horticulturae

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Review

Viral Infection Control in the Essential Oil-Bearing Rose Nursery: Collection Maintenance and Monitoring

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Abstract: Viral diseases affecting the essential oil rose, which is a valuable object of agricultural production, may have a significant negative impact on the economic value of this crop. Hence, the study and control of potentially dangerous viruses is essential to improving the quality of cultivars of this raw plant material, to enable production of valuable derivatives. The diversity of viruses affecting *Rosa* L. plants manifests itself in their conditional division into those that are specific to this crop, and those that are hosted by other plants. Representatives of both groups are found in different countries, however, a low number of viruses identified have been thoroughly studied through the use of experimental methods. In particular, with regard to many viruses, the issue of their spread remains open. The viruses infecting *Rosa* L. plants along with other crops are described in the literature in detail, as the range of hosts they affect is rather wide and well-studied. It is also possible to single out the three most significant viruses affecting this host—*Prunus necrotic ringspot virus*, *Apple mosaic virus* and *Arabis mosaic virus* which individually, or collectively, cause viral diseases that manifest themselves in mosaic symptoms. The most likely mechanisms for the spread of the *Rosa* L. species viruses are vegetative propagation procedures and transmission by various pests. These presumptions underlie viral infection control methods, including a well-thought-out planting scheme and provision of accurate plant care, which considers plant disinfection, disease monitoring associated with diagnostics and obtaining virus-free material through biotechnology techniques.

Keywords: essential oil rose; *Rosa* L.; viruses; pests; the European Plant Protection Organization; *Ilarvirus*; *Nepovirus*; *Prunus necrotic ringspot virus*; *Apple mosaic virus*; *Arabis mosaic virus*; viral mosaic; viral genome

Citation: Seitadzhieva, S.; Gulevich, A.A.; Yegorova, N.; Nevkrytaya, N.; Abdurashytov, S.; Radchenko, L.; Pashtetskiy, V.; Baranova, E.N. Viral Infection Control in the Essential Oil-Bearing Rose Nursery: Collection Maintenance and Monitoring. *Horticulturae* **2022**, *8*, 629. https://doi.org/10.3390/horticulturae8070629

Academic Editor: Jiatao Xiè

Received: 28 June 2022
Accepted: 9 July 2022
Published: 12 July 2022

1. Introduction

Essential oil roses belong to the family Rosaceae, genus *Rosa*. The essential oil rose plant is an important agricultural crop due to its high economic value. Essential oil is obtained mainly from four types of roses: *R. damascena* Mill., *R. alba* L., *R. gallica* L. and *R. centifolia* L. The highest quality oil is extracted from the Bulgarian rose *R. kazanlika* (*Rosa damascena* Mill. *f. trigintipetala* Dieck) which is a form of *Rosa damascena* Mill. [1,2].

The rose petals account for the main content of essential oil in the essential oil rose plant (about 93% of the total content in the flower). The rose flower derivatives include rose water (hydrolate), absolute oil (absolute), concrete and extract used in the perfume and toiletry industry and medicine [3,4]. The origin of the essential oil rose is associated with Iran and Syria [5,6]. Currently, the centres of cultivation of this crop are Bulgaria [7]

and Turkey [8], it is also grown commercially in Saudi Arabia [9], Egypt [10], India [11], Russia [12], Georgia, China, Algeria, Spain, France, Italy and Morocco [2,13].

The essential oil rose derivatives of domestic production can claim competitiveness vis-à-vis the global reference producers, provided that the international standards of processing practice are observed. Hence, the need arises for breeding new high-performance cultivars [14,15]. This is the challenge facing the Research Institute of Agriculture of Crimea, the owner of a unique gene pool collection of essential-oil, spicy, aromatic and medicinal plants [16]. The collection specimens are a source material for the studies carried out by the Essential-Oil and Medicinal Crops Selection Division. The Institute is the owner and originator of five essential oil rose cultivars included in the State Register of Selection Achievements Authorized for Use of the Russian Federation [17].

Apart from the agro-technical crop care measures, essential-oil rose cultivation involves pest and pathogen control [18,19]. The rose-specific viral diseases are widely spread in the countries where this crop is grown. They impair the rose habit, its decorative and economic value, affect the vegetative and generative parts of plants and blunt the plant health and viability, even to the point of its death [20,21]. Detection of viral diseases, developing means and techniques for their prevention and control, as well as cultivation of new cultivars most resistant to infections are the important challenges facing the plant material growing process.

This article reviews the studies focused on the viral diseases of plants falling under genus *Rosa* L., including essential oil roses.

2. Overview of the *Rosa* L. Species Viruses

Studies focused on the essential oil rose-specific viruses are rare in the literature while the information available addresses the viral pathologies specific to *Rosa* L. plants only in general. The basic reference source to be relied upon in this context is the European and Mediterranean Plant Protection Organization Global Database [22]. The documents of this organization provide the certification scheme for *Rosa* L. species and hybrids tested for pathogens [23]. A detailed list of the *Rosa* L.-specific viruses is given in the works by various authors. The data from these sources is presented in Table 1.

Table 1. List of viruses infecting *Rosa* L. plants.

Virus	Acronym	Reference
Prunus necrotic ringspot virus	PNRSV	[20,23–27]
Apple mosaic virus	ApMV	
Arabis mosaic virus	ArMV	
Tobacco streak virus	TSV	
Tobacco ringspot virus	TRSV	
Tomato ringspot virus	ToRSV	
Tomato spotted wilt virus	TSWV	
Tomato yellow ring virus	TYRV	
Strawberry latent ringspot virus	SLRSV	
Blackberry chlorotic ringspot virus	BCRV	
Raspberry ringspot virus	RpRSV	
Cherry necrotic rusty mottle virus	CNRMV	
Impatiens necrotic spot virus	INSV	
Apple chlorotic leaf spot virus	ACLSV	
Apple stem grooving virus	ASGV	
Iris yellow spot virus	IYSV	
Rose rosette virus	RRV	
Rose leaf curl virus	RoLCuV	
Rose spring dwarf-associated virus	RSDaV	
Rose yellow vein virus	RYVV	
Rose cryptic virus-1	RoCV-1	
Rose yellow mosaic virus	RoYMV	
Rosa rugosa leaf distortion virus	RrLDV	

Table 1. *Cont.*

Virus	Acronym	Reference
Rose yellow leaf virus	RYLV	
Rose chlorotic ringspot virus	RoCRSV	
Rose necrotic mosaic virus	RoNMV	
Rose leaf rosette-associated virus	RLRaV	
Rose colour break virus	RCBV	

It should be noted that *Tobacco ringspot nepovirus* and *Tomato ringspot nepovirus* presented in Table 1 are included in the Uniform List of Quarantine Objects of the Eurasian Economic Union [28].

As seen from Table 1 a fairly large number of viruses infecting *Rosa* L. species are known; they may be divided into only the viruses hosted by *Rosa* L. plants and those whose host plants are represented by other crops. Table 2 presents the viruses hosted by *Rosa* L. plants.

Table 2. Viruses specific to *Rosa* L. plants.

