Danger of phytoplasma diseases for fodder crop cultivation

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Abstract. In a number of Russian regions phytoplasma diseases can be of great importance in the cultivation of forage crops. Nevertheless, phytoplasma diseases are still often considered as a result of the action of abiotic or other factors. The aim of the research was to monitor the distribution of different groups of phytoplasmas on cultivated and wild-growing species of promising forage plants in different regions of Russia. The material for the research was freshly cut or dried parts of cultivated and wild plants with symptoms of phytoplasma infection, collected in different regions of Russia. To detect and identify phytoplasmas, PCR and RFLP analysis were used. Seven genetic groups of phytoplasmas: 16SrI, 16SrII, 16SrIII, 16SrVI, 16SrXII, 16SrXIV and 16SrX were identified in 47 species of plants from 18 families, including 24 (51%) species of cultivated and 23 wild plant species collected in 17 administrative units of the Russian Federation. The greatest harm phytoplasma diseases cause on wheat and barley cultivated for grain fodder, Russian bromegrass, alfalfa, clover and other legumes, sugar and fodder beets, potatoes, as well as fruits used for animal feed. The harmfulness of phytoplasma diseases increases in areas to the south of the Middle Volga region and in dry years. This should be taken into account when developing regional recommendations for the cultivation of forage crops.

1. Introduction

The cultivation of forage crops not only solves the problem of the fodder base of animal husbandry, but, enriching agrobiogeocenoses with the species diversity of cultivated plants, contributes to the stability of crop production, soil reclamation, increasing of crop production, optimizing the phytosanitary state of crops in the conditions of the prevalence of cereals, the introducing abandoned arable land into circulation and other. The gross volumes of fodder cultivation: beets, fodder wheat, grasses and other plant feeds for farm animals are always several times higher than the output of natural food products and raw materials for food processing enterprises. The commercial productivity of livestock breeding is determined by the quality of feed by 45-65%, which implies an expansion of the sowing of forage crops, an increase in their seed industry and the quality of products. In addition to fodder corn, fodder grains and grasses, table varieties of many vegetable and fruit crops are also used for animal feed. Fodder and sugar beets, rutabagas, carrots, fodder turnips, potatoes, Jerusalem artichoke, as well as fruit and pumpkin fruits are used as feed for many types of farm animals, and there are many natural types of grasses on pastures.

One of the problems of fodder production is the susceptibility of cultivated crops to diseases and pests, which reduce seed productivity and the quality of fodder. In a number of regions, phytoplasma diseases are the most dangerous, combining a pathogen (phytoplasma) and an insect carrier
(leafhoppers, psyllids, bugs) in a pathological process, which complicates their control. Phytoplasma diseases not only reduces the productivity of the vegetative mass of plants, which is expressed in dwarfism, witches’-broom and decline, but also reduces seed productivity caused by the sterility of reproductive organs due to flower modifications: phyllody, virescence and proliferation. In the conditions of rainfed steppe agriculture, phytoplasma diseases often lead to epidemics, in this connection there is a need to reduce the time use of perennial crops, to search for and introduce alternative types of forage crops, which weakly affected by phytoplasma.

The aim of the research is to determine the species composition of cultivated and wild-growing forage plants affected by phytoplasmas, to determine the group affiliation of phytoplasmas in some regions of the Russian Federation in order to clarify regional recommendations for the cultivation of forage crops, to characterize the symptoms of phytoplasmic infection of plant species.

2. Methods
Total DNA was extracted from 0.3-0.5 g of previously finely cut and frozen leaf veins, using the method described by Maixner et al. [1], with minor modifications or Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA) as manufacture recommended [2]. Phytoplasma DNA was detected by nested PCR with a pair of primers P1 / 16S-SR in the first amplification and R16F2n / R16R2 in the second [3, 4]. The resulting products were digested with restriction endonucleases, DNA fragments were separated by electrophoresis, and RFLP profiles were used to identify a group (subgroup) of phytoplasmas [5, 6].

