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Aphid transmitted viruses of raspberry in Norway: detection, occurrence, and vector association

Bijaya Sapkota
M.Sc. Plant Sciences

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Abstract

Raspberry (*Rubus idaeus* L.) is the high value horticultural crop, which is susceptible to multiple viruses, including aphid borne viruses, that lead to yield loss. Thus, proper identification of relevant viruses and their possible vector is essential to reduce disease and loss.

Symptomatic samples of red raspberries including aphid samples from three different counties (Vestland, Agder and Viken) of Norway were analyzed for the presence of four important aphid-transmitted raspberry viruses: black raspberry necrosis virus (BRNV), raspberry leaf mottle virus (RLMV), raspberry vein chlorosis virus (RVCV) and rubus yellow net virus (RYNV). Altogether, 95 leaf samples and 31 aphid samples from three main cultivated cultivars (Glen Ample, Glen Mor and Veten) and samples of wild raspberry plants were tested by RT-PCR, using virus-specific primers. BRNV was the most prevalent virus in leaf samples in all counties, comprising 89% of infection followed by RLMV (28%) and RVCV (8%). BRNV and RVCV were found in all selected counties, but RLMV was detected only in Vestland. The obtained result showed a high proportion of mixed infections, the most common involving BRNV and RLMV (24.3%), followed by the mixed infection of BRNV and RVCV (1.4%). Furthermore, all 3 main cultivars and samples of wild species of raspberry were detected with at least one of the viruses mentioned. Apart from this, 13 out of 31 aphid samples which include both *Amphorophora (Am.) idaei* and *Aphis (Ap.) idaei.* were found infectious with BRNV and RLMV.

For the first time, BRNV and RLMV were detected in *Ap. idaei*. So, to gather more information in biology of this aphid species and its role in BRNV transmission, virus free *Ap. idaei* were reared in culture rooms of NIBIO and were transferred to virus free raspberry plants using different acquisition and inoculation time periods. *Ap. idaei* acquired BRNV after at least one hour and lose it after at least five hours of starvation. However, the transmission of BRNV by *Ap. idaei* was not proven. This thesis will contribute to a better understanding of virus diseases and their vectors in raspberry production in Norway.

Abbreviations

ANNOVA	Analysis of Variance
BLAST	Basic local alignment search tool
BRNV	Black raspberry necrosis virus
CAS	Czech academy of sciences
COI	Cytochrome oxidase subunit 1
DMART	Duncan's Multiple Range Test
DNA	Deoxyribose nucleic acid
dNTP	deoxyribonucleoside triphosphates
EDTA	ethylenediaminetetraacetic acid
FAOSTAT	Food and agriculture organization corporate statistical database
NCBI	National center for biotechnology information
NIBIO	Norwegian institute of bioeconomy research
PARC	Pacific agri-food research centre
RLMV	Raspberry leaf mottle virus
RMD	Raspberry mosaic disease
RNA	Ribonucleic acid
RVCV	Raspberry vein chlorosis virus
RYNV	Rubus yellow net virus
SSIV	Superscript IV
TBE	Tris-borate-EDTA

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1. Introduction

1.1 Background

Raspberry (*Rubus idaeus* L.) is an economically important perennial crop with the annual production of 895,771 tonnes in 2020 worldwide (FAO, 2022). Due to its high nutritional, dietary, and medicinal values, raspberry consumption has soared in Europe and North America recently (Bobinaitė, 2016). Raspberries are almost always in short supply despite a high demand for them (Bojkovska et al., 2021). In Norway, raspberry production accounts around 2400 tonnes, with an estimated value of around US$ 16 million (around 157 million NOK) in 2019 (FAO, 2022). Raspberry and other *Rubus spp.* are particularly susceptible to many pathogens including viruses (Wilson, 2014).