Virus	Symptoms	Virus Spread Mechanism	Country	Reference
Rose leaf curl virus	Pronounced leaf stunted growth and curling	Not specified	Pakistan	[29]
	Dwarfing, leaf distortion and leaf curling	Not specified	India	[30]
Rose rosette virus	Shoot elongation and colouring from light pink to dark purple; thorn proliferation; leaf elongation, distortion and red pigmentation; petioles shortening; reduced flowering; lateral buds coming out of dormancy, growing and colouring red.	Eriophyid mite *Phyllocoptes fructiphylus*	USA	[31]
	Leaf curling and puckering; flower distortion; persistent red pigmentation	Not specified	India	[32]
	Excessive thorn production; "witch's broom" rosetting; abundance of lateral shoots; shoots coloring red; leaves and flowers mottling or distortion; lateral shoot growth.	Eriophyid mite *Phyllocoptes fructiphilus*	USA	[33]
Rose spring dwarf-associated virus	Rosetting; leaves shortening with vein clearing or netting; shoot zigzag growth pattern.	Aphids *Metapolophium dirhodum* and *Rhodobium porosum*	USA	[34]
	Yellow vein chlorosis	Aphid *Rhodobium porosum*	Chile	[35]
	Not specified	Aphid *Rhodobium porosum*	Turkey	[36]
	Leaf rosetting	Aphid *Metapolophium dirhodum*	New Zealand	[24]
	Not specified	Not specified	China	[37]
	Not specified	Not specified	Turkey	[38]
Rose yellow vein virus	Not specified	Not specified	New Zealand	[24]
	Mosaic and vein yellowing	Grafting	USA	[39]
	Vein banding, central vein chlorosis	Not specified	Turkey	[40]
Rosa rugosa leaf distortion virus	Leaf stunted growth and distortion; pale circular lines appearing only on early spring growth	Not specified	USA	[41]
	Leaf distortion and stunted growth; pale circular lines appearing only on early spring growth	Grafting	USA	[39]
	Vein yellowing	Not specified	Turkey	[38]
Rose color break virus	Deformed, flecked and streaked petals	Sap-transmission and use of infected budwood	Egypt	[27]
Rose yellow leaf virus	Leaf premature yellowing and senescence	Not specified	USA	[41]
	Leaf premature yellowing and senescence	Grafting	USA	[39]

Table 2. *Cont.*

Virus	Symptoms	Virus Spread Mechanism	Country	Reference
Rose cryptic virus 1	Not specified	Not specified	USA	[42]
	Leaf mottling and necrosis	Not specified	New Zealand	[24]
	Leaf banding, mottling and distortion	Not specified	UK	[43]
	Mottling	Not specified	Turkey	[38]
Rose transient mosaic virus	Leaf mosaic and yellowing	Not specified	Minnesota, the USA	[41]
Rose leaf rosette-associated virus	Leaf rosette ("witch's broom" symptom) formed by dense small leaves on branches; clearly noticeable decay, destruction and, finally, dieback of plants	Not specified	China	[44]
	Not specified	Not specified	USA	[45]
Rose necrotic mosaic virus	Mosaic, necrotic streaks, leaf distortion	Not specified	USA	[41]
Rose partitivirus	Not specified	Not specified	Canada	[46]
Rose yellow mosaic virus	Yellow mosaic; ring mosaic; premature leaf senescence and dark-brown rings on canes	Not specified	Minnesota, the USA	[41]
	Yellow mosaic; premature leaf senescence	Grafting	Minnesota, the USA	[39]
	Yellow chlorotic spots	Not specified	Japan	[47]
Rose Chlorotic Ringspot Virus	Chlorotic ringspots and mosaic symptoms	Not specified	Minnesota, the USA	[41]
Rose virus A	Leaf distortion; mosaic symptoms	Not specified	California, the USA	[48]

The data presented in Table 2 suggests that the spread mechanism for many *Rosa* L.-specific viruses is not known while the symptoms of the plant viral diseases are common in certain cases.

Table 3 presents the viruses for which *Rosa* L. is one of the host plants.

Table 3. Viruses non-specific to *Rosa* L. plants.

Virus	Symptoms	Virus Spread Mechanism	Country	Reference
Apple chlorotic leaf spot virus	Not specified	Vegetative propagation and grafting	Greece	[49]
Apple stem grooving virus	Leaf rosette	Sap-transmission, transmission on grafting and via infected seed material [27]	China	[50]
Blackberry chlorotic ringspot virus	Leaf rosette	Sap-transmission, transmission on grafting [27]	China	[44]
	Not specified		USA	[51]
Cherry necrotic rusty mottle virus	Chlorosis and necrotic spots on leaves	Transmission via budding and grafting [27]	India	[52]
Impatiens necrotic spot orthotospovirus	Small necrotic spots; leaves yellowing; ringspots; necrotic streaks; wilting and dwarf symptoms	Transmitted by thrips *Frankliniella occidentalis*; sap-transmission; use of infected budwood [27]	Iran	[53]
Iris yellow spot orthotospovirus	Chlorotic and necrotic symptoms	Thrips, sap-transmission [27]	Iran	[54]
Raspberry ringspot virus	Mosaic; chlorosis; leaves curling and distortion; stunted growth	Nematodes, sap-transmission [27]	Germany	[55]
Strawberry latent ringspot virus	Yellow flecking in young leaves and reduction in leaflet size	Nematodes, sap-transmission; grafting [27]	India	[56]
Tomato ringspot virus	Banded chlorosis wrinkling; malformation and chlorotic spots on leaves	Nematodes (*Xiphinema* spp.), sap-transmission, grafting [27]	Iran	[57]
Tomato spotted wilt orthotospovirus	Necrotic spots and leaves marginal necrosis	Thrips, sap-transmission, grafting [27]	Iran	[58]

More information is available on the transmission routes of the viruses that are not specific to *Rosa* L. plants, since these phytopathogens have been studied in more detail in other crops. Nevertheless, the data presented in Tables 2 and 3 suggests that, in general, the viral infections affecting rose plants are superficialized, which is confirmed by the scarcity

of data on symptomatic manifestations and transmission routes of these phytopathogens. The economic cost caused by viruses is assessed by the effect they produce on the normal growth and vital activity of plants. The effect produced by the virus on plants is studied based on the disease manifestations. In general, viruses are characterized as causing a systemic disease in plants, when a phytopathogen moves from the primary point of the infection entry to other parts of the plant (Figure 1).

