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DIVERSE PHYTOPLASMAS ASSOCIATED WITH DISEASES IN VARIOUS CROPS IN RUSSIA — PATHOGENS AND VECTORS

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Abstract

Over a long-term survey (2006-2014), we detected that at least 22 species of cultivated plants from 10 families (*Amaranthaceae*, *Apiaceae*, *Asteraceae*, *Brassicaceae*, *Cucurbitaceae*, *Fabaceae*, *Vitaceae*, *Poaceae*, *Rosaceae*, *Solanaceae*) were infected with phytoplasma. Most of the plant species are herbaceous plants used as food, commercial, and forage crops, including vegetables (tomato *Solanum lycopersicum*, potato *Solanum tuberosum*, pepper *Capsicum annuum*, carrots *Daucus carota*, horseradish *Armoracia rusticana*), cereals (wheat *Triticum aestivum*, barley *Hordeum vulgare*), legumes (chickpeas *Cicer arietinum*, kidney beans *Phaseolus vulgaris*, beans *Vicia faba*), gourds (gourd *Cucurbita pepo*), oilseeds (rapeseed *Brassica napus*), essential oil (coriander *Coriandrum sativum*, tarragon *Artemisia dracunculus*), fodder (alfalfa *Medicago sativa*) and commercial (sugar beet *Beta vulgaris*) crops. Phytoplasma was also detected in fruit trees and shrubs (pear *Pyrus communis*, cherry *Prunus* subg. *Cerasus*, apple *Malus domestica*, raspberry *Rubus idaeus*, grapes *Vitis vinifera*). Leaves, roots, or fruits of infected plants with characteristic symptoms of phytoplasma infection were collected in six economic regions of the Russian Federation including Western Siberia, Volga, Northern, North Caucasus, Central and Central-Black Soil region. In total, phytoplasmas belonging to eight groups or subgroups, i.e. 16SrI, 16SrI-C, 16SrII, 16SrIII, 16SrVI-A, 16SrVI-C, 16SrX, and 16SrXII-A, were found in infected plants in these six economic regions. Phytoplasma diversity differed in each region. The most diverse phytoplasma populations were found in the Volga and Central regions, which contained six and five phytoplasma groups or subgroups, respectively. Some phytoplasmas caused disease in multiple plant species, while certain crops were infected by multiple phytoplasmas. For example, both 16SrVI-A and 16SrXII-A caused diseases in 12 plant species, 16SrI caused disease in seven species, and 16SrIII caused disease in six ones, while potato was infected by six phytoplasma groups or subgroups. In general, there were 4 phytoplasma groups/subgroups: 16SrIII, 16SrVI 16SrI-A and 16SrXII-A almost equally represented in cultural plants in the Central Economic Region. In more southern areas, such as Volga, Central Black Earth and North-Caucasian, the stolbur subgroup (16SrXII-A) phytoplasma dominated. Phytoplasma subgroup 16SrVI-A occurred predominantly in the Western Siberia Region.

Keywords: *Amaranthaceae*, *Apiaceae*, *Asteraceae*, *Brassicaceae*, *Cucurbitaceae*, *Fabaceae*, *Vitaceae*, *Poaceae*, *Rosaceae*, *Solanaceae*, phytoplasma, phytoplasma diseases, phytoplasma groups 16SrI, 16SrII, 16SrIII, 16SrVI, 16SrX, 16SrXII, economic regions of Russia

In 1926, A.A. Yachevskiy described potato Witch broom [1]. In the end of 1920s, the studies of the disease which caused tomato fruit lignification started in Crimea (stolbur is a distorted Ukrainian word «stovbur», or trunk; it was introduced as a scientific international term stolbur to describe a group of 16SrXII phytoplasmas) [2]. Despite the confirmation of the fact that the disease is transmitted by grafting a diseased plant to a healthy one [3], the disease was associated with abiotic factors for a long time. In 1945, a *Solanaceae* stolbur vector, the hopper *Hyalesthes obsoletus* Sign., was identified [4], which made it possible to