Virus is the second most important plant pathogen group worldwide after fungal plant pathogens (Vidaver & Lambrecht, 2004), and causes the economic loss of billions of US dollars worldwide annually (Jeong et al., 2014; Sastry & Zitter, 2014). Viruses are rarely transmitted through the direct contact between the plants, but through vectors, mechanical injury, and propagation (Mattew & Hull, 2002). Aphids are well known vector of various plant viruses including raspberry viruses (Martin et al., 2013). Raspberry is infected with multiple viruses among which, aphid transmitted viruses are responsible for most of the decline, especially black raspberry necrosis virus (BRNV), raspberry leaf mottle virus (RLMV) and rubus yellow net virus (RYNV) all of which cause raspberry mosaic disease, RMD (Alford, 2007; Converse et al., 1987). RMD is used to describe a range of diseases caused by the various combination of viruses transmitted by aphids (Wilson, 2012). The viruses of RMD complex are transmitted by *Amphorophora (Am.) agathonica* in North America and *Am. idaei* in Europe (Martin et al., 2013). Beside these viruses, raspberry vein chlorosis virus (RVCV) which is transmitted by *Aphis (Ap.) idaei* (Martin et al., 2013) is another important aphid borne virus which is studied in this paper.

Several aphid transmitted viruses (mainly BRNV) have been found to be prevalent in raspberry plants in Norway (NIBIO, unpublished data). The raspberry production and yield both seems decreasing over the last few years (Fig 1). Apart from this, Nordic environment have a greater threat of climate change (Uleberg, 2016) which can cause important changes in the interaction between viruses, vectors and their host plants posing risks of new strains of viral disease and

outbreak (Amari et al., 2021). Thus, a better virus management strategy is necessary to develop the healthy raspberries in Norway.

Vector borne viruses have a close relationship with their vector, which is their primary means of transmission to a new host (Lightle & Lee, 2014). *Am. idaei* which is the important aphid vector in Europe have been well studied because of its effectiveness in transmitting the viruses in the RMD complex. However, there is a lack of recent studies on the status and transmission mode of *Ap. idaei* in raspberry (Martin, 2013). Literatures suggest that the *Ap. idaei* is able to transmit RVCV only (Cadman & Hill, 1947; Cadman, 1952a; Stace-Smith, 1961;). However, in recent research BRNV was detected in this aphid by RT-PCR, no transmission role has yet been proven (Personal communication, kappa project).

NIBIO, in collaboration with Biology Center CAS, Czech Republic, is working on the project called "Healthy berries in a changing climate: development of new biotechnological procedures for virus diagnostics, vector studies, elimination and safe preservation of strawberry and raspberry" (NOBERRYVIRUSCZ / also called KappaBerry in Norway) for the period 2021-2024 within the bilateral KAPPA program (Personal communication, Kappa project). In this period, the raspberry (and strawberry) samples from different parts of Norway have been collected each year to get the knowledge of virus prevalence. On the basis of this knowledge, they are working on the best management option. This thesis was within the framework of this project. An assessment included in this research helps to overview the aphid transmitted raspberry viruses of Norway. Furthermore, it can help to uncover the role of aphid vectors in broader terms, which can contribute in developing new approaches to control aphid populations.

1.2 Objectives

- To survey the occurrence and distribution of aphid transmitted raspberry viruses in raspberry and aphids in selected counties of Norway.
- To investigate the potential of aphid *Ap. idaei* as a vector of BRNV.

