

Viruses in natural populations of wild hop in the south of Western Siberia

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Wild hops are abundant in the southern regions of Western Siberia, Russia, where the natural conditions are favorable for growing commercially valuable varieties. The genetic diversity present in wild hop populations serves as a valuable source of beneficial genes for developing new genotypes. However, before harnessing these traits, it's crucial to test the wild hops for the presence of harmful phytopathogenic viruses, which tend to accumulate in natural repositories. This study aimed to evaluate the prevalence of viral and viroid infections in wild hop populations in the Altai Territory and the Altai Republic. Plant material from wild hops (*Humulus lupulus* L. 1753) was collected during expeditions from July 15 to August 15 in both 2022 and 2023, covering 18 districts. Using DAS-ELISA and real-time RT-PCR, we assessed 243 samples for the presence of 16 viruses and 1 viroid. The frequency of infected plants varied based on the sampling location and the specific pathogen. Notably, three particularly damaging hop viruses – *Hop latent virus* (HpLV), *Apple mosaic virus* (ApMV), and *Arabidopsis mosaic virus* (ArMV) – were identified, infecting 24.5%, 8.2%, and 5.5% of the studied plants, respectively. HpLV was found throughout the region, primarily in the moderate-arid steppe and forest-steppe, while ApMV was detected in three adjacent districts with a frequency ranging from 11.2% to 45.5%. ArMV was found in half of the plants in a single location. Additionally, minor viruses such as *Strawberry latent ringspot* (SLRSV), *Tobacco necrosis virus* (TNV), and *Tobacco ringspot virus* (TRSV) appeared sporadically in different zones. Potato mosaic viruses S and Y, as well as *Potato leafroll virus* (PLRV), were widely distributed in wild hop populations, with high frequencies in all natural zones except the foothills. *Potato virus M* (PVM), *Potato virus A* (PVA), and *Potato virus X* (PVX) were common in the steppe and forest-steppe zones. It's important to note that the presence of viral infection in plants did not necessarily correlate with the expression of disease symptoms. Notably, *Hop mosaic virus* (HpMV), *Petunia asteroid mosaic virus* (PetAMV), *Prunus necrotic ringspot virus* (PNRSV), and *Potato spindle tuber viroid* (PSTVd) were not detected. The existence of natural reservoirs of viral infection poses a threat to commercial hop cultivars in areas where they coexist. As such, it is imperative to monitor and control the spread of these dangerous viruses.

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Keywords

DAS-ELISA, genetic resources, *Humulus lupulus* L., real-time RT-PCR, virus detection, wild hop

Introduction

According to modern concepts, the genus *Humulus* L. 1753 belongs to the family Cannabaceae and includes 3 species (*H. lupulus* L. 1753, *H. japonicus* Sieb. Zucc. 1846 and *H. yunnanensis* Hu 1936). The origin of hop remains a subject of debate to this day. N.I. Vavilov (1992) attributed hop to the Mediterranean origin of cultivated plants, the range of which spread to northern Europe, where it was introduced into cultivation. However, the prevalence of three species of the genus in China suggested that hop originated in Asia. Then it spread west to Europe and east to North America (Small 1978; Murakami et al. 2006b). In addition, the hypothesis of the ancient Laurasian origin of hop in the New World with subsequent migration to the Old World, where it diverged into two species, is discussed (Boutain 2014). Determining the origin of wild hop is of great importance in terms of its use as a genetic resource for the improvement of commercial cultivars. The centers of origin of species tend to be characterized by the greatest genetic diversity (Steele 2011).

Common hop is the most widespread and economically important. The wild hop *H. lupulus* grows along the banks of rivers and creeks, in damp broad-leaved forests, in shrub thickets, as well as around clearings and along roadsides in the temperate zone of the Northern Hemisphere. Based on the analysis of intraspecific variability of the species, 5 varieties were identified. *H. lupulus* var. *lupulus* E. Small 1978 lives in Europe, *H. lupulus* var. *cordifolius* (Mig.) Maxim. 1879 grows predominantly in Japan, and *H. lupulus* var. *neomexicanus* A. Nels. et Cockerell 1903, var. *pubescens* E. Small 1978 and var. *lupuloides* E. Small 1978 comprise North American wild hops (Small 1978). The varieties are characterized by a number of distinctive morphological characteristics relating to the structure of the leaf blade and the number of hairs on the shoots. European wild hops differ significantly from other subspecies in the morphological features of the structure of the leaves, which are most often 3–5 lobed and only at the tops of the shoots the leaves are whole, heart-shaped. Medium-sized cones with a high content of lupulin are formed on the upper part of the shoots. North American and Japanese wild hops have similar morphologies, suggesting they are genetically closely related (Zanoli and Zavatti 2008).