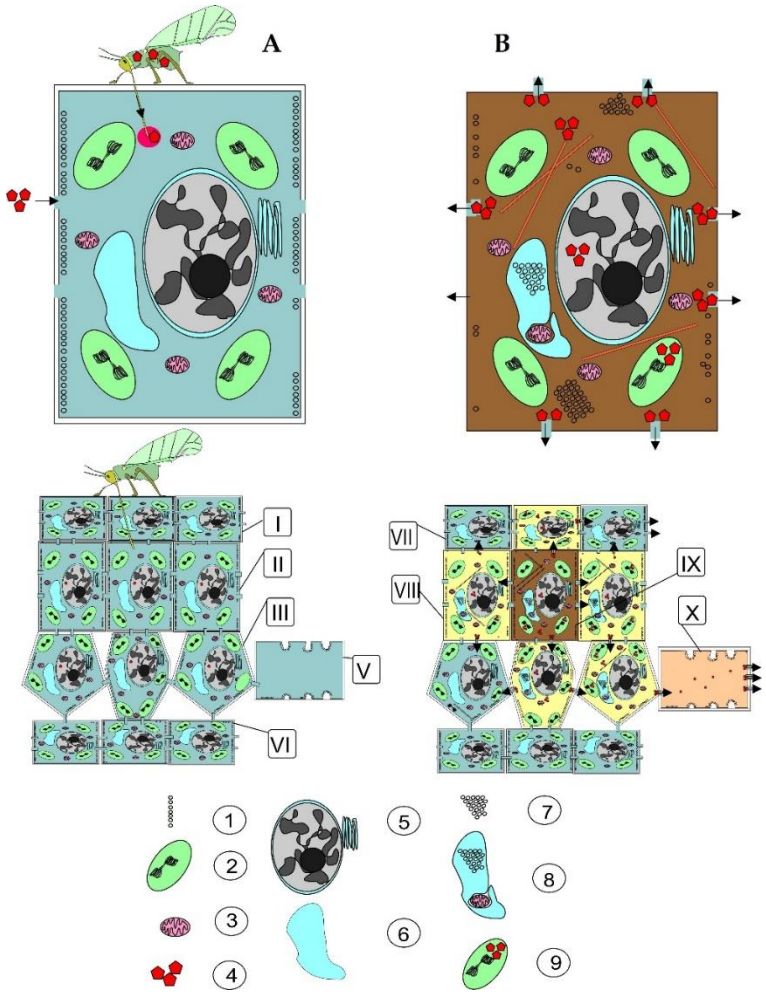

Figure 1. Features specific to viral disease progression from inoculation to viral spread in tissues and other plant organs (**A**) Virus entry: infiltration of viral particles or nucleic acid into the cytoplasm via a biological object or sap-transmission; infiltration into the plant cell from another infected cell via plasmodesma. (**B**) Further progression of a viral infection: specific damage to cellular compartments whereby the virus uses the protein synthesis and RNA/DNA synthesis systems of infected cells (nucleus, plastids and pro- and eukaryotic ribosomes); successive spread of the virus by near and far transport via plasmodesmata as well as by means of the phloem transport, subject to the nature of the virus, the speed at which it spreads and the ways it spreads. (**I**) The upper epidermis (**II**) The columnar parenchyma (**III**) The spongy parenchyma (**IV**) The vascular system (**V**) The lower epidermis (**VI**) The viral infection manifestation in the upper epidermis (**VII**) Progression of a viral infection in the columnar parenchyma (**VIII**) Progression of a viral infection in the spongy parenchyma (**IX**) Viral infection-caused death of cells (**X**) Virus entry into the vascular system (**1**) The cytoskeleton (**2**) The chloroplast (**3**) The mitochondrion (**4**) Viral particles (**5**) The nucleus with the reticulum elements (**6**) The vacuole (**7**) Viral proteins in the form of crystals (**8**) The vacuole with viral crystals and invagination (**9**) Crystal and viral particle formation in the plastid.

Localized infection with viral diseases manifests itself as discoloration of the lamina. This type of symptom is not so significant for essential oil roses, but is important for the diagnostic detection of viruses. Examples of such symptoms include chlorosis (chlorophyll decay or deficiency), increased chlorophyll concentration in some areas of old leaves, necrotic lesions, ringspots. Systemic symptoms include stunted growth; mosaic (alternating light- and dark-green areas); yellowing (complete leaves yellowing); ringspots in leaves and fruits caused by the tissues yellowing or the surface cells destruction; necrotization of large groups of cells, organs or even the whole plant; malformation (distortion of various organs, overgrowth, tumours) [59,60].

For all the diversity of viral symptoms, many of them are similar to those caused by other pathogens which makes diagnosis difficult (Figure 2).

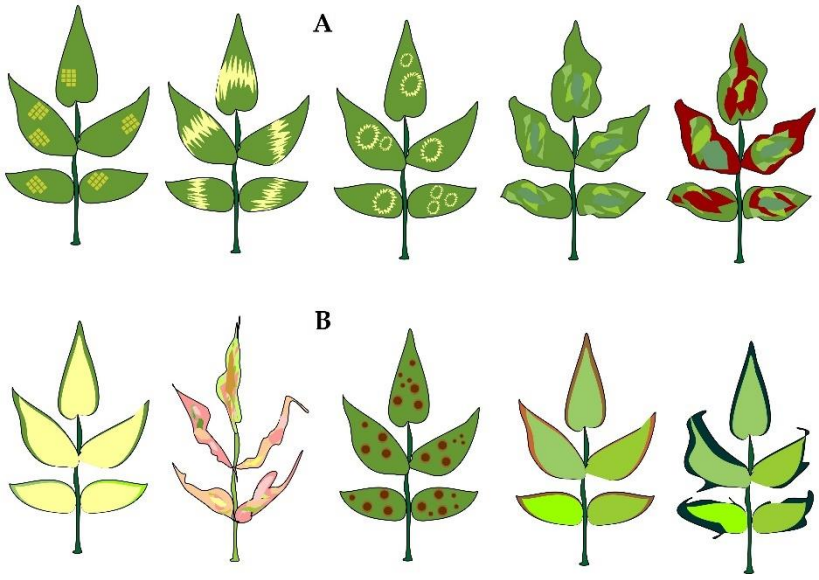

Figure 2. (**A**) Symptoms of viral diseases affecting *Rosa* L. species (mosaic, ringspot, wrinkling, bronzing). (**B**) Similar symptoms of viral and other diseases affecting *Rosa* L. (chlorosis of various origin resulting from a viral infection and nutritional deficiency; effects of the plant treatment with plant growth regulators and/or auxin herbicides or viral wilting; bacterial, fungal or viral spots; marginal effects caused by viral streak disease or micronutrients deficiency or imbalance; leaf development upon viral infection or impaired hormonal balance).

Based on the articles by various authors, it may be concluded that the most typical and common manifestation of the viral diseases affecting *Rosa* L. plants is viral mosaic caused by such pathogens as *Prunus necrotic ringspot virus*, *Apple mosaic virus* and *Arabis mosaic virus* [8,39,41,61–65]. Its hallmark is that the infection has a mono- or mixed nature, that is, it is triggered by a single virus or a group of viruses. Moreover, manifestations of one or several viruses may be different: chlorotic lines; ringspots; vein clearing and banding; leaf mottling during vegetation; yellow netting and yellow mosaic; oak leaf pattern; leaf distortion and curling; necrosis; flower distortion; flower size reduction; shrinkage in the plant stem at the grafting point [24,26,63,64].

Signs of a viral infection may also vary wildly, being subject to the air temperature and the time of year. They often manifest themselves in spring and early summer. For example, bands along the leaf veins may come up during lengthy hot periods. At times only a certain part of the plant may have lesions while in some cases infected parts of the plant manifest no symptoms. The disease results in reduced flowering, impaired winter survival, premature leaf fall and increased vulnerability to low temperatures. At the same time some infected plants do not manifest any symptoms at all [26,64].

Descriptions of the viruses causing mosaic in rose plants are presented in Table 4; details of their genetic structure are given in Appendix A.

Table 4. Description of the viruses causing mosaic in rose plants.