2. Literature

2.1 Raspberry

Raspberry is a soft fruit perennial crop which belongs to Rosacea family under *Rubus* genus. There are about 250 species within *Rubus* genes (Mabberley, 2017) but among them European red raspberry, North American red raspberry and black raspberry are grown commercially in large scale (Bobinaitė, 2016). Raspberry plants have the life span of about 10-15 years whereas black raspberry plants have a shorter commercial lifetime (2-3 harvest seasons) (Bobinaitė, 2016). Raspberries have perennial roots and crowns whereas, stems (canes) are biennial. Raspberries can be divided into two types i.e., primocane fruiting cultivars and floricans fruiting cultivars which is basically termed as annuals and perennial cultivars (Jennings, 1998). They have an aggregate fruit composed of individual drupelets with very pleasant taste. They are a good source of many nutrients, minerals, vitamins, fatty acids, and different polyphenolic phytochemicals. Besides this, raspberries contain high level of antioxidants, dietary fiber and fructose which contribute to numerous health benefits (Bobinaitė, 2016). In raspberries, temperature between 18°C to 21°C are ideal for leaf growth and temperature between 24°C to 26°C are ideal for root growth (Perasović, 2013).Both root and shoot are sensitive to high temperature (Jennings, 1998). Due to chilling requirement and inability to tolerate hot summer, raspberries do not do well in southern climates (Perasović, 2013).

2.1.1 Raspberry production in Norway

With the production of 2060 tonnes per year in 2020, Norway is one of the important raspberries producing countries in Europe (FAO, 2022). The trend in production as well as yield of raspberry production in Norway has now been decreasing in the last five years (Fig 1). Currently , three summer fruiting cultivars dominate the raspberry industry in Western Europe, Glen Ample from the James Hutton Institute, Tulameen from the Pacific Agri-Food Research Centre (PARC) in Canada, and Octavia from East Malling Research (Perasović, 2013). The Norwegian market is dominated by the Scottish-bred cultivar Glen Ample, released in 1996, replacing the hardy Norwegian variety Veten (Heiberg et al., 2002). Bøthun & Heiberg (2004) describes the different berry production regions of Norway. According to this paper, the main growing area for raspberries in Norway is in the west, in the fjord district of Sogn og Fjordane (recently, named as Vestland) where the climate has proved to be very suitable for raspberry production, with high yield and fruit quality and little winter damage. In this paper, it is also

described that as we go further north along the coastline, summers get cooler, but winters are similar to those in the main growing district. Glen Ample is performing well in organic outdoor production as far north as Brønnøysund in Nordland (65º N) (Bøthun & Heiberg, 2004). Rain and wind, however, are annually damaging the harvest, reducing its yield. The use of plastic tunnels can be a better option. Production in tunnels has made it possible to grow raspberries during winter in Southern Europe and extend the harvest season in Northern Europe (Oliveira et al., 1996; Allen & Raffle, 2000).