Early attempts to create a hierarchical model of the relationships between these varieties did not produce clear results. However, they made it possible to separate North American taxa from Asian and European varieties (Small 1978), which was confirmed by subsequent molecular genetic studies (Murakami et al. 2006b). At the same time, samples of Russian hops from the Caucasus and Altai regions were separated from the European cluster (Murakami et al. 2006a). Studies of wild hop populations in various regions of the world using SSR, RAPD, AFLP and ISSR markers have confirmed previously identified genetic diversity (Mafakheri et al. 2020; Dabbous-Wach et al. 2021; Calvi et al. 2023) with its morphological and biochemical diversity (Patzak et al. 2010; Riccioni et al. 2021; Paguet et al. 2023).

Cultivation of *H. lupulus* over a long period of time has resulted in the emergence of a large number of commercial cultivars. However, breeders often have to work within narrow genetic boundaries. The situation is due to the fact that producers of successful beer brands, in an effort to

preserve valuable recipes, introduce new cultivars that are functionally or organoleptically similar to previous genotypes. As a result, the same alleles migrate from one cultivar to another, which leads to depletion of the gene pool. In addition, the clonal selection method used in hop breeding also contributes to the reduction in cultivar diversity. For example, hop cultivars from Europe present less genetic diversity compared to wild forms from North America (Bassil et al. 2008). The high level of polymorphism of numerous wild hop loci creates the prerequisites for the active involvement of wild germplasm in breeding programs. This will expand the limited genetic base of modern cultivars (Jakse et al. 2004; Murakami et al. 2006b; Patzak et al. 2010; McCallum et al. 2019; Rodolfi et al. 2022).

Wild hops are widespread throughout the Altai Territory and the Altai Republic of the Russian Federation, the natural and climatic conditions of which are suitable for its growth. The proximity of these areas to China, the likely center of origin of the species, suggests the potential value of the region's populations. However, along with the obvious benefits, such populations pose a certain threat to industrial hop plantations as natural reservoirs of pathogens, including viruses and viroids. Viral and viroid infections affecting hops occur throughout the world in hop growing areas, creating obstacles to successful cultivation of the crop (Davis et al. 2021; Patzak et al. 2021). The spread of viruses in wild hop populations in the south of Western Siberia, Russia, has not previously been assessed.

The most dangerous hop viruses include three representatives of the genus *Carlavirus*, *Hop mosaic virus* (HpMV), *Hop latent virus* (HpLV), and *American hoplatent virus* (AHLV). In addition, the group of the most harmful hop pathogens combines *Apple mosaic virus* (ApMV) and *Prunus necrotic ringspot virus* (PNRSV) from the genus *Ilarvirus*, as well as a nepovirus, *Arabis mosaic virus* (ArMV) (Pethybridge et al. 2008; Sastry et al. 2019). They have a significant impact on the yield and secondary metabolism of hop plants, which leads to a decrease in the level of bitter acids and essential oils in the cones, which are valuable components in the production of beer (Mishra et al. 2020). The possible presence of these pathogens in wild nature poses a threat to further spread among cultivars, increasing the likelihood of potential economic damage. In addition, so-called minor viruses with a low frequency of spread and weak infectious ability have been described. It is believed that in certain pathosystems the status of the diseases they cause may change, which determines the need to control their spread. This group of pathogens includes various mosaic viruses (*Alfalfa mosaic virus* (AMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Petunia asteroid mosaic virus* (PetAMV)), as well as *Strawberry latent ringspot virus* (SLRSV), *Tobacco ringspot virus* (TRSV), *Tobacco necrosis virus* (TNV) (Pethybridge et al. 2008; Gargani et al. 2017; Sastry et al. 2019). Information regarding their harmfulness is limited; there are only descriptions of symptoms, including deformation and perforation of leaves, various types of chlorosis and necrosis, and shortening of internodes (Yu and Liu 1987; Pethybridge et al. 2008).