3. Results and Discussion
For 14 years of monitoring of phytoplasma diseases carried out at VNIIF, phytoplasmas of 10 groups were detected in 122 plant species from 36 families. Some of these species: cereals, root crops, fruits and grasses are important components of the feed base of animal husbandry, both in stalls and grazing animals in various forms of farms. Table 1 shows 47 plant species from 19 families in which phytoplasmas belonging to 7 taxonomic groups were identified. Plant samples were collected in 14 provinces: Arkhangelsk, Astrakhan, Belgorod, Volgograd, Vologda, Voronezh, Kaluga, Leningrad, Moscow, Novosibirsk, Orenburg, Rostov, Samara, Tambov, as well as in the Republic of Tatarstan, the Krasnodar and Stavropol Territories. Of these, 23 wild species from 14 families can be considered promising for introduction into perennial poly-species crops of forage grasses and tinning of artificial pastures.

Table 1. Phytoplasma in plant species cultivated or promising for forage production.

| Plant species                          | Family      | Taxonomic group / subgroup |
|---------------------------------------|-------------|-----------------------------|
| **Grain fodder crops**                |             |                             |
| *Cicer arietinum* L. (chickpea)       | Fabaceae    | 16SrVI-A                    |
| *Hordeum vulgare* L. (spring barley) | Poaceae     | 16Srl                       |
| *Triticum aestivum* L. (winter wheat)| Poaceae     | 16SrI-C                     |
| *Vicia faba* L. (broad bean)         | Fabaceae    | 16SrIII-A, 16SrVI-A         |
| **Perennial herbs**                   |             |                             |
| *Alopecurus pratensis* L. (meadow foxtail)| Poaceae   | 16SrI                       |
| *Bromus inermis* Leyss. (Russian bromegrass)| Poaceae | 16SrXII-A, 16SrI            |
| *Lolium perenne* L. (perennial ryegrass )| Poaceae   | 16SrI                       |
| *Poa pratensis* L. (meadow bluegrass)| Poaceae     | 16SrXIV, 16SrI              |
| **Perennial legumes**                 |             |                             |
| *Lupinus polyphyllus* Lindl. (large-leaved lupine)| Fabaceae | 16SrI-C, 16SrIII            |
| *Medicago sativa* L. (alfalfa sowing) | Fabaceae    | 16SrIII-A, 16SrVI-A, 16SrXII-A|
| *Melilotus albus* Medik. (white sweet clover) | Fabaceae | 16SrI-C, 16SrIII-B, 16SrVI-A, 16SrXII-A|
It was shown that in China, wheat (Triticum aestivum L.) and other cereals 
(including durum wheat, T. durum Desf. var. durum) increases the prevalence of the disease, as well as the vicinity of fields of perennial grasses and winter leafhopper (Psammotettix striatus L.). Early sowing of winter wheat and late sowing of spring cereals increases the prevalence of the disease, as well as the vicinity of fields of perennial grasses and winter cereals [7]. It was shown that in China, wheat (Triticum aestivum L.) was infected with phytoplasm
of subgroup 16SrI-S, in India with phytoplasma of subgroup 16SrXI-B, and in Serbia with phytoplasma of subgroup 16SrXII-A. Phytoplasmas of the subgroup 16SrI-A, 16SrI-B and 16SrI-L were found on oats (*Avena sativa* L.) in Lithuania; subgroups 16SrI-B in barley (*Hordeum vulgare* L.) in Canada, the same phytoplasma subgroup was detected in corn (*Zea mays* L.) in Peru, Mexico, Nicaragua, Costa Rica, Belize, Brazil, Colombia and India, while phytoplasmas of subgroup 16SrI-L in Poland and of subgroup 16SrXII-A in Serbia were observed [8].