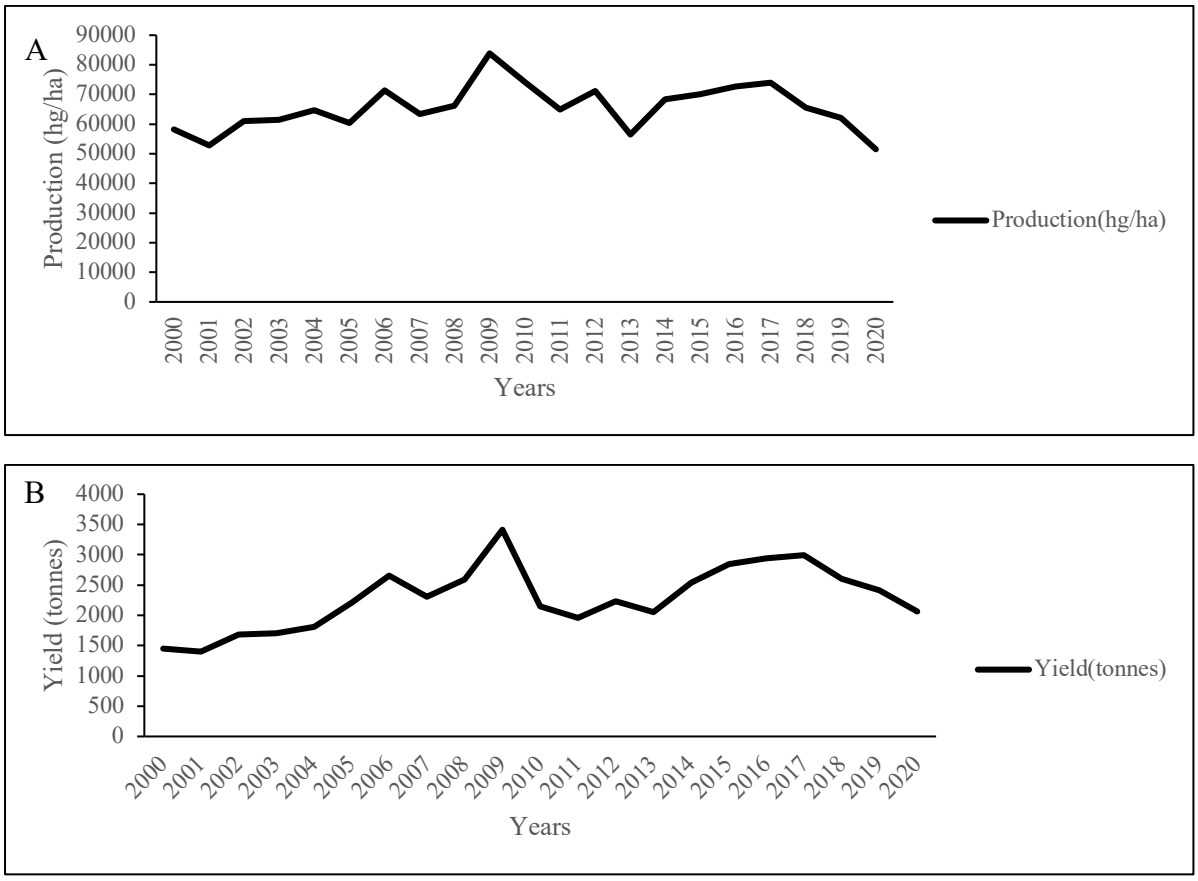


Fig 1. A. Total raspberry production in Norway. B. Total raspberry yield in Norway.
Source: (FAOSTAT).

2.2 Plant viruses of *Rubus*

Plant viruses are submicroscopic infectious obligate parasites of plant cells that generally results in disease within their plant hosts (Matthews & Hull, 2002). They are responsible for considerable losses by infecting the plants and reducing the quality and/or quantity of harvested plant products, including raspberry (Matthews & Hull, 2002). Till now, 22 plant viruses from different families and genera are known to infect raspberry (Table 1).

Table 1. List of all 22 different viruses that are known to infect Raspberry.

Virus Name	Family
Apple mosaic virus (ApMV)	*Bromoviridae*
Arabis mosaic virus (ArMV)	*Secoviridae*
Blackberry virus Y (BVY)	*Potyviridae*
Black raspberry necrosis virus (BRNV)	*Secoviridae*
Cherry leaf roll virus (CLRV)	*Secoviridae*
Cherry rasp leaf virus (CRLV)	*Secoviridae*
Cucumber mosaic virus (CMV)	*Bromoviridae*
Raspberry bushy dwarf virus (RBDV)	unassigned
Raspberry latent virus (RpLV)	unassigned
Raspberry leaf blotch virus (RLBV)	*Fimoviridae*
Raspberry leaf curl virus (RpLCV)	unassigned
Raspberry leaf mottle virus (RLMV)	*Closteroviridae*
Raspberry ringspot virus (RpRSV)	*Secoviridae*
Raspberry vein chlorosis virus (RVCV)	*Rhabdoviridae*
Rubus yellow net virus (RYNV)	*Caulimoviridae*
Sowbane mosaic virus (SoMV)	*Solemoviridae*
Strawberry latent ringspot virus (SLRSV)	*Secoviridae*
Strawberry necrotic shock virus (SNSV)	*Bromoviridae*
Tobacco ringspot virus (TRSV)	*Secoviridae*
Tobacco streak virus (TSV)	*Bromoviridae*
Tomato black ring virus (TBRV)	*Secoviridae*
Tomato ringspot virus (ToRSV)	*Secoviridae*