The purpose of the study was to assess the spread of viral infections among plants of wild hop populations in the Altai Territory and the foothills of the Altai Republic of Russia.

Materials and methods

The expedition to collect plant material from wild hops *H. lupulus* took place from July 15 to August 15, 2022 and 2023. The Altai Krai (Russia) occupies the south-eastern part of Western Siberia, located in the basin of the upper reaches of the Ob River between 50° and 55° N and 77° and 87° E. The natural zones of the region are represented by the Kulunda and Aleisk steppes, the Priob forest-steppe, the forest-steppe of the Salair foothills and the Altai foothills. Thus, the region contains almost all natural zones of Russia: steppe, forest-steppe, taiga and mountains.

The collection route covered all natural zones of the Altai Krai including steppe, forest-steppe and foothills (Fig. 1). In addition, we examined the foothill region of the Altai Republic (Russia) adjacent to the southeastern border of the Altai Krai. A total of 14 locations were surveyed in 2022 and 11

locations in 2023, 243 accessions were collected, at least 8 plants per population. Transportation of accessions was carried out in individual plastic bags placed in a thermal container TKM-10 (LLC Termo-Kont MK, Russia) at a temperature of +4 °C.

Laboratory tests were carried out to detect infection with 16 pathogens. A combined sample of leaves taken from different layers of each plant (lower, middle and upper) was used for analysis. Samples were assessed for infection by 9 viruses and 1 viroid (HpLV, PNRSV, TRSV, PVS (*Potato virus S*), PVA (*Potato virus A*), PVM (*Potato virus M*), PVX (*Potato virus X*), PVY (*Potato virus Y*), PLRV (*Potato leaf roll virus*) and PSTVd (*Potato spindle tuber viroid*) by real-time RT-PCR. RNA was extracted with DiamondDNA reagents (Russia), according to the manufacturer's instructions. The study was performed using a QuantStudio5 DNA amplifier (Thermo Fisher Scientific, USA). Diagnostic kits and thermal cycling conditions were developed by the company "Synthol" (Russia). Testing for the presence of 6 viruses (HpMV, ApMV, ArMV, PetAMV, SLRSV and TNV) was carried out by DAS-ELISA. Diagnostic kits from BIOREBA AG (Switzerland) were used.

The incidence of the virus was assessed by the proportion of infected samples (%) relative to the number of plants tested. Laboratory studies were carried out at the Altai Center for Applied Biotechnology of Altai State University (Barnaul, Russia). The significance of differences in the infection of hop plants by various viruses was assessed by ANOVA at $P < 0.05$.