3.2. Broad bean and chickpea
Symptoms: chlorosis, anthocyanin, small curled leaves and lack of beans. In the West Siberian region of the Russian Federation, these cultures were affected by phytoplasma of the 16SrVI group/16SrVI-A subgroup. In the Central region, phytoplasma of the 16SrIII group was found in horse beans. In Western Siberia, the harmfulness was 80-100%, which led to an almost complete loss of seed productivity [9]. Phytoplasma of the 16SrI group was isolated from beans in Cuba, while phytoplasma of the 16SrII group was detected in Sudan, Saudi Arabia and Iran and the 16SrIII group in Spain. Phytoplasma of the 16SrII group has been isolated from chickpeas in Sudan, Oman, Pakistan, India and Australia [10].

3.3. Alfalfa
The most harmful is the alfalfa witch's broom, widespread from the latitude of Tatarstan to the southern regions of the European Russia, as well as in Asian part of Russian Federation. Plants become dwarf with many thin shoots and small leaves, and do not form generative organs. The harmfulness and prevalence of the disease increases by 10-20% every year of the existence of the herbage. The mass of roots decreases, nodules with nitrogen-fixing bacteria are absent. Seed plots of 1-2 years of use have low yields, puny seeds have low germination, old-growth plots do not form seeds at all. Forage crops mainly yield one mowing. There are varieties that are moderately resistant to phytoplasmas: Tulunskaya hybrid, Flora, Omskaya, etc. [9]. The alfalfa witch's broom has been reported in Asia, Europe, America and Australia and is associated with genetically diverse phytoplasmas, including such groups/subgroups as 16SrI, 16SrII-C and 16SrII-D, 16SrIII-B, 16SrV-B, 16SrVI, 16SrVII-C and 16SrXII-A [11].

3.4. Clover species
Symptoms of infection: dwarfism, edge and interveinal yellowing and redness, chlorosis, small leaves and inflorescences, phylloidy, virescence of flowers, sterility. The harmfulness is very high, it increases by the 2-3rd years of use. Losses of green mass yield up to 80-90%, seeds yield up to 100%. The protein content decreases and the nutritional value of the forage is deteriorated. Phytoplasmas found on leguminous herbs in Russia belonged to four taxonomic subgroups: 16SrI-C, 16SrIII-B, 16SrVI-A, 16SrXII-A. The name of the diseases that cause the first three of them is directly related to clover: clover phyllody (CPh), clover edge yellowing (CEY) and clover proliferation (CP). The CPh (16SrI-C phytoplasma) and CYE (16SrIII-B phytoplasma) diseases prevailed mainly in the Northern and Central Economical Regions, while the CP disease (16SrVI-A phytoplasma) prevailed mainly in the West Siberian Economical Region [12].

By our data, five species of the family Cicadellidae (Aphrodes bicinctus, Cicadula quadridotata, Empoasca pteridis, Euscelis incisus and Sonronius binotatus) and one of the Aphrophoridae family (Philaenus spumarius) that inhabited on leguminous were carriers of phytoplasmas. There are resistant and tolerant varieties (tetraploid and early maturing): Meteor, memory Lisitsyn, etc. [9]. Phytoplasmas of the aster yellows group / the clover phyllody subgroup 16SrI-C were found in Canada, Germany and China. Phytoplasmas of 16SrI-C and 16SrIII-B subgroups was detected in Lithuania and Italy. Phytoplasma of the clover proliferation group 16SrVI-A was found in clover in Canada, and the 16SrXII-B subgroup in Australia [10].
3.5. *Russian bromegrass*
Symptoms: the plant is undersized with chlorotic or reddish leaves, the panicle is reduced or absent. As a result of long-term use of fodder crops, the number of infected plants increases and the productivity of vegetative mass and seeds decreases. Often, after summer mowing, bald spots with no grass stand are formed in the fields, which partly grow only in autumn with a decrease in temperature and an increase in precipitation. Harmfulness exceeds 50%, increases with increasing years of crop use.

3.6. *Perennial ryegrass* and *meadow foxtail*
Symptoms: stunting, chlorosis of the leaves and increased bushiness, absence or underdevelopment of ears, decreased forage and seed productivity.