justify the protective recommendations against the disease [5]. A virus was considered as stolbur causal agent for many years, although it was not isolated. As late as in 1967, in Japan, with the electron-microscopic study of phloem tissue sections from the plants with the signs of jaundice, polymorphic particles similar to animal mycoplasmas were discovered, which were similarly named mycoplasma-like organisms [6]. They belong to class *Mollicutes*, the bacteria lacking cell walls. In 1994, to designate the pathogenic mycoplasmas, the term phytoplasma was accepted, and since 2004, the name of *Candidatus* Phytoplasma was used as a genus name [7]. Within the genus, phytoplasma groups are distinguished. Phytoplasmas are attributed taxonomically by the pattern of restriction fragments of the amplified 16S ribosomal RNA gene sequence given the 97.5 % DNA sequence identity within the group [8-11]. To date, phytoplasma diversity includes more than 30 groups with a variety of sub-groups, and the list of them is constantly growing. To characterize strains, other genes encoding ribosomal protein, membrane protein, *secY*, *tuf*, *vmp1*, etc. are analyzed [12-16].

The main crops most affected by phytoplasma diseases in Russia are potato and tomato. We have published the results of the research on potato phytoplasma diseases in Russia earlier, within the scope of the International Scientific and Technical Center project (ISTC, 2006-2012) [17]. However, the range of phytoplasma host plants is unusually wide. Over the past 25 years, the phytoplasma nature has been confirmed using the sequence of gene encoding 16S ribosomal RNA as the marker for a large number of plant diseases, and more than 1000 this gene sequences have been deposited in GenBank [18].

It is noteworthy that such studies have not been performed in Russia until 2006, and the etiology of many prevalent harmful diseases has not yet been established yet.

The purpose of this study was to detect phytoplasma infections in various crops, identify phytoplasma taxonomic attribution using molecular methods and to investigate the association of these pathogens to the six economic regions of Russia, which would make it possible to assess the potential risks in crop cultivation.

Technique. The study was performed in 2006-2014. Leaves, fruits, flowers, and roots of infected plants with characteristic symptoms of phytoplasma infection (redness, yellowing, whitening, wrinkling and curling of leaves, proliferation of shoots, modification of appearance and virescence of flowers, beard and softening of root-crops, modification of fruit appearance) collected in six economic regions of the Russian Federation (Western Siberian, Volga, Northern, North Caucasus, Central, and Central Black Soil regions) were the samples. In addition, phytoplasmas were detected in *Hemiptera* vector insects caught in stationary plots in the Moscow and Samara regions.

Plant samples (0.5 g), frozen at -20°C were triturated in a porcelain mortar with 3 ml of CTAB-buffer. DNA was isolated using a commercial kit DNeasy Plant Mini Kit (Qiagen N.V., Germany) according to manufacturer's instructions. To detect phytoplasmas in two-round (nested) PCR the following pairs of primers were used: P1/16S-SR for the first amplification [19] and R16F2n/R16R2 for the second one [20]. We used 35 PCR cycles under the following conditions: denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes, product synthesis at 72°C for 3 minutes (7 minutes at the final stage). Diluted first PCR amplification product (1.8 kbp) was used as template in the second (nested) PCR. To identify phytoplasmas belonging to a group (subgroup), the products obtained after nested PCR were treated with restriction endonucleases AluI, MseI, HhaI, HpaII, and TaqI (each separately) according to the manufacturer's instructions (Fermentas, Lithuania). DNA fragments obtained by restriction were separated electrophoretically in 5 % polyacrylamide gel. The resulting RFLP-profiles (restriction fragment

length polymorphism) were compared with the published real or virtual restriction maps [9, 11].

Total nucleic acid was extracted from insects individually according to the recommendations [21]. PCR was performed as described above.

Results. Phytoplasmas isolated during the regional survey from various plant species and attributed to group or subgroup using PCR and the restriction fragment length polymorphism analysis are summarized in Table 1 (systematized for economic regions and further for belonging to the group and family).