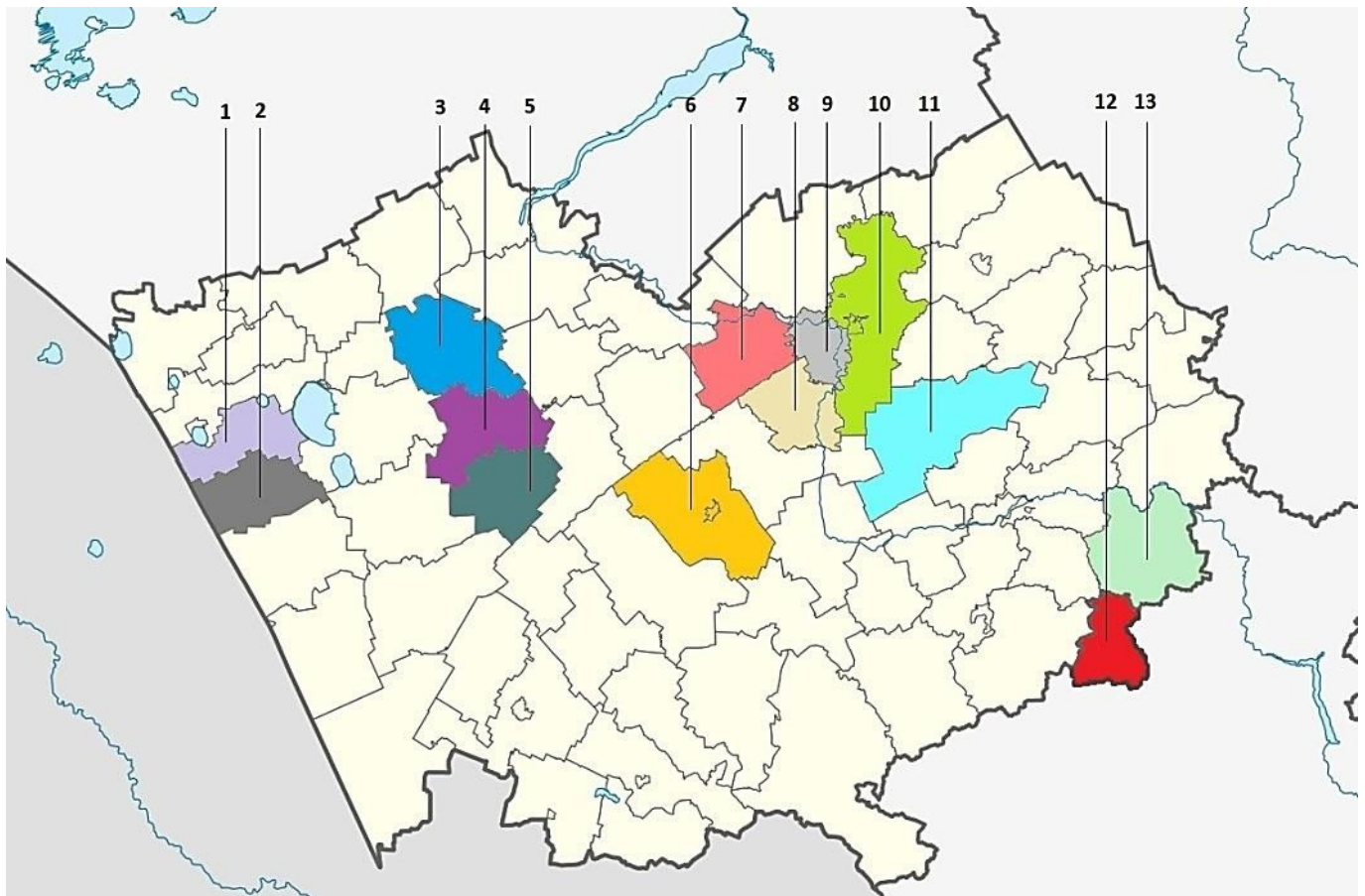


Figure 1. Location diagram of the districts of the Altai Krai and the Altai Republic examined for the presence of viruses in natural hop populations in 2022: 1 - Tabunyskiy district; 2 - Kulundinskiy district; 3 - Bayevskiy district; 4 - Zavyalovskiy district; 5 - Romanovskiy district; 6 - Aleyskiy district; 7 - Pavlovskiy district; 8 - Kalmankinskiy district; 9 - Barnaul and Nauchny Gorodok; 10 - Pervomayskiy district; 11 - Troitskiy district; 12 - Maima district (the Altai Republic); 13 - Krasnogorskiy district.

Result

The expedition route made it possible to cover all natural areas of the region. Wild hops were found throughout most of the surveyed area, mainly in places where there was access to moisture. They formed extensive thickets in the floodplains of rivers, near creeks, on open forest edges, and along the sides of roads overgrown with bushes. Hop vines grew by climbing up the natural supports of bush stems and tree branches (Fig. 2).



Figure 2. Wild common hop: A – Maima district, Altai Republic, village Verkh-Karaguzh, near a creek, willow forest (52°04.60'N, 86°07.29'E); B – Pervomayskoye district, Altai Krai, bank of the Losikha River, floodplain forest (53°35.03'N, 84°40.74'E).

On the leaves of hop plants growing in various natural zones of the region, mosaic symptoms in the form of rings, as well as yellow and pale green spots were observed (Fig. 3), which presumably indicated the presence of viral infections. Considering the large number of viruses that infect hops (Pethybridge et al. 2008; Sastry et al. 2019), as well as the availability of test kits, the analysis included both biological agents that are widespread and harmful to this crop, and those that have not previously been studied on hops, but have common vectors.