3.7. *Bluegrass*
Symptoms: systemic chlorosis, proliferation of secondary shoots, bushiness, small leaves and their discoloration, shortening of rhizomes and stolons, dwarfism, inflorescence necrosis, plant death. In the early stages of the disease, light green and yellow stripes may be seen on the leaves. In the Samara region, signs of the disease occur in spring and autumn and are expressed in the withering away or drying out of diseased plants with the formation of bald spots in the grass stand. The prevalence of the disease in the Samara region was 5-30%.

Other perennial grasses, growing on pastures in conditions of species diversity, although they are affected by phytoplasmas with similar symptoms, nevertheless are not as widespread as in monoculture, or have a focal character.

In Lithuania, phytoplasmas of several subgroups of the aster yellows group were found in different species of forage grasses: for example, *Bromus inermis* Leyss was infected with phytoplasma of the 16SrI-B subgroup, *Lolium multiflorum* L. with phytoplasma of the subgroup 16SrI-L, and *Poa pratensis* L. and *Festuca arundinacea* (Arizona fescue) – by phytoplasma of subgroup 16SrI-C [13]. In Poland, phytoplasma of subgroup 16SrI-B, was detected in grasses that are a frequent component of pasture grasses: *Elymus repens* (L.) Gould, *Lolium perenne* L., *Echinochloa crus-galli* (L.) Beauv. (cocksbur grass), *Polygonum aviculare* L. (birdweed), *Viola arvensis* Murray. (field pansy), *Matricaria perforata* Mérat (scentless false mayweed) and *Stelaria media* (chickweed) [14]. In India, among forage crops and herbs infected with phytoplasmas were also species of plants known in Russia. The phytoplasma of the 16SrI group was detected in *Zea mays* L., *Cucurbita pepo* L. and *Solanum tuberosum* L., the phytoplasma of the 16SrII group was contained in the plants *Beta vulgaris* L., *Daucus carota* L., *Cicer arietinum* L. and *Amaranthus* spp.

3.8. *Potatoes*
Symptoms are characterized by small upper leaves, chlorosis or redness, and curling of the lower leaves. Airy sessile micro-tubers can form in the leaf axils and at the base of the shoots. Underground tubers are small, often numerous, flabby, ugly, can give threadlike sprouts or form ‘beads’ of microtubers connected by stolons. In some types of phytoplasma infections, germination of young tubers without a dormant period can be observed. Harmfulness is maximum and reaches 20-100%, when cultivated without irrigation in drought conditions, when early colonized by vectors, or infection transmitted by tubers. Phytoplasmas belonging to 9 groups – 16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, 16SrVIII, 16SrXII, 16SrXIII and 16SrXVIII – have been found on potatoes all over the world. In European countries (Belgium, Greece, Serbia, Montenegro and Romania), phytoplasma of the 16SrXII-A (‘Ca. P. solani’) subgroup of columnar is recognized as the most harmful and predominant on potatoes. The following cixiid species are known as its insect vectors: *Hyalesthes obsoletus*, *Pentastiridius leporinus*, *Reptalus panzeriand*, and *R. quinquecostatus* [16].
3.9. Root crops
Symptoms: beets are characterized by small, corrugated leaves, reduced size, and sometimes ugliness of roots; on carrots, a decrease in the size of leaves and root crops, chlorosis and reddening of leaves, excessive formation of secondary root hairs and enlarged lentils on the roots, sometimes ugliness and softening of the root crop. Harmfulness: 20-80% and even greater decrease in marketable yield.

Phytoplasmas of the following groups were found in beets: 16SrI (Hungary), 16SrII (India, Iran, Saudi Arabia), 16SrIII (Chile), 16SrXII (France). In France, the cixiida Pentastiridius leporinus L is the vector of beet disease, which also carries γ-3 proteobacteria. In some years, beet phytoplasma disease was widespread and harmful. So, in Hungary (1999, 2000) phytoplasma of aster yellows was detected in 60% of the examined plants. In France, beet yellows had epidemics from 1991 to 1992 and from 1996 to 1998, resulting in a loss of sugar content with serious economic consequences. In 1992, the decrease in income was more than 50% from an area of 1000 hectares. In Chile, the phytoplasma wilt of beets led to a loss of 100% of the yield [17].