1. Taxonomic belonging of phytoplasmas identified in crops from various economic regions of Russia (field studies, 2006-2014)

Plant family	Plant species	Phytoplasma groups or subgroups
West Siberian region		
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrIII
<i>Brassicaceae</i>	<i>Brassica napus</i> L. (spring rapeseed)	16SrVI-A
<i>Fabaceae</i>	<i>Cicer arietinum</i> L. (chickpeas)	16SrVI-A
<i>Fabaceae</i>	<i>Medicago sativa</i> L. (alfalfa)	16SrVI-A
<i>Fabaceae</i>	<i>Phaseolus vulgaris</i> L. (kidney beans)	16SrVI-A
<i>Fabaceae</i>	<i>Vicia faba</i> L. (beans)	16SrVI-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrVI-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrVI-C
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A
Volga region		
<i>Poaceae</i>	<i>Hordeum vulgare</i> L. (barley)	16SrI
<i>Poaceae</i>	<i>Triticum aestivum</i> L. (wheat)	16SrI-C
<i>Rosaceae</i>	<i>Prunus</i> subg. <i>Cerasus</i> (Mill.) A. Gray (cherry)	16SrI
<i>Rosaceae</i>	<i>Rubus idaeus</i> L. (raspberry)	16SrIII
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrIII
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrIII
<i>Cucurbitaceae</i>	<i>Cucurbita pepo</i> L. (gourds)	16SrVI-A
<i>Solanaceae</i>	<i>Solanum lycopersicum</i> L. (tomato)	16SrVI-A
<i>Rosaceae</i>	<i>Pyrus communis</i> L. (<i>P. domestica</i> Medik.) (pear)	16SrX
<i>Apiaceae</i>	<i>Armoracia rusticana</i> G. Gaertn., B. Mey. & Scherb (horseradish)	16SrXII-A
<i>Apiaceae</i>	<i>Daucus carota</i> subsp. <i>sativus</i> (Hoffm.) Arcang (carrots)	16SrXII-A
<i>Fabaceae</i>	<i>Medicago sativa</i> L. (alfalfa)	16SrXII-A
<i>Rosaceae</i>	<i>Malus domestica</i> Borkh. (apple)	16SrXII-A
<i>Rosaceae</i>	<i>Prunus</i> subg. <i>Cerasus</i> (Mill.) A. Gray (cherry)	16SrXII-A
<i>Rosaceae</i>	<i>Pyrus communis</i> L. (<i>P. domestica</i> Medik.) (pear)	16SrXII-A
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum lycopersicum</i> L. (tomato)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A
<i>Vitaceae</i>	<i>Vitis vinifera</i> L. (grapes)	16SrXII-A
Northern region		
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrI
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrII
<i>Fabaceae</i>	<i>Medicago sativa</i> L. (alfalfa)	16SrIII
<i>Fabaceae</i>	<i>Vicia faba</i> L. (beans)	16SrIII
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A
North Caucasian region		
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrIII
<i>Apiaceae</i>	<i>Daucus carota</i> subsp. <i>sativus</i> (Hoffm.) Arcang (carrots)	16SrXII-A
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum lycopersicum</i> L. (tomato)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A
Central Black Earth region		
<i>Amaranthaceae</i>	<i>Beta vulgaris</i> L. subsp. <i>vulgaris</i> (sugar beet)	16SrI
<i>Amaranthaceae</i>	<i>Beta vulgaris</i> L. subsp. <i>vulgaris</i> (sugar beet)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A
Central region		
<i>Asteraceae</i>	<i>Artemisia dracunculus</i> L. (tarragon)	16SrI-C
<i>Apiaceae</i>	<i>Coriandrum sativum</i> L. (coriander)	16SrI
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrI
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrI
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrII
<i>Fabaceae</i>	<i>Cicer arietinum</i> L. (chickpeas)	16SrIII
<i>Rosaceae</i>	<i>Fragaria</i> × <i>ananassa</i> (Duchesne ex Weston) Duchesne ex Rozier (Garden strawberry)	16SrIII
<i>Rosaceae</i>	<i>Rubus idaeus</i> L. (raspberry)	16SrIII
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrIII
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrIII

<i>Apiaceae</i>	<i>Daucus carota</i> subsp. <i>sativus</i> (Hoffm.) Arcang (carrots)	16SrVI-A
<i>Asteraceae</i>	<i>Artemisia dracuncululus</i> L. (tarragon)	16SrVI-A
<i>Rosaceae</i>	<i>Rubus idaeus</i> L. (raspberry)	16SrVI-A
<i>Solanaceae</i>	<i>Capsicum annuum</i> L. (pepper)	16SrVI-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrVI-A
<i>Apiaceae</i>	<i>Coriandrum sativum</i> L. (coriander)	16SrXII-A
<i>Solanaceae</i>	<i>Solanum tuberosum</i> L. (potato)	16SrXII-A

In Western Siberia (Novosibirsk region), samples infected by 16SrVI group phytoplasma (subgroup 16SrVI-A named «clover proliferation subgroup») were identified among the species of *Fabaceae*, *Brassicaceae*, and *Solanaceae* families. Phytoplasmas from groups 16SrIII (X-disease), 16SrXII (subgroup 16SrXII-A, the *Solanaceae* stolbur infectious agent), and subgroup 16SrVI-C (found in potatoes for the first time) were also identified in potato plants in this region [17]. That is, potato phytoplasmas were more diverse taxonomically.

Composition of phytoplasmas infected crops in the Volga region was much richer and included 14 species belonging to 7 families. Phytoplasmas isolated were more diverse taxonomically, including members of five groups, among which stolbur prevailed (subgroup 16SrXII-A phytoplasma). Stolbur phytoplasma was found in 10 crop species. In 2008, stolbur was identified in 18 tomato samples from the Astrakhan region. Along with grapes, *Solanaceae* (potato, tomato, pepper), alfalfa, horseradish and carrots, fruit trees (apple, cherry, and pear) were infected by stolbur in the Samara region. Another phytoplasma from 16SrX group was found in pears. This pathogen causes a disease of pear drying. Group 16SrIII phytoplasma infected some potato and pepper plants, and the same phytoplasma was detected in raspberry. Barley, wheat, and cherry were infected by group 16SrI phytoplasma.

The samples from the North Caucasus economic region included tomatoes and peppers from the Rostov region, potatoes and tomatoes from the Krasnodar and Stavropol regions, and carrot from the Rostov region. All samples were infected with stolbur (subgroup 16SrXII-A phytoplasma), with an exception of potato where the samples infected by group 16SrIII phytoplasma were found along with the ones infected by stolbur.

Potato samples from the Tambov and Lipetsk regions of the Central Black Earth area were infected by 16SrXII-A phytoplasma, sugar beet samples from the Voronezh and Belgorod regions had the symptoms of a disease of unknown etiology. Stolbur group phytoplasma infected all sugar beet samples from the Voronezh region and a part of samples from the Belgorod Region analyzed (Fig., h), another Belgorod samples contained phytoplasma 16SrI.

Despite the small number and species monotony of the Northern Economic Region samples tested (potato and 2 legume species), phytoplasmas from four different groups were isolated, of which three groups (16SrI, a very rare in the Russian group 16SrII, and subgroup 16SrXII-A) were found in potato. Legumes were infected by 16SrIII group phytoplasma.

In the Central economic region, there was no such species diversity of the crops infected by phytoplasmas as in the Volga region, but the phytoplasmas isolated were not less diverse. The symptoms of infected plants are shown in the Figure (see a-g). Phytoplasmas from 16SrI, 16SrIII and 16SrVI groups were detected in the area at about the same rate, while the stolbur group phytoplasma was less frequent. Like in the Northern economic region, 16SrII phytoplasma was detected just once in 2009 in two potato samples

The variety and predominance of these or other phytoplasma groups is determined by the diversity and predominance of vectors, which is enhanced by the conditions favorable to their habitat. Thus, in 1974, the northern border of

Solanaceous stolbur ran approximately along the 52nd parallel, that is south of the Samara region, which is probably associated with the brown planthopper habitat [22]. Subsequently, another stolbur vector species *Pentastiridius leporinus* L. which also belonged to family *Cixiidae* was identified in the central area of the Samara region [23].