A laboratory RT-PCR study of plant material collected in 2022 revealed the presence of 7 viruses with varying frequencies (Table 1). Statistically significant differences in the results obtained were revealed both depending on the place of collection of the material and the infection with a specific virus. The F-test values exceeded the critical ones at $P < 0.05$ (F-tests were 2.52 and 31.52, respectively). The total proportion of infected plants was high and reached 95.5 %. All natural populations, with the exception of samples collected in the Maima district of the Altai Republic, were 100 % infected. PNRSV and PSTVd were not found. The most frequently detected pathogens were potato mosaic viruses PVY and PVS, the infection rate of which reached 93.3 %. These pathogens were present in almost all populations in 100 % of plant samples. The frequency of PLRV infection was slightly lower. The overall infection rate was 82.2 %, ranging from 50.0 % to 100 %. This pathogen was not detected only in the population of the foothill zone of the Altai Republic. The

prevalence of PLRV in dry and arid zones was lower than in more humid areas. At the same time, PVM and PVA, on the contrary, were more regularly diagnosed in the dry steppe zone.

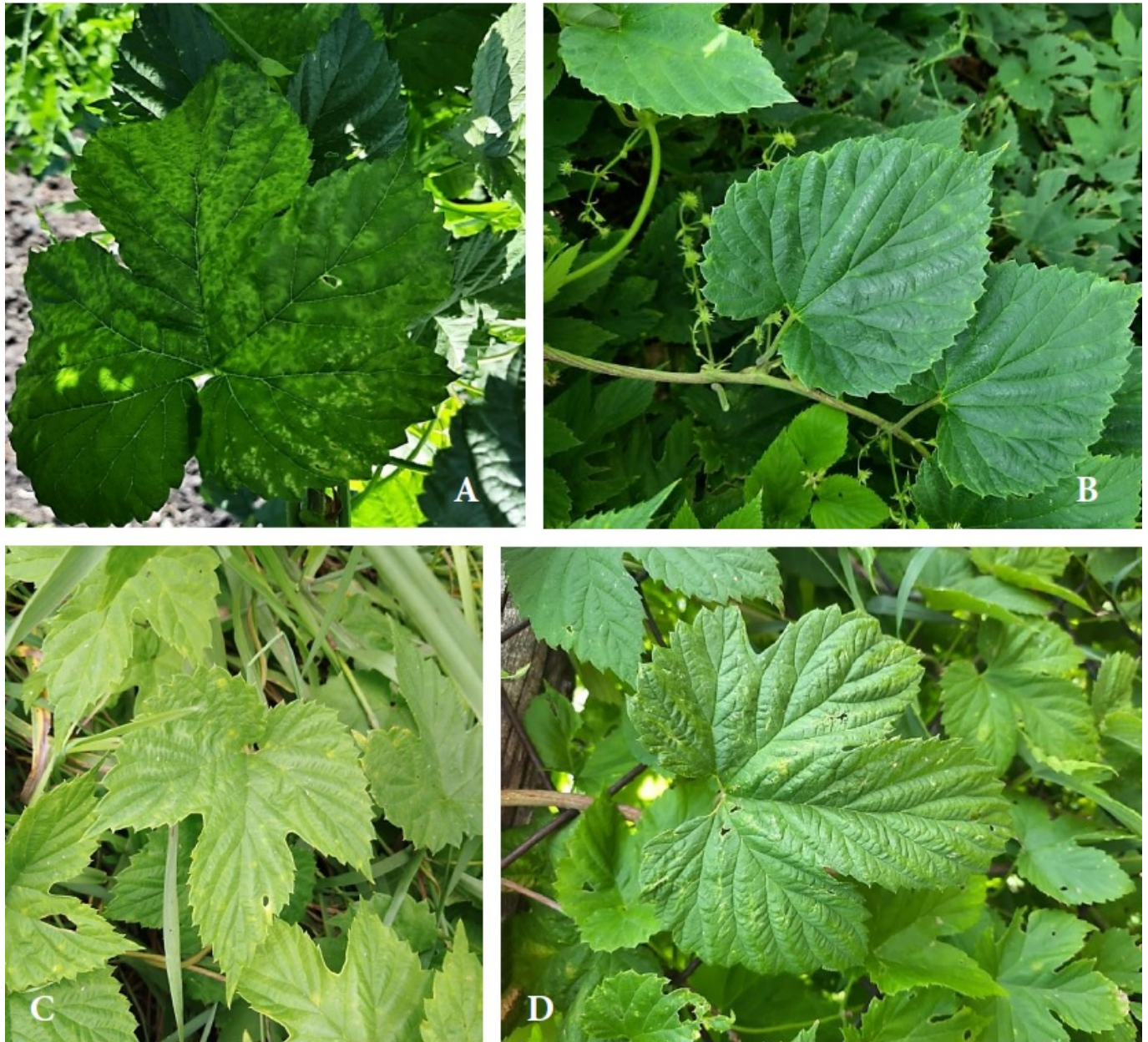


Figure 3. Mosaic symptoms on the leaves of wild hops growing in the Altai Krai.

District	Total incidence	TRSV	PVM	PVS	PVY	PVX	PVA	PLRV
Tabuny (dry steppe)	100	0	100	100	100	0	50.0	50.0
Kulunda (dry steppe)	100	0	66.7	100	100	33.3	66.7	66.7
Zavyalovo (arid steppe)	100	0	50.0	100	100	0	100	50.0
Aleysky (moderate-arid steppe)	100	55.5	100	100	100	0	33.3	100
Baevo (moderate-arid)	100	0	0	100	100	33.3	33.3	66.7

steppe)								
Romanovo (steppe)	100	0	66.7	100	100	0	66.7	66.7
Pavlovsk (forest-steppe)	100	0	0	100	100	0	100	100
Kalmanka (forest-steppe)	100	0	0	100	100	0	0	100
Barnaul	100	33.3	0	100	100	0	0	100
Nauchny Gorodok (forest-steppe)	100	0	100	100	100	12.5	37.5	100
Pervomaiskoye (forest-steppe)	100	44.4	100	100	100	0	33.3	100
Troitskoye (forest-steppe)	100	20.0	20.0	80.0	100	0	0	100
Krasnogorsk (mountain forests)	100	0	0	100	100	0	0	100
Maima (mountain forests)	33.3	0	0	33.3	0	0	0	0
Total	95.5	8.9	48.9	93.3	93.3	6.7	40.0	82.2

Table 1. Virus incidence in natural populations of wild hop in the Altai Krai and Altai Republic (2022), %

The minimum proportion of positive wild hop samples was detected for PVX. This pathogen appeared sporadically in different locations, infecting several plants. The described agents are among the most dangerous, widespread viruses found in potato growing areas. They are transmitted by the green peach aphid *Myzus persicae* Sulzer 1776 (Makarova et al. 2017), which is also a vector for a number of hop viruses (Pethybridge et al. 2008). To our knowledge, cases of infection of wild hops by potato viruses, with the exception of PVM, have not previously been reported.