The following phytoplasma groups / subgroups were found on carrots: 16SrI-B / rplI-B and 16SrI-A / rpl-A (USA), 16SrI-A (Canada, Israel), 16SrI-A / rplI-A and 16SrI-B / rpl-B (Serbia), subgroup 16SrI-A in mixed infection with Spiroplasma citri Saglio (Spain), 16SrI-B (Great Britain), 16SrI-A (Finland), 16SrI-A (Lithuania), 16SrI-B / rplI-L (Iran), 16SrIII (Israel, Japan), 16SrV (Israel), 16SrVI-A (USA), 16SrIX (Italy) and 16SrXII-A (Serbia, Spain, Hungary, Italy). In Serbia, leafhoppers Macrosteles quadripunctulatus Kirschbaum and M. sexnotatus Fallén are considered to be potential vector of phytoplasma 16SrI-A and 16SrI-B, and M. laevis Ribaut is considered to be vector of the phytoplasma of stolbur 16SrXII-A [16].

3.10. Fruit trees
Similar symptoms are typical for apple-tree and pear tree: chlorotic or reddened, twisted leaves, a decrease in the size of fruits, which often do not reach maturity and have low consumer qualities. The apple tree is characterised a proliferation of shoots, an increase in the size of stipules and peduncles. Decline and dieback of shoots is characteristic of pears. Trees infected with phytoplasma resume shoot growth in autumn and show secondary and autumn flowering, which reduces their winter hardiness. The harmfulness exceeds 50%.

Phytoplasma diseases of the apple tree are widespread in most European countries and in Turkey. The harmfulness of the disease, depending on the variety, rootstock, environmental conditions and technology, ranges from 50% to 80%. The carriers of the 16SrX-A apple proliferation phytoplasm are psyllids: Cacopsylla melanoneura Förster (Italy) and C. picta Förster (Germany).

Phytoplasma disease "pear decline" was observed in many European countries, as well as in the USA, Iran, Turkey and Chile. In the 1960s, about two million pear trees died in California (USA). The carriers of the 16SrX-C phytoplasma are pear psyllids: Cacopsylla pyri Linne, C. pyricola Förster, and C. pyrisuga Förster. The disease can lead to the dieback of the tree in a year after infection.

In addition, nonspecific phytoplasmas of subgroups 16SrI-B, 16SrI-C, and 16SrXII-A were found in apple trees in the UK and Czech Republic, and of subgroups 16SrI-B, 16SrXII-A, and 16SrX-A in pears in Croatia and Hungary [18].

4. Conclusions
Thus, in some regions of Russia, as in other regions of the world, phytoplastic diseases of fodder plants are widespread and highly harmful, that determines their economic importance in the production of fodder. For 14 years of monitoring carried out at VNIIF, phytoplasmas of 7 groups (16SrI, 16SrII, 16SrIII, 16SrVI, 16SrXII, 16SrXIV, 16SrX) were detected in 47 plant species from 18 families collected in 14 provinces, as well as in the Republic Tatarstan, the Krasnodar and Stavropol regions. Some of these species, representing cereals, root crops, fruits and grasses, are important components of the feed base of animal husbandry, both in stalls and grazing animals in various forms of farms. Of these, 23 wild species from 14 families can be considered promising for introduction into perennial poly-species crops of forage grasses and tinning of artificial pastures. Phytoplasma diseases
of wheat and barley grown for forage, Russian bromegrass, alfalfa, clovers and other legumes, sugar and fodder beets, potatoes, as well as fruits used for animal feed, in the years of epidemics can cause a decrease in their quality and yield by 20-100%. The harmfulness of phytoplasm disease increases in the regions located south of the Middle Volga region, especially in dry years. This should be taken into consideration when developing regional recommendations for the cultivation of forage crops. To prevent harm from phytoplasm diseases, it is required to carry out a phytosanitary assessment of their spread, paying attention to regional seasonal conditions, using molecular genetic methods of analysis. Research and application of poly-species crops of forage grasses, and determination of the economically correct temporal period of their usage is promising.

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