* sp. ir *Cicadella viridis*, 16SrIII-B kamienas – *A. ribauti*, 16SrIII-P – *Aphrodes* sp. ir *E. incisus* vabzdžiuose. Tai patvirtina hipotezę, kad Lietuvoje šie vabzdžiai gali pernešti minėtas fitoplazmas. Šių duomenų pagrindu buvo atlikti baltuosiuose dobiluose 16SrI-C pogrupio fitoplazmų pernešimo *E. incisus* vabzdžiais eksperimentai, kurių rezultatai patvirtino, jog *E. incisus* lietuviškas biotipas juose gali pernešti Lietuvoje aptinkamas 16SrI-C pogrupio fitoplazmas. Putinių cikadų trijose rūšyse: *Aphrophora alni*, *Lepyronia coleopterata* ir *Philaenus spumarius*, aptikta fitoplazmų įvairovė parodė, kad šie ksilemos audinių sultimis mintantys vabzdžiai, besimaitindami ant fitoplazmomis infekuotų augalų, praryja ir floemos ląstelių turinį. Tai leidžia daryti prielaidą, kad kai kurie ksilema mintantys vabzdžiai atsitiktinai galėtų tapti fitoplazmų pernešėjais.

Reikšminiai žodžiai: *Cercopoidae*, *Cicadellidae*, cikadėlės, citoplazma, pernešėjai.