TRSV is classified as a minor pathogen of hop, which, as a rule, has a limited range in Europe (Sastray et al. 2019). It is unevenly distributed throughout our region and was identified in 4 surveyed populations. Despite the total low proportion of infected plants, its occurrence in certain areas was high, reaching 50.5 %. The virus is known to be transmitted by nematodes non-persistently or mechanically by inoculation of sap. We were also unable to find information on the prevalence of *Tobacco ringspot virus* in hop plantings in Russia.

A feature of the spread of viruses in wild populations surveyed in 2023 was the detection of HpLV, an economically important and dangerous pathogen of hops. This can slow down plant growth and reduce yields (Ziegler et al. 2014). The total incidence was 24.5 %. The infection was detected in all locations, with the exception of the Kulunda, Mayma districts and Nauchny Gorodok, located near Barnaul. The maximum number of positive plant samples was found in hop populations growing in the moderate-arid steppe (Rebrikha and Topchikha districts) and in the forest-steppe (Pervomaiskoe district, Fig. 4). The detection of the virus in the Charysh district is of great concern, since industrial hop plantations are located here. Wild hops can serve as a reservoir for infection, threatening to spread to commercial cultivars. As the name suggests, HpLV is latent in nature, which makes it difficult to assess its manifestation. In addition, season and leaf age influence the development of infection (Ziegler et al. 2014). However, it has been found in hop gardens in North America, Australia, China, and Japan. HpLV is reported to be widespread in Europe (Seigner et al. 2014). Thus, in Poland it has been registered in various cultivars in all hop growing regions (Przybyś 2020). The virus usually develops asymptotically, but sometimes chlorotic spots appear

on the leaves. HpLV is transmitted by various species of aphids (*M. persicae*, *Phorodon humuli* Schrank 1801, *Macrosiphum euphorbiae* Thomas 1878). It can also be transmitted mechanically by contact (Pethybridge et al. 2008). Because sampling was predominantly carried out from plants forming thickets, the virus could have spread through contact between damaged intertwining bines. It is likely that, along with the wide distribution of vectors, this may also be the reason for the high frequency of infected plants we found.