Virus	Taxonomic Affiliation	Host Plants	Geographical Region/Country	Symptoms
Prunus necrotic ringspot virus	Family: Bromoviridae Genus: *Ilarvirus* [22]	Apple tree (*Malus domestica*); white mulberry (*Morus alba*); red mulberry (*Morus rubra*); sour cherry tree (*Prunus cerasus*); oriental cherry (*Prunus serrulata*); sweet cherry (*Prunus avium*); almond (*Prunus dulcis*); peach tree (*Prunus persica*); Japanese plum (*Prunus salicina*); garden plum (*Prunus domestica*); apricot tree (*Prunus armeniaca*); Japanese apricot (*Prunus mume*); common hop (*Humulus lupulus*); rose (*Rosa* spp.) [22,25,66–73]	Africa: Algeria, Egypt, Morocco, South Africa, Tunisia. America: Argentina, Brazil, Canada, Chile, Mexico, USA, Uruguay. Asia: China, India, Iran, Israel, Jordan, Japan, Korea, Lebanon, Saudi Arabia, Syria. Oceania: Australia, Fiji, New Zealand. Europe: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Latvia, Malta, Moldova, Montenegro, Netherlands, Poland, Russia, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Turkey, Ukraine, Great Britain [22].	Chlorotic and necrotic ringspots, mottling and vein banding develop on leaves. Stems are covered with necrotic stripes. Flowers are distorted due to a significant diameter reduction, loss of fresh and dry weight as well as petal discoloration and decrease in number [62,66,70,71,73]
Apple mosaic virus	Family: Bromoviridae Genus: *Ilarvirus* [22]	Some representatives of the chestnut tree genus (*Aesculus*); birch tree *Betula*); *Prunus* (apricot, peach, cherry, plum, cherry plum); *Rosa; Rubus* (raspberry, blackberry, blackcurrant); hawthorn (*Crataegus*); wormwood (*Artemisia vulgaris*); hazel (*Corylus avellana*); strawberry (*Fragaria ananassa*); common hop (*Humulus lupulus*); apple tree (*Malus domestica*); common pear (*Pyrus communis*); redcurrant (*Ribes rubrum*); wild clary (*Salvia verbenaca*); rowan (*Sorbus aucuparia*) [8,22,74,75]	Africa: Algeria, Ethiopia, Kenya, Morocco, South Africa, Tunisia, Zimbabwe. America: Argentina, Brazil, Canada, Chile, Mexico, USA, Uruguay; Asia: China, India, Japan, Jordan, Lebanon, Syria. Europe: Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, Great Britain. Oceania: Australia, New Zealand [22].	Leaves turn wrinkled and distorted and manifest linear patterns, chlorotic ringspots, mottling and vein banding [65,74].
Arabis mosaic virus	Family: Secoviridae Genus: *Nepovirus* [22]	Grapevine (*Vitis vinifera*); apricot (*Prunus armeniaca*); sweet cherry (*Prunus avium*); plum (*Prunus domestica*); almond (*Prunus dulcis*); cherry laurel (*Prunus laurocerasus*); peach (*Prunus persica*); rhubarb (*Rheum rhabarbarum*); raspberry (*Rubus idaeus*); black elderberry (*Sambucus nigra*); representatives of the genus *Gladiolus*; celery (*Apium graveolens*); horseradish (*Armoracia rusticana*); common beet (*Beta vulgaris*); strawberry (*Fragaria ananassa*); common hop (*Humulus lupulus*); lettuce (*Lactuca sativa*); olive (*Olea europaea*) [22,76]	Africa: Egypt, South Africa. America: Canada, Chile, Mexico, Peru, USA. Asia: Kazakhstan, India, Iran, Japan, Lebanon, Syria. Oceania: Australia, New Zealand. Europe: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Moldova, Netherlands, Norway, Poland, Russia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, Belarus, Great Britain [22,76].	Chlorotic spots on the first leaves followed by mottling and ringspots. Later on the developing leaves show little to no symptoms [27].

3. Spread of *Rosa* L. Plants' Viruses

Control of rose plant-affecting viral diseases involves, in the first instance, the study of the pathogen transmission routes. However, as mentioned above, there is only a conjectural concept of transmission mechanisms of viruses affecting *Rosa* L. plants as experimental studies focused on these issues are very few. For example, ArMV is spread by the nematode *Xiphinema diversicaudatum* Micol. but this data is related to the crops which are the main virus host plants, while no data on *Rosa* L. plant infection have been reported [76–79]. ApMV is presumably transmitted via grafting, including root grafting, and infected sap when pruning [74]. PNRSV can be transmitted via cuttings from an injured plant during vegetative propagation [62].

Moury et al. [25] reported that the progression of PNRSV-caused infections in greenhouse grown roses is very slow or non-existent since in this study only 1% of the plants manifested symptoms two years after they were planted (in the absence of special precautions such as the plant isolation or disinfection of the tools used for pruning). Therefore, it can be concluded that the main source of the roses' infection with this virus is grafting in which one of the participants is infected. Furthermore, the difficulty in studying the viral mosaic may be ascribed to its duration and latent progression [25].

The study by Golino et al. [61] considered *Rosa* L.-affecting viruses transmission routes experimentally. As a result, the following hypotheses were set forth. The virus transmission via the rose seeds from an infected mother plant to sprouts does not occur or is a rare thing; infected pollen is ineffective in spreading viruses to recipient plants; virus transmission via cutting tools is unlikely. Wherein, a significant field spread of two rose mosaic viruses, PNRSV and ApMV, between infected and healthy roses growing close together was observed in experimental fields. This may be due to the fact that root grafting where the roots of the plants growing close together grow and fuse forming vascular links between the plants could be a mechanism for viral transmission.

Sertkaya [63] suggests that the rose mosaic virus could be transferred to these plants initially from an infected stone fruit crop via grafting, and then spread from one rose cultivar to another via infected rootstocks.

The data obtained from the studies carried out by the Research Institute of Agriculture of Crimea showed that various pests affect the essential oil rose. Among these, the green rose aphid (*Macrosiphum rosea* L.) and rose leaf cicada (*Edwardsiana rosae* L.) may be suspected vectors of viruses [80]. Since rose propagation is carried out by cutting, the virus transmission from parent plants and via instruments is likely [4,81,82].

Whereas there are a few works confirmed by experimental studies, the data presented in Tables 2 and 3 suggests a significant contribution on the part of various pests in the spread of viruses affecting *Rosa* L. species (Figure 3).

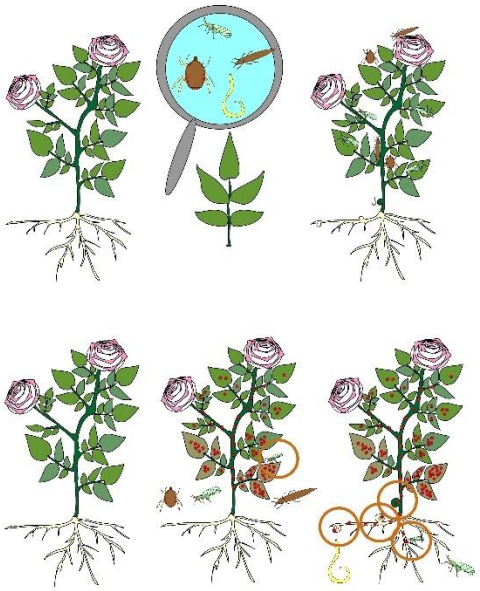

Figure 3. Pest vectors of the viruses affecting *Rosa* L. plants (nematodes, aphids, mites and thrips). The infection spreads from the bottom-up the phloem as the plant grows. For this reason, viral particles may be present in an outwardly healthy part of the plant.