Source: (Martin et al., 2013; Tan et al., 2022)

2.2.1 Black raspberry necrosis virus (BRNV)

Black raspberry necrosis virus, BRNV (species: *Black raspberry necrosis virus*, genus: *Sadwavirus*, family: *Secoviridae*) was first descried by Stace-Smith (1955b). It has two viral RNA molecules encapsulated in 30-nm spherical particles (Martin et al., 2013). McGavin et al. (2010) identified the complete nucleotide sequence of a UK strain of the BRNV, by amplification and sequencing of virus RNA from infected plants. BRNV is distributed worldwide and often occurs rapidly in Britain (Cadman & Fisken, 1958; Jones & Murant, 1972;

Jones & Roberts, 1977). The host range of the virus is restricted to Rubus species where it causes chlorosis along the leaf veins (Halgren, 2007; Martin et al., 2013). On its own, BRNV induces indistinct symptoms, or no symptoms, in the red raspberry cultivars tested in Britain but it causes apical necrosis in shoots of the indicator species *R. henryi* and *R. occidentalis* (Jones & Jennings, 1980a). Jones & Murant (1972) reports the mechanical transmission, but with difficulty, in one herbaceous plant i.e., *Chenopodium quinoa*. In nature, it is transmitted by the raspberry aphids, *Am. agathonica* in North America and *Am. idaei* in Europe in a semi-persistent manner (Stace-Smith, 1955b; McMenemy, 2009). A combination of meristem culture and thermotherapy is commonly used to eliminate BRNV from mother plants contaminated with the virus (Baumann, 1982). In vitro thermotherapy of explants without meristem tip culture was sufficient to eliminate this virus (Cheong et al., 2014).

2.2.2 Raspberry leaf mottle virus (RLMV)

Raspberry leaf mottle virus, RLMV (species: *Raspberry leaf mottle virus*, genus: *Closterovirus*, family: *Closteroviridae*) was first described by Cadman (1951). It have a very large RNA genome packaged into a long, flexuous particle (Martin et al., 2013). It is widespread in raspberry in Great Britain and in many European countries (Cieślińska, 2021). RLMV has been experimentally transmitted to several Rubus species and cultivars tested have been found susceptible (Jones and Jennings, 1980b). In nature, It is transmitted by aphid *Am. idaei* in Europe and cause tip necrosis in black raspberry but can be differentiated from BRNV because they are symptomless in most red raspberry cultivars (Martin et al., 2013). Infected raspberry plants can be free from infection with this virus by thermotherapy (Chambers, 1954; Jordovic, 1963).

2.2.3 Rubus yellow net virus (RYNV)

Rubus yellow net virus (species: *Rubus yellow net virus*, genus: *Badnavirus*, family: *Caulimoviridae*) was first isolated and described from naturally infected Himalaya blackberry in British Columbia which was later found in raspberry as a component virus of the raspberry mosaic disease complex and named as RYNV by Stace-Smith (1956). RYNV is common and has been known for many years in North America and Europe (Cadman, 1961). Particles of RYNV are bacilliform, 80 to 150 nm long and 25 to 31 nm wide, as visualized in thin sections of infected raspberry leaves (Jones & Roberts, 1976; Stace-Smith & Leung, 1976). Infected plants develop a net-like chlorosis along the veins, giving the plant a pale green appearance

whereas, some of the leaves are slightly cupped downward (Stace-Smith, 1955a). RYNV is readily transmitted by the aphid vectors, *Am. agathonica and Am. idaei*, but it has not been transmitted mechanically (Converse et al., 1970; Stace-Smith & Jones, 1978). Unlike other viruses transmitted by *Amphorophora species*, it is usually not inactivated by exposure to an air temperature of 37°C for several weeks (Converse, 1966; Jones & Roberts, 1976), but it can be eradicated from small meristem tip cuttings following treatments at 37° to 39°C for 4 to 14 weeks (Mellor & Stace-Smith, 1979).