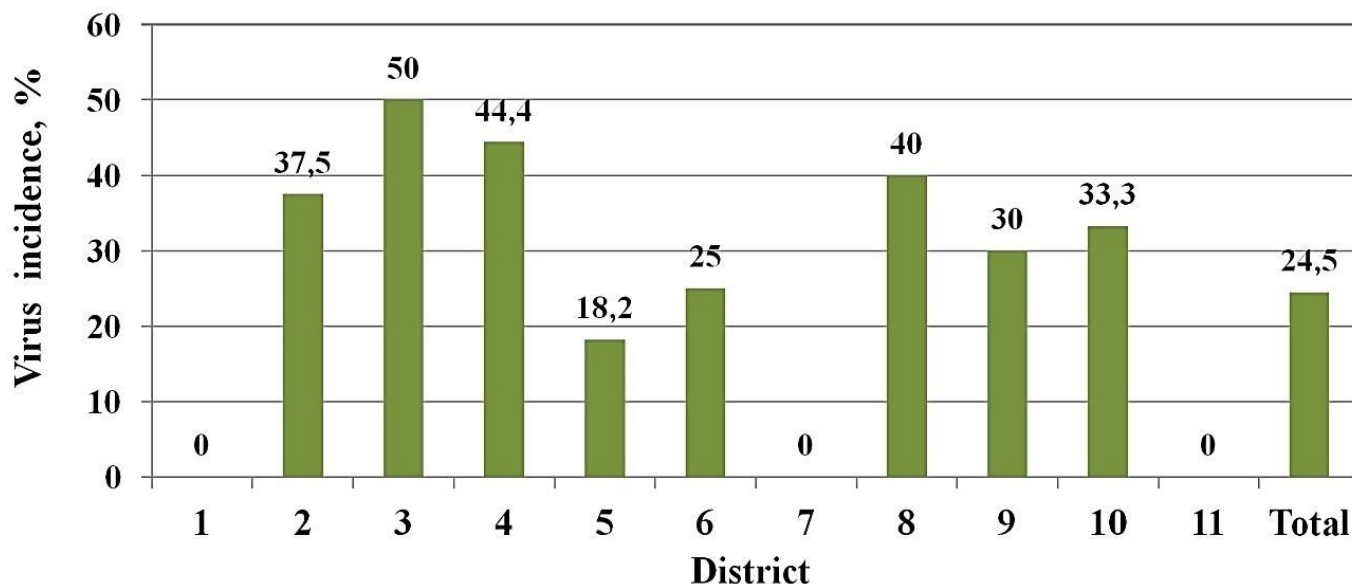


Figure 4. Prevalence of HpLV in wild hop in the Altai Krai and Altai Republic (2023): 1 - Kulunda district; 2 - Zavyalovo district; 3 - Rebrikha district; 4 - Topchikha district; 5 - Pavlovsk district; 6 - Barnaul; 7 - Nauchny Gorodok; 8 - Pervomaiskoye district; 9 - Kosikha district; 10 - Charysh district; 11 - Maima district.

All potato viruses detected in the previous season were also found in wild hops collected in 2023. The only exception was TRSV, which could not be identified. However, the frequency of infected plants was slightly lower. PVY continued to dominate. The proportion of plants affected by PVX tripled (18.2 %), while PVS was much less common (15.5 %). The incidence of PLRV also decreased significantly (19.1 %).