The pests described for *Rosa L.* plant as confirmed and potential vectors of viruses are listed in Table 5.

Table 5. Pests of *Rosa* L. plant (potentially) contributing to the spread of viruses.

Pest	Mechanism of Damage to the Plant	Infection Symptoms	Reference
	Mites		
Phyllocoptes fructiphilus	Mites overwintering on plants and feeding on plant tissues; transfer by insects, wind and with clothes	Not specified	[83]
Tetranychus urticae	Not specified	Leaves mottling and drying	[84]
	Toxic substances injected by insect	Leaves yellowing; reduced photosynthesis; petals darkening and falling	[85]
	Not specified	White tiny flecks at the points of puncture by the mite's mouthparts on the upper surface of the leaf; plants pale yellowing; dull foliage; leaves and buds inlacing with a cobweb	[86]
	Mites feeding on the cell content mainly on the lower surface of the lamina causing destruction of the epidermis and underlying cells	Light spots on the upper surface of leaves; leaves turning yellow-brown and drying out	[87]
	Female mites wintering under the plant debris and the bark of shrubs; colonizing young leaves in spring; weaving a web and laying eggs while feeding	Leaves yellowing, distortion and drying; buds failing to open.	[88]
	Nematodes		
Meloidogyne hapla	Not specified	Leaves yellowing and prematurely falling; small shoots; reduced productivity and quality of flowers (the stem length and the flower size); symptoms of mineral deficiency; roots bearing galls; necrosis, segments dying-off, bark reducing and failing, roots shrinking and cracking	[89]
	Not specified	The root system distortion; leaf chlorosis; the stem size decreasing	[90]
	Sedentary internal parasites cutting tunnels in the plant root and creating permanent feeding sites without leaving them	Giant cells developing at the feeding site; hyperplasia of the cortical and vascular parenchyma; retarded meristematic activity in the root tips	[26]
Xiphinema diversicaudatum	Migratory external parasites feeding outside the root system	Galls caused by the cortical cells' hyperplasia developing at the feeding site; cells growing in size two–three fold; retarded meristematic activity	[26]
	Thrips		
Frankliniellaoccidentalis	Not specified	Retarded or stunted growth of leaves and transmission of certain plant viruses (for instance, *Tomato spotted wilt virus*).	[91]
	Immature and adult specimens feeding on the plant tissues by means of their piercing-sucking mouthparts; damage caused by females' saw-like ovipositor used for laying eggs in leaves, petioles, flower bracts and petals	Surface damage followed by necrotic spots; impaired photosynthesis capacity	[92]
Frankliniella tritici	Not specified	Buds turning brownish; petals curling up	[84]
Thrips tabaci	Not specified	Impaired decorative value of leaves and flowers; white flecks on leaves and buds; leaves tarnishing, turning from green to various shades of brown and falling; decreased intensity of flowers' colour and brightness; silvery dots on the petals developing into stripes	[86]

4. *Rosa* L. Plants Viruses Control

The rose plant vulnerability to various diseases is due to its vegetative propagation (grafting, bud-grafting, cutting grafting, clonal micropropagation), whereby the infection is transmitted from a mother plant to a vegetative progeny [93,94] (Figure 4). When selecting cuttings for vegetative propagation or grafting, lignified young shoots from rose bushes not affected by pests and diseases are used. For propagation by cutting or in vitro clonal micropropagation, healthy plants that do not show the following damages are selected (Figure 4): changes in shape; shoot or flower deformations; changes in the leaf colour; no

manifestations of marginal necrosis, spotting, chlorosis and mosaic which are specific to viral infections (Figure 2A), and raise doubts about the sources of damage (Figure 2B).

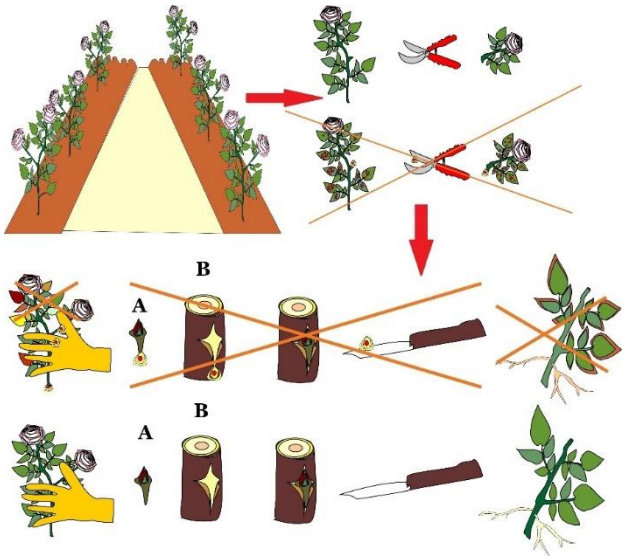

Figure 4. The most common causes of the viruses spread in nurseries and farms. (**A**) Scion. (**B**) Rootstock. For propagation, only intact fragments of cuttings of rose bushes are selected. Infected fragments can cause inevitable multiplication of infected and defective material resulting in a severe epiphytoty and viral spread in the nursery.

Due to the impossibility of visual identification of a viral infection, the following indirect precautions are taken to prevent viral diseases: work clothing and tool disinfection; plant residue destruction; disposal of the plants that may be a source of viruses (weed plants and cultivated plants showing signs of infection); control of insects and/or mites and/or nematodes as potential viral vectors; compliance with the spatial isolation regulations (in cases where several species of host plants are located in one area); use of virus-free planting material; observance of quarantine regulations in case of product expansion; obtaining virus-free planting material from reliable sources and/or its preliminary verification [59].

According to da Silva et al. [64], it is possible to remove the plant parts showing specific symptoms. However, this will not interfere with further progression of pathology since the plant is infected systemically and the signs of infection may manifest themselves on other organs or parts of the plant over time. Perennial plants showing clear signs of infection should be removed completely. However, in case of their spatial isolation from virus-free plants, cultivation does not pose a significant risk. If this is the case, it is important to disinfect the tools used for pruning or bud-grafting even in the absence of clear signs of infectious plant sap activity. It is also advisable to practice mixed planting where roses are planted next to other plants which are attractive to insect vectors in this way reducing the likelihood of infection spread [95].