RMD is induced by infection with a complex of viruses (Table 2). In Canada, BRNV and RYNV together reported to induce raspberry mosaic disease whereas, in Europe additional RLMV and raspberry leaf spot virus (RLSV) were involved (Stace-Smith, 1956*)*. Prior to their genetic similarities, RLMV and Raspberry leaf spot virus (RLSV) were regarded as separate viruses because of their different symptoms, but now, they are considered to be identical isolates (McGavin & MacFarlane, 2010). Yield losses due to RMD in some red raspberry cultivars were 11 to 14% in British Columbia (Freeman & Stace-Smith, 1970*)* and 39% in Maryland (Converse, 1963*)*.

Table 2. Particle morphologies of viruses probably involved in the raspberry mosaic disease complex (RMD).

Virus	Morphology	Dimensions	Reference
RYNV	Unenveloped bacilliform particle with rounded ends	80-150 * 25-31 nm	(Stace-smith & Leung,1976; Jones & Roberts, 1976)
BRNV	Isometric	25-30 nm	(Jones & Murant, 1972)
RLMV	Possibly isometric	30 nm	(Jones, 1976)

2.2.4 Raspberry vein chlorosis virus (RVCV)

Raspberry vein chlorosis virus, RVCV (species: *Raspberry vein chlorosis virus*, genus: *Cytorhabdovirus*, family: *Rhabdoviridae*) was first described by Cadman (1952a). It is the bacilliform virus with enveloped particles, measuring c. 430-500 x 65-80 nm in sections of raspberry. (Stace-Smith & Lo, 1973). It is common in Europe (Cadman, 1952a), Canada (Stace-Smith, 1961) and New Zealand (Cadman & Stace-Smith, 1970). In most cultivars the virus induces a chlorosis of the minor leaf veins to form a yellow net pattern (Martin et al.,

2013). The virus has few hosts apart from *R. idaeus* however it has been transmitted by grafting to loganberry (*R. loganobaccus*) and by aphids to *Fragaria vesca* (Stace-Smith, 1961). This virus is transmitted by *Ap. idaei* but not by the other main aphid vectors of Rubus viruses (Cadman & Hill, 1947; Cadman, 1952a; Stace-Smith, 1961). Unlike most other aphid-borne viruses occurring in Rubus, it is not inactivated in infected raspberry plants by exposure to 37°C for several months (Stace-Smith, 1960).

2.3 Insect transmission of plant viruses

Movement of plant viruses between plant hosts is an essential requirement for virus survival and proliferation and for expansion of disease epidemics (Wilson, 2013). A diverse range of virus vectors have been discovered where, insects, mites and nematodes are considered the most common (Dietzgen et al., 2016). Watson & Roberts (1939) proposed a system to classify transmission of plant pathogens by insects. They classified viruses into 2 groups, non-persistent and persistent, to recognize the differences in the length of time that vectors, once having acquired viruses, remain inoculative. Later, they are further divided and classified under 4 categories 1) non-persistent, 2) semi-persistent, 3) circulative persistent and 4) propagative persistent (Berger & Ferriss, 1989).

Non-persistent transmission is the most common association found between plant viruses and their aphid vectors (Nault, 1997). Apart from aphid, no other biological vector transmits the plant virus in a non-persistent manner (Nault, 1997). Viruses that are transmitted non-persistently are rapidly acquired from the plant and are transmitted (inoculated) to plants equally fast (Table 3). These viruses are acquired during the brief probes (or tasting; host finding behavioral response where the aphid determines the suitable host) into the epidermal cells of the virus source plant prior to settled feeding from the phloem (Namba, 1962). Potyviruses, carlaviruses, fabaviruses, alfamoviruses, carlaviruses, cucumoviruses and maculuraviruses have virus species that can be transmitted in a non-persistent manner (Wilson, 2014). Semi-persistent virus requires a longer acquisition and transmission access period than non-persistently transmitted viruses (Table 3). Member of caulimoviruses, closteroviruses and sequiviruses can be transmitted in this manner by aphid vectors (Wilson, 2014). In the circulative persistent transmission manner, viruses enter and circulate within, but doesn't replicate in their aphid vector (Kennedy et al., 1962; Sylvester, 1958). Viruses from two main families i.e., *Luteoviridae* and *Nanoviridae* are transmitted in this manner (Wilson, 2014).