Testing 110 samples of wild hops using DAS-ELISA for infection with three harmful hop viruses (HpMV, ApMV and ArMV) and 3 minor viruses (SLRSV, TNV and PetAMV) revealed their presence in 20.0 % of samples (Table 2). HpMV and PetAMV were not detected. The overall contamination of the material with ApMV was low (8.5 %). However, the pathogen was found in three locations. The number of affected samples was 1–5 per population. The maximum incidence of the virus reached 45.5 % (Pavlovsk district), which is an alarming signal. It has been reported that the pathogen can reduce the yield of commercial cultivars and alpha acid content by up to 32 % and 16 %, respectively. ApMV has a wide host range, affects a variety of plant species and can spread at high rates (Grimová et al. 2016). The virus is transmitted mainly mechanically, which threatens neighboring plants. Symptoms of the disease include ring-shaped chlorotic spots (Fig. 3A). However, according to our data, not only hops with visible symptoms of the disease, but also asymptomatic plants showed a positive reaction. The external manifestation of signs of the disease largely depends on temperature. Cool weather and rapid temperature changes contribute to visible damage to leaf tissue (Pethybridge et al. 2008).

District	Total incidence	ApMV	ArMV	SLRSV	TNV
Kulunda (dry steppe)	12.5	0	0	12.5	0
Zavyalovo (arid steppe)	12.5	0	0	0	12.5

Rebrikha (moderate-arid steppe)	20.2	0	0	20.0	0
Topchikha (moderate-arid steppe)	22.2	11.1	0	22.2	0
Pavlovsk (forest-steppe)	54.5	45.5	54.5	0	0
Barnaul	50.0	37.5	0	0	12.5
Nauchny Gorodok (forest-steppe)	0	0	0	0	0
Pervomaiskoye (forest-steppe)	20.0	0	0	20.0	0
Kosikha (forest-steppe)	30.0	0	0	30.0	0
Charysh (mountain forests)	8.3	0	0	8.3	0
Maima (mountain forests)	0	0	0	0	0
Total	20.0	8.2	5.5	10.0	1.8

Table 2. Virus incidence in natural populations of wild hop in the Altai Krai and Altai Republic (2023), %

More than half of the plant samples collected in the Pavlovsk district is infected with ArMV. The virus has not been detected in other wild populations. ArMV is considered an extremely dangerous pathogen for hops, causing severe diseases, including spidery hop, split leaf blotch, and hop chlorotic disease. The main vector of the virus is the nematode *Xiphinema diversicaudatum* Micoletzky, 1927. Some ArMV strains reduce hop yields by up to 75 % (Pethybridge et al. 2008).

We also detected two species of minor viruses, SLRSV and TNV, appearing sporadically in different locations. The maximum presence of SLRSV was observed in the Kosikha district, where every third plant was infected. TNV was identified in the Zavyalovo district and in Barnaul. The proportion of affected plants was small and amounted to 12.5 %. These viruses were previously discovered in Europe on commercial cultivars. Information about their harmfulness is scarce (Sastry et al. 2019). However, they also require control. Their status, relative to other viral diseases, is expected to change under certain conditions.

Conclusion

Wild hops are widespread throughout the Altai Krai and the bordering foothill district of the Altai Republic. Natural populations were found along the entire expedition route in 18 areas. Testing of plant samples using ELISA and RT-PCR for the presence of 15 viruses and 1 viroid revealed a wide distribution of various pathogens. The frequency of infected plants varied depending on the sampling location and the virus. Among the most dangerous hop pathogens are HpLV, ApMV and ArMV. HpLV infected 24.5 % of the samples tested. Most often it was found in areas located in the zone of moderate-arid steppe and the forest-steppe zone. The virus incidence reached 50 %. ApMV was detected in 3 districts with a frequency of 11.2–45.5 %. ArMV was found in half of the plants in one location (Pavlovsk district). The group of minor viruses included SLRSV, TNV, and TRSV. They appeared sporadically in different areas with varying incidences. In all wild populations, concomitant viruses were found that share common vectors with hop pathogens. They infected 33.3–100 % of plants. PVY, PVS and PLRV were the most frequently diagnosed. The presence of mosaic pathogens PVA, PVM and PVX ranged from 6.7 to 48.9 %. HpMV, PetAMV, PNRSV and PSTVd were not detected. Thus, wild hops are prone to the accumulation of a variety of viruses, including those that are economically important for hop growing. The presence of natural reservoirs of infection poses a threat to commercial cultivars in areas where they grow together (Charysh district). The use of wild hops as sources of useful traits for breeding requires their

preliminary testing for the presence of the most dangerous pathogens.

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