4.1. Traditional Methods of Viruses Control

Measures to propagate essential oil roses implemented for example, by the Research Institute of Agriculture of Crimea include, among others, systemic precautions to prevent the spread of pests and diseases. The essential oil roses cultivation technology includes providing optimal conditions for their growth; implementing care measures at the right time; weed, insect and pest control, as well as the observance of quarantine regulations and preventive treatment of plants relocated from different sites of the nursery. In the selected area, the predecessor culture is harvested, and the stubble is broken as deep as 8–10 cm. Upon the emergence of weeds above ground repeated stubble breaking is carried out with the application of an herbicide (glyphosate), at a dose of 4–6 mL/ha^{-1}. In October, fertilizers such as ammophos at a dose of 200–400 kg/ha^{-1} are applied, and if possible,

organic fertilizers are used; finally, trench ploughing as deep as 40 cm is carried out. From March onwards, the field surface autumn fallow is to be maintained and the soil surface is to be cleared of weed seeds and vegetative rudiments; the area is cultivated three to five times. Prior to planting essential oil rose seedlings, the soil is harrowed as deep as 18 cm. Roses are planted in October-November (as well as during frost-free periods in winter) according to the scheme 3.0 × 0.85 m with a density of 4000 plants/ha^{-1}. For planting, selected conditioned plantlets previously dipped in a mash of clay and cow manure are used. The care for non-bearing plants (during the next year after planting) focuses, mainly, on intensive weed, disease and pest control. As weeds germinate, mechanical inter-row tillage is carried out as deep as 10–16 cm for 3–5 times. If weeds are dense inter-row weed pulling is carried out. In October–November, seedlings are underplanted manually, in the required quantity. In the second year after planting, in February–March, bushes are pruned along with the culling of bushes manifesting signs of infection and deformation and collecting samples for the diagnostic laboratory. Since the essential oil rose is used both for obtaining oil and producing jam, syrups and soaps, the care for plants during the harvesting period is limited to weeding, fertilizing and watering, without the use of chemical crop protection products that may affect the quality of the essential oil rose derivatives. The crop protection interventions involving the use of herbicides, fungicides or insecto-acaricides can be carried out only a month before or after the harvesting. Therefore, the identification of phytopathological damage and phytosanitary control, during the flowering period (from late May to early July) is limited to detecting and culling infected plants, along with intensive weeding. The strongest six or seven shoots are left on the plant, two of them located in the centre of the bush are cut 30–35 cm high from the soil surface, and the rest are cut 20–25 cm high from the soil surface. All the damaged and weak shoots are cut at the level of the soil surface. Plants manifesting obvious viral damage are discarded and burned. Agronomists inspect the plots on a weekly basis under the routine procedure for plant care, pruning and weeding. When finding suspicious spots and deformations, the location of the infected plant is noted; the disease manifestations in bushes are photographed and sent to the laboratory. The rose bushes manifesting clear signs of obvious disease symptoms are removed from the area to prevent disease spread. However, regular inspection and culling of low-quality material at all stages of plant cultivation does not rule out the presence and accumulation of a viral load. For this reason, planting material renewal is most effective if in vitro collection materials are used where valuable genotypes are preserved and multiplied by clonal micropropagation [4,96].

Figure 5 presents integrated data on traditional measures to control viral infections.

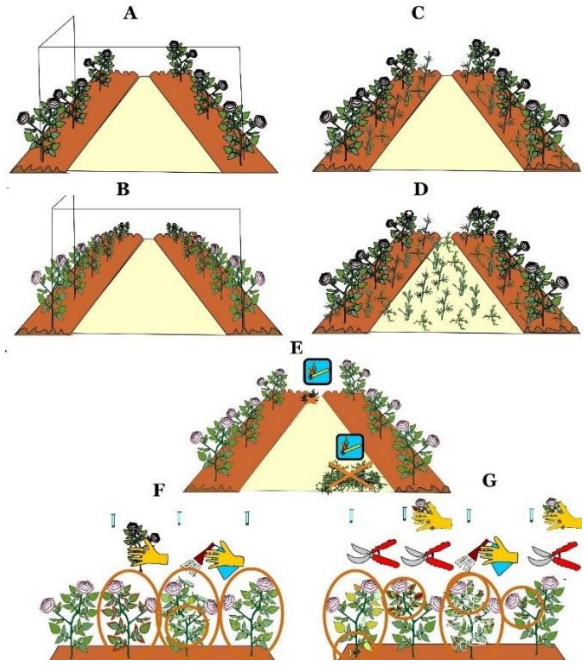

Figure 5. Compliance with the spatial isolation regulations and preventive agronomic practices applied to control viral diseases. (**A**) Plant distancing. (**B**) Row distancing. (**C**) Reducing the frequency of weeding to prevent the contact of pests with healthy plants. (**D**) Spacing other plants between rows. (**E**) Well-timed removal and disposal of weed plants. (**F**) Visual inspection, primary diagnosis, treatment and testing of the plants showing visual signs of damage. (**G**) Treatment and testing of the plants showing signs of a viral infection, removal of the infested plants.

4.2. Biotechnological Methods for Viruses Control

Virus-free planting material is produced through the use of biotechnological techniques: apical meristem culture methods including the use of thermotherapy or chemotherapy [97].

Apical meristem culture is based on the concept that the meristem is so structured that its upper layers (an apical meristem) give rise to cover tissues while its lower layers give rise to the conduction system. Due to the fact that these layers are compartmentalized the ability of a virus to penetrate the upper layers via the conduction system is limited. Viruses move along the vascular system at a higher speed; however, it is presumed that viral particles can slowly make their way into the upper layers via the plasmodesmata connecting the meristematic cells. Another reason for the absence of viruses in certain parts of the meristem is that cell division and virus multiplication and spread, occur at different rates. This is also the reason for the presence of certain viruses and strains in various parts of the meristem [98]. Clonal in vitro propagation is based on apical meristem culture. It is a cell engineering method whereby, within a short time, valuable cultivars are multiplied and introduced into production and virus-free planting material is produced. This method is also well-known for essential-oil roses [94,99,100].

Thermotherapy is based on inhibiting virus reproduction or preventing the viral particles' penetration into the re-growing parts of a plant by means of a high temperature, whereby the integrity of cell compartments are not impaired, and the damage to plant cells and tissues is minimized. There are a few variations of this method: (1) Hot water dipping. This method is applied to the resting parts of a plant (tubers, buds, cuttings). These parts are dipped in hot water as they are able to survive higher temperatures than actively vegetating plants. Upon thermotherapy the plant fragments are dried a little in the air, preferably in aseptic conditions. (2) The dry-air process is preferable for vegetating parts of a plant, whereby, they are exposed to warm air at temperatures of 35–40 °C over

several weeks. This method does not impair plant health and helps to produce virus-free shoot apexes, subsequently grafted on the rootstock or rooted [98,101].

Chemotherapy can be successful in combination with thermotherapy and apical meristem culture [98]. The best-known antiviral agent is ribavirin that inhibits replication of multiple animal and plant viruses [102]. The studies carried out by Yegorova [103] to look at the effect of virazole (ribavirin) identified features specific to the essential oil rose explants' morphogenesis in vitro, subject to the agent's concentrations in the culture medium. The chemotherapy conditions optimization, centres around the empirical identification of the virus-inhibiting agent's concentration and exposure time, taking into account the explant type and the explant treatment method. It is critical to minimize the negative impact of the agent on the explant. For two months the meristems and apexes isolated from developing shoots were exposed to chemotherapy with virazole at concentrations of 20.0–25.0 mg/L. A decrease in the number of leaves, buds and developing explants, as well as the shoot length, by 1.2–2.7 times compared to the control group was observed. At the same time, on further micropropagation of viable shoots, the development of the plants grown with chemotherapy scarcely differed from the control group. This points to the possibility of using virazole during the stated period, and in the empirically identified concentration, for essential oil rose chemotherapy when carrying out sequential cultivation of the meristems and shoot apexes.

Mitrofanova et al. [98] described a model system for viral elimination in flower crops comprised of the following basic elements: screening the plants for viruses, thermo- or chemotherapy, apical meristem culture, the adapted plants retested for viruses. This model involves the following stages:

- Mother plant diagnosis using test plants, electron microscopy, ELISA and PCR techniques.
- In case the plant is infected, thermotherapy in vitro, or in vivo at 37 °C for 4–15 weeks, or chemotherapy with virucides in vitro.
- Plant tissue culture growth and plant regeneration on artificial nutrient media over 14–20 weeks.
- Regenerated plants adaptated in vitro at 15–20 °C over 3–4 weeks.
- The adapted plants retested using the test plants, ELISA and PCR techniques.
- Obtaining of virus-free plants and their certification.

5. Key Points and Current Prospects for Viral Disease Control in Essential Oil Rose Cultivation

Compliance with the regulations for agricultural machinery maintenance and operation, an ongoing monitoring of damage to plants, and comprehensive diagnostic interventions (including ELISA and PCR-based diagnostics of phenotypically identified manifestations of the diseases affecting a proposed planting material) may help prevent significant damage to plants and inhibit viral infection spreading. However, these interventions are economically feasible and appropriate only in the case of a significant decrease in productivity caused by disorders in the shoots, buds and flower development (apparent only in cases where the plants are affected by 'viruses causing disorders in the plants' development). Such manifestations are described for *Prunus necrotic ringspot virus* [71], *Rose leaf curl virus* [29], *Rose rosette virus* [31], *Rose leaf rosette-associated virus* [44], *Impatiens necrotic spot virus* [53], *Raspberry ringspot virus* [55].

Currently, a negative impact of viral diseases on rose essential oil production and its qualitative indicators, remains unevaluated, which testifies to the relevance of such studies [8]. Viral infection monitoring remains an expensive and not readily available or affordable intervention. On the other hand, a moderate ignorance of such damage can be considered, as the adverse effects of a number of viral diseases such as losses in the essential oil quantity and quality may be less significant compared to the implementation of a full range of antiviral interventions involving the use of expensive assessment and monitoring methods, with participation of external experts. Interventions for essential oil rose viral infection control may include up-to-date genetic engineering methods, such as CRISPR-Cas technology. This will

enable in the longer term, a total block of one of the phases of viral assembly or replication, by inhibiting the activity of the cell systems used by the virus for replication, transport or other key mechanisms of the infection process [104,105]. It is seen as feasible for essential oil roses as clonal crops. For example, antisense technology, RNA interference-based technology or modulation of the activity of cell processes, particularly, the processes causing dissociation of the coat protein from the viral nucleic acid could be used [106,107].

6. Conclusions

Care for essential oil roses as valuable agricultural crops requires the availability of sophisticated schemes for preventing and managing various diseases, including viral infections. Research activities carried out by the Research Institute of Agriculture of Crimea, as the owner of a unique collection of essential oil crops, focus on implementing an effective system for protecting these valuable plants against pathogens. The literature provides scarce evidence of the viral diseases specific to essential oil roses, however, the affiliation to genus *Rosa* L. suggests typical manifestations of viral diseases in representatives of this taxon. There are *Rosa* L. viruses specific to this crop and typical of other plant species. They cause both localized damage (leaf discoloration) and systemic changes (impaired plant health). The best-known manifestation of the *Rosa* L. plants' viral diseases is the rose viral mosaic caused by three pathogens (PNRSV, ApMV and ArMV) which may be present in plants individually or collectively. Living organisms contribute significantly to the viral infections' spread. Aphids, nematodes, mites and thrips are the experimentally proven and suspected vectors of viral infections in *Rosa* L. species. However, their role in certain cases is superficialized, which leaves open the issue of many known viruses' transmission. The essential oil rose viral infections control is complicated by the lack of a general technique for eradication of the viruses. Therefore, indirect methods based on the disinfection and disposal of the materials potentially infected with viruses, and production of virus-free plant material as well as standard and up-to-date techniques emerging from molecular biology and biotechnology are used. Unfortunately, an important limitation in plant viral disease control is the high costs for virus detection and inactivation with the use of up-to-date methods, which are more accurate and effective as compared to the classic approaches. Therefore, development of both efficient and economically feasible techniques for essential oil rose viral disease control is the goal of scientific research carried out in this area.

Author Contributions: Conceptualization, S.S., E.N.B., N.N., N.Y. and S.A.; methodology, E.N.B., N.N. and N.Y.; software, S.S.; validation, E.N.B., A.A.G. and L.R.; research, S.S., E.N.B. and S.A.; resources, S.S. and E.N.B.; data curation, E.N.B. and A.A.G.; writing—original draft preparation, S.S. and A.A.G.; writing—review and editing, S.S., E.N.B., A.A.G., N.N. and S.A.; visualization, S.S.; supervision, S.S. and E.N.B.; project administration, V.P. and L.R.; funding acquisition, V.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was carried out with the support of the Ministry of Education and Science of the Russian Federation under scientific projects No FNZW-2022-0006 (Research Institute of Agriculture of Crimea); 0431-2022-0003 (All-Russia Research Institute of Agricultural Biotechnology) and 122011400178-7 (Tsitsin Main Botanical Garden of Russian Academy of Sciences).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Genetic Structure of the Mosaic Viruses

Appendix A.1. Prunus Necrotic Ringspot Virus

The functional singularity of this virus is due to the features specific to RNAs making up its whole genome, particularly, the smallest RNA-3. PNRSV like all other representatives of the family Bromoviridae has a genome comprised of three segments of positive-sense single-stranded RNA: RNA-1, RNA-2 and RNA-3. RNA-1 and RNA-2 are monocistronic and encode the non-structural proteins involved in viral RNA synthesis. RNA-3 is bi-

cistronic and encodes the movement protein and the coat protein where the movement protein is translated directly from RNA-3 while the coat protein is translated from sub genomic mRNA-4) [25,69,70,108,109]. The features of the PNRSV genomic RNAs are presented in Table A1.

Table A1. Characteristics of the genomic RNAs of *Prunus necrotic ringspot virus*.

RNA Type	RNA Chain Length (Number of Nucleotides)	Encoded Protein
RNA-1	3332	Replicase P1 protein Methyltransferase/helicase)
RNA-2	2594	Replicase P2 protein RNA-dependent RNA polymerase
RNA-3	1951	Movement protein P3a and the coat protein

The methyltransferase/helicase domain is conserved and contains several sequence motifs which are retained in the ilarviruses and are essential to the P1 protein functionality. The RNA-dependent RNA polymerase domain, for its part, contains eight conserved motifs essential to the positive-sense RNA-viruses' replication [73].

The study of the PNRSV genome was initially based on the common features of the representatives of genus *Ilarvirus* which lies in the fact that the coat protein (hereinafter referred to as CP) is the initiator of the viral genome replication in host plants. If this is indeed the case, a specific interaction of the N-terminal part of CP with the 3′-terminal sequences of the viral RNA-3 occurs.

It was assumed that the hairpins flanked by the AUGC sequences near the 3′-termini of the genomic RNAs, were responsible for the specific binding with CP. The experimentally induced mutations in the AUGC-box impaired the RNA ability to bind with CP [110,111]. The functional area for the genome activation in the CP structure is the zinc-finger domains comprised of a complex of four amino acids (two histidines and two cysteines) and zinc ions [112].