Propagative persistently transmitted viruses enter, circulate, and are retained by their vectors for extended period, probably for the life of the insect (Nault, 1997). Generally, member of *Rhabdoviridae* and *Reoviridae* falls under this group (Wilson, 2014).

Table 3. Characteristics of insect transmission by plant viruses.

Characters	**Non persistent**	**Semi persistent**	**Circulative persistent**	**Propagative persistent**
Feeding period required for acquisition	Seconds-minutes	Minutes-hours	Hours-days	Minutes-days
Feeding period required for transmission	Seconds-minutes	Minutes-hours	Hours-days	Minutes-hours
Site of virus association in vector	Stylet tip	Foregut or stylet	Uptake from gut, circulates within hemocoel	Invade various insect tissues.
Capable of replication in the vector	no	no	no	Yes

Source: Wilson, 2013.

2.3.1 Aphid vectors of raspberry viruses

Aphids are the most common vector of plant viruses, vectoring over 60% of transmitted viruses (Matthews, 2002). There are around 300 species of aphids identified as a vector (Tan et al., 2022). It is evident that aphids and viruses are intimately connected, which reflects the biology of the aphids and their behavior while feeding (Wilson, 2013). They have piercing and sucking type of mouth and may transmit the viruses in all four modes of transmission described in table 3. (Matthews, 2002; Wilson, 2013). However, most species of aphids transmit virus through the stylet-borne, non-persistent mechanism (Butter, 2018). Altogether 8 different aphid vectors of raspberry viruses have been identified till date i.e., *Am. idaei*, *Am. rubi*, *Am. agathonica*, *Ap. idaei*, *Ap. rubicola*, *Macrosiphum euphorbiae*, *Macrosiphum fragariae* and *Myzus ornatus* (Converse et al., 1987; Martin et al., 2013; Tan et al., 2022).

2.3.1.1 *Aphis idaei*

Ap. idaei is pale in color and their body size measures from 0.76 mm to 2.96 mm (Borowiak-Sobkowiak, 2005). Overwintering Egg hatches at the end of March and in the beginning of April in Poland (Borowiak-Sobkowiak, 2005) whereas in Temperate region, it may extend up to beginning of May (Rautapää, 1967). After hatching, it takes about a month to form fully matured fundatrices (Dicker, 1941). Dependent on different environmental factor i.e., temperature and humidity. They have 8-11 generation of life cycle (Borowiak-Sobkowiak, 2005; Grigorov, 1965; Rautapää, 1967). All the progeny after second generation produces wings which usually occur during June/July (Dicker, 1941). Dicker (1941) studied the complete life cycle of Ap. idaei and observed High population density is also observed at the same time. The offspring of the winged viviparous females are all apterous. They are smaller than the previous wingless forms, of a uniform pale yellow and quite as inactive. From August until the production of sexual forms in October only apterous viviparous females are produced. Apterous oviparous females and apterous males are both progeny of the apterous viviparous females. Sexual forms first appear in October, while some aphides are still reproducing viviparously. By November, only sexual forms can be found, and these persist in diminishing numbers until about the middle of December when the canes are completely defoliated. Winged individuals are important in term of virus transmission (Converse, 1987).

2.4 Plant virus detection and identification tools

There are many diagnostic tools for the identification of a virus inducing plant disease which includes biological indexing, microscopic observation, serological methods, and molecular techniques (PCR, qPCR, sequencing). All these methods vary in their specificity of viral identity, the rapidity of gaining results, the sensitivity of the assay and the reliability of detection. In certain situation, disease can be recognized by visual observation of symptoms. BRNV related symptoms can occasionally be observed in sensitive red raspberry cultivars under field conditions in the spring and early summer by the appearance of veinal chlorotic spots (Converse, 1987). RLMV like symptoms can be observed by the presence of characteristic angular chlorotic spots on the leaves (Converse, 1987). This can be appropriate in some instances, although symptom production may require the presence of more than one virus, like RYNV (Stace-Smith, 1955a) and can vary depending on variety, seasonal or environmental conditions and plant nutritional status. Biological indexing includes mechanical inoculation and grafting. For some viruses, mechanical inoculation of infected plant extracts to

indicator plants may result in better identification of particular virus. This approach is often referred to as sap testing, and uses a variety of herbaceous indicator plants, including *Chenopodium quinoa*, various tobacco species, and cucumber. Many berry viruses, such as RLMV (Converse et al., 1987), are not mechanically transmissible, requiring alternative testing approaches, such as grafting. Microscopy can detect unknown or uncharacterized pathogens, but it requires sophisticated and expensive equipment and significant expertise to undertake assays. For RYNV, use of electron microscopy for detection and identification is difficult because such particles are rarely seen in leaf dip preparations, and electron microscopy of thin sections of infected tissue is too laborious to be useful for routine identification (Stace-Smith, 1955a). Serological test like ELISA will only detect viruses to which the antiserum reacts and thus have limited capacity to screen for unknown virus. Apart from theses diagnosis tools, molecular technique produces the most sensitive and robust assays. Polymerase chain reaction (PCR) provides the most sensitive of the regularly used virus detection tests, including aphid borne viruses of raspberry. For the development of a PCR test it is necessary to have prior information of the nucleic acid sequence of the virus and, if the virus genome is RNA, a reverse transcription step is necessary. PCR is the laboratory technique used to make millions of copies of a particular segment of DNA. It amplifies, or copy, a specific DNA target from a mixture of DNA molecules. It is performed in 3 steps: denaturation where, high temperature (95 °C) is provided to denaturant the DNA segment, annealing (at 5 °C less then melting temperature of primer) where complementary sequences have an opportunity to hybridize, and extension (72 °C) where the DNA polymerase extend the segment. A range of these different tools is presented in table 4. The choice among all these methods is crucial and depends on the conclusions sought and the clues collected in the field (Wilson, 2014).

Table 4. comparison of different diagnostic tools in plant virology. The more '+' sign each receives, the better the testing system addresses the criterion.

	Biological indexing	**Transmission Electron Microscopy (TEM)**	**Serological test**	**PCR, RT-PCR**	**Real time PCR**	**Next generation sequencing**
Required virus-specific information or antibodies.	No	No	Yes	Yes	Yes	No
Sensitivity of assay	+	+	+ + +	+ + + +	+ + + + +	+ + + +

Specificity of assay	+	+	+ + +	+ + +	+ + +	+ + + + +
Adaptability to high-throughput testing	+	+ +	+ + + +	+ + + +	+ + + +	+
Requirement of sophisticated equipment	+	+ + + +	+ +	+ + +	+ + + +	+ + + + +
Rapidity of diagnostic results	+	+ + +	+ +	+ + +	+ + + +	+ + + +
Diagnostic value of virus identification	+	+	+ +	+ +	+ +	+ + + + +

Source: Wilson, 2014

3. Materials and methodology

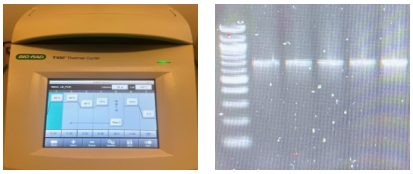
3.1 Surveillance of raspberry viruses in Norway

3.1.1 Survey and sampling

Sampling site.

Symptomatic plant sample in field.

Symptomatic leaf sample



Virus detection using RT-PCR technique