Plants from the Central region of Russia infected by phytoplasmas from various taxonomic groups and subgroups: a — *Capsicum annuum* (pepper, fruit), b — pepper (leaves), c — *Fragaria ananassa* (garden strawberry), d — *Cicer arietinum* (chickpeas), e — *Vicia faba* (kidney beans) (a-e — group 16SrIII); f — *Artemisia dracuncululus* (tarragon) (on the left — healthy plant, on the right — infected plant), g — *Solanum tuberosum* (potato) (subgroup 16SrI-C); h — *Beta vulgaris* (sugar beet, leaves) (stolbur group 16SrXII) (2006-2012).

2. Presence and identification of phytoplasmas detected in *Hemiptera* insects (Samara region, 2011-2012)

Cicada species	Family	Insects			Phytoplasma groups or subgroups
		caught	checked	infected	
2011					
<i>Dictyophara europaea</i> L.	<i>Dictyopharidae</i>	12	12	2	16SrIX
<i>Empoasca pteridis</i> Dahlbon	<i>Cicadellidae</i>	82	20	0	
<i>Euscelis obsoletus</i> Kbm.	<i>Cicadellidae</i>	3	3	0	
<i>Hyalesthes obsoletus</i> Sign.	<i>Cixiidae</i>	17	8	5	16SrXII-A
<i>Pentastiridius leporinus</i> L.	<i>Cixiidae</i>	25	11	3	16SrXII-A
<i>Psammotettix striatus</i> L.	<i>Cicadellidae</i>	22	22	3	16SrIII
Total		161	76	13 (17.1 %)	
2012					
<i>Dictyophara europaea</i> L.	<i>Dictyopharidae</i>	8	4	1	Not identified
<i>Empoasca pteridis</i> Dahlbon	<i>Cicadellidae</i>	17	15	0	
<i>Euscelis obsoletus</i> Kbm.	<i>Cicadellidae</i>	11	8	0	
<i>Hyalesthes obsoletus</i> Sign.	<i>Cixiidae</i>	11	3	1	16SrXII-A
<i>Kelisia guttula</i> Germ.	<i>Delphacidae</i>	43	2	0	
<i>Laodelphax striatella</i> Fallen	<i>Delphacidae</i>	5	2	0	
<i>Macrosteles laevis</i> Rib.	<i>Cicadellidae</i>	10	7	1	Not identified
<i>Pentastiridius leporinus</i> L.	<i>Cixiidae</i>	2	2	0	
<i>Psammotettix striatus</i> L.	<i>Cicadellidae</i>	113	5	2	Not identified
Total		220	48	5 (10.4 %)	

Hemiptera insects, potential phytoplasma vectors, were caught in the Samara region in 2011 and 2012, and identified (Table 2). Of possible studied vectors, 17.1 % in 2011 and 10.4 % in 2012 were viroforous, with the only phytoplasma found in planthopper *H. obsoletus* Sign. (subgroup 16SrXII-A) identified of

those five isolated. Group 16SrIX phytoplasma was found in European lantern fly *Dictyophara europaea* in 2011, which requires clarification, as we have not been found the plants infected with this phytoplasma. For other cicadas, the amounts of PCR products were insufficient for RFLP-analysis. In general, half of phytoplasmas identified in insects within 2 years belonged to stolbur group, six of them were from *H. obsoletus* Sign., and three ones were found in *Pentastiridius leporinus* L. (both species belong to family Cixiidae).

The proportion of viroforous insects in the Moscow region was lower than in the Samara region: 5.5 % in 2011 and 1.8 % in 2012 with larger samples (128 and 276 specimens) and considerable species differences [17, 24].