Based on this data, Guo et al. [108] identified the complete nucleotide sequence of PNRSV RNA-3. It was established that it consists of 1943 nucleotides and has two large open reading frames (hereinafter referred to as ORF). The 5′-proximal ORFa begins with the 174th nucleotide and ends with the 1023–1025th nucleotides, while the 3′-proximal ORFb begins with the 1100th nucleotide and ends with the 1772–1774th nucleotide. The 5′-proximal ORF3a encodes the movement protein P3a; the 3′-proximal ORF3b encodes CP.

The 3′-noncoding region of 169 nucleotides, called the 3′-NCR, was studied also. Its terminal sequence consisting of 18–23 nucleotides is common for the representatives of genus *Ilarvirus*. Presumably, this particular region is able to form the hairpins flanked by the AUGC-boxes which constitute the binding sites with a high affinity for CP. Based on the data obtained, the authors presumed that this particular common structural feature of the 3′-NCR RNAs of the genus *Ilarvirus* representatives, may be responsible for the specific interaction with the coat protein resulting in the genome activation.

When investigating the CP gene structure, it was discovered that its N-termini includes motifs of the above-mentioned zinc-finger domains involved in binding the genomic RNA when the genome replication is encapsidated and activated.

The study of the coat protein is essential to virology research. The diversity of plant viruses is due to their genetic variability that exists behind the virus isolates. The viruses' diversification analysis is very important for developing techniques for viral pathologies management and control. In this respect the virus coat protein gene due to its singularity and multi-functionality is one of the most common molecular markers for investigating the genetic diversity and molecular evolution of plant viruses [113].

In 1997 Sánchez-Navarro and Pallás [114] carried out a comparative phylogenetic analysis of the coat protein sequences in all the representatives of the family Bromoviridae.

Their findings showed a very close affinity between CP PNRSV and the ilarviruses ApMV and TSV. It was also noted that PNRSV and ApMV are closely related, both with regard to the amino acid sequence of their coat proteins, especially taking into account that both viruses have a very similar spectrum of natural hosts (fruit trees from the genus *Prunus*).

Appendix A.2. Apple Mosaic Virus

The ApMV genome structure is similar to that of other ilarviruses and is presented in Table A2.

Table A2. Characteristics of the genomic RNAs of *Apple mosaic virus*.

RNA Type	RNA Chain Length (Number of Nucleotides)	Encoded Protein
RNA-1	3476	Methyltransferase/helicase)
RNA-2	2979	RNA-dependent RNA polymerase
RNA-3	2056	Movement protein and the coat protein

In 1994 Sánchez-Navarro and Pallás [115] identified the complete nucleotide sequence of the ApMV subgenomic RNA-4 with a view to investigating the genetic affinity of ApMV with other representatives of genus *Ilarvirus*. This sequence comprises 891 nucleotides and one ORF beginning with the 43–45th nucleotide and ending with the 721–723rd nucleotide. The ORF encodes the coat protein, having in its structure a motif rich in cysteine and histidine and forming a zinc finger tetrahedral zinc complex. The N-terminal domain of the coat protein is cationic while the C-terminal domain is negatively charged. The N-terminal domain binds with the 3′-terminal region of RNA, while the C-terminal acid domain may interact with the replicase complex and enable its contact with the genomic RNA.

3′-regions of RNA-4 comprise several hairpin structures flanked by the AUGC sequence. The CP binding with this region initiates a cycle of replication. Thus, the RNA-4 secondary structure confirms the assumption that "the genome activation" process is a common mechanism for ilarviruses.

In 1995 Shiel et al. [116] identified the complete nucleotide sequence of ApMV RNA-3. It is 2056 bases long and comprises two ORFs. One ORF encodes the movement protein. The other encodes CP and is transcribed into sub genomic RNA-4. The 5′-noncoding region of RNA-3 comprises a 15-base sequence, suggestive of the internal control region of the eukaryotic tRNA gene promoters.

The 3′-termini of all the ilarviruses end with the AUGC sequence essential to the coat protein recognition [110,111]. In contrast, ApMV RNA-3 ends with the AGGC tetranucleotide instead of the AUGC sequence. Nevertheless, the AGGC tetranucleotide is also present in the line of 18 bases above the 3′-terminal.

In 2000 Shiel and Berger [117], in a continuation of their work, described ApMV RNA-1 and RNA-2. RNA-1 comprises 3476 nucleotides and encodes one large polypeptide comparable to the methyltransferase-like and helicase-like domains present in many plant RNA-viruses. ApMV RNA-2 is made up of 2979 bases and encodes the RNA-dependent RNA polymerase.

Appendix A.3. Arabis Mosaic Virus

Arabis mosaic virus includes a positive-sense genome composed of two RNAs the translation of which results in two polyproteins performing the function of predecessors. Both the RNAs are polyadenylated at the 3′-terminal and have a covalently attached viral protein VPg at the 5′-terminal. RNA-1 encodes a protease which breaks down polyproteins into functionally active units. The end products of the RNA-1 activity include 1A, 1B, 1CVPg (VPg), 1Dpro (proteinase) and 1Epol (polymerase). The RNA-2 activity results in the end products as follows: 2A (involved in RNA-2 replication), 2BMP (the movement protein) and 2CCP (the coat protein) [79,118].

Gao et al. [113] stated that polyprotein P1 encoded by RNA-1 breaks down into six proteins identified as X1 (functions are unknown), X2 (a putative protease cofactor), NTB (nucleotide triphosphate-binding protein), VPg, Pro (3C-like proteinase) and Pol (RNA-dependent RNA polymerase). Polyprotein P2 encoded by RNA-2 is broken down by RNA-1-encoded protease into three functional fragments: the homing protein (2A), the movement protein (MP) and the coat protein (CP).

Although ArMV is regarded as one of the viruses causing rose mosaic, the literary sources lack the data concerned with experimental study of the ArMV isolated specifically in rose plants, as was previously the case with PNRSV and ApMV. This is due to the fact that this virus is associated with grape viral diseases [76].

In 2001 Wetzel et al. [118] cloned and sequenced RNA-2 of ArMV-NW isolates affecting grapevine. It was established that the complete sequence of ArMV-NW RNA-2 comprises 3820 nucleotides except for the poly(A) tail. The analysis of the putative open reading frames (ORF) showed the availability of one large ORF (from 296 to 3626 nucleotides). The study of amino acid sequences identified the availability of putative Cys/Ala and Arg/Gly proteolytic cleavage sites for the ArMV-NW polyprotein.

Moreover, in the 5′-noncoding regions there were conserved repeats capable of forming hairpins present in RNA-2 from other isolates identified. Similar structures were found in the 5′-noncoding regions of other nepoviruses, however, their role is not yet clear. The study of RNA-2-encoded polyprotein showed the availability of three domains corresponding to the RNA activity products: N-terminal, central and C-terminal.

In 2003 Wetzel [79] presented the structure of RNA-1 of ArMV-NW isolates. The complete nucleotide sequence of RNA-1 comprises 7334 nucleotides except for the poly(A) tail. There is one ORF composed of 228-7079 nucleotides. Conserved sequences comparable to the stem-loop structures identified in the 5′-noncoding regions of RNA-2 [118] were found as well in the 5′-noncoding regions of RNA-1. The analysis of RNA-1-encoded polyprotein, identified motifs of the viral protease cofactor domain, NTP-binding domain, viral protease domain and RNA-dependent RNA polymerase domain.

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