Until 2010, stolbur infection in potato and tomato in the Samara region exceed 20-30 % rarely [23] and was due to the spread of root planthopper *Pentastiridius leporinus* L. After the very drought year of 2010, brown planthopper had favorable conditions for its habitat in the agrocenoses of the central zone of the Samara region; and now brown planthopper is regularly detected (due to warming, larvae are likely to successfully hibernate). In the period from 2011 to 2014, Solanaceae stolbur epiphytoticities were observed annually, and traditional mass potato planting by individuals have been discontinued in many parts of the Samara region.

Along with potato and tomato phytoplasmas which cause the greatest concern in Russia, there are threats of other crops damage. Thus, a problem of carrot damage emerged in the Central, Volga and North Caucasus economic regions. In carrot variety Kaskad samples with the symptoms of red leaf and root softness delivered from the Rostov region in 2007, the stolbur group phytoplasmas (16SrXII) have been detected. In 2009, 16SrVI-A subgroup phytoplasma was found in carrot from the Moscow region in the plants with leaf twisting, redness and yellowing, bearded fruit. Stolbur group phytoplasma was also identified in carrot plants with chlorotic edge-colored leaves and fibrous roots in the Volga economic region (Samara Province).

In the USA, there are two types of Umbelliferae (carrot in particular) phytoplasma diseases caused by genetically various phytoplasmas. The 16SrI group pathogen is most typical, and 16SrVI-A subgroup phytoplasma is less common. Phytoplasma from group 16SrI prevailed in carrot in Serbia, but subgroup 16SrXII-A phytoplasma has also been found [27]. In the Russian areas of sugar beet cultivation, phytoplasma diseases are also found, often referred to as the diseases of unknown etiology. No less dangerous fruit tree and grape phytoplasma widespread in European countries [28, 29] have emerged in Russia. A dangerous Bois noir (wood blackening) disease is caused by *Candidatus Phytoplasma solani* (stolbur subgroup 16SrXII-A) in grapes. Interestingly, in the Crimea, where for the first time in the former USSR stolbur was found in tomatoes, the disease caused by the same pathogen has not been reported in grapes until 2012, but since 2012, it detected every year in the areas planted in 2005-2011 with material imported from different European countries [30]. The latter circumstance provides not only for the need to monitor phytoplasma diseases using molecular techniques for the early detection of phytoplasma groups and subgroups not found in the Russian Federation previously, but also indicates the desirability of their biotyping using gene analysis (including 16S rRNA genes).

Thus, phytoplasmas were found in 22 crop species from 10 botanical families (cereals, grain legumes, oilseeds and fodder, vegetables, herb, root, technical, fruit, berry crops) in six economic regions of Russia. Phytoplasmas found in the plants belonged to six taxonomic groups: 16SrI, 16SrII, 16SrIII, 16SrVI, 16SrX, and 16SrXII. Phytoplasma diversity in the Northern and Central economic regions was roughly similar: there were groups 16SrI, 16SrII, 16SrIII,

and 16SrXII, and in the Central region there was group 16SrVI as well. In Western Siberia, subgroup 16SrVI-A phytoplasma from group 16SrVI found in other Russian regions, was predominant, but subgroup 16SrVI-C phytoplasma has been reported as well. In the Voronezh, Belgorod, Samara, Rostov regions and Krasnodar Krai, 16SrXII phytoplasma prevailed, but phytoplasmas of groups 16SrI and 16SrIII have also been found. Phytoplasma 16SrX was found in pear in the Samara region. In the examination of *Hemiptera* insects five species of infection vectors were identified in the Samara region in 2011-2012: *Dictyophara europaea*, *Hyalesthes obsoletus*, *Macrosteles laevis*, *Pentastiridius leporinus*, and *Psammotettix striatus*. Leafhopper *Psammotettix striatus* was infected with phytoplasma of group 16SrIII, planthoppers *Hyalesthes obsoletus* and *Pentastiridius leporinus* were the phytoplasma 16SrXII-A (stolbur group) vectors. Attribution of phytoplasma found in European lantern fly *Dictyophara europaea* to group 16SrIX requires clarification as we have not found this phytoplasma in the plants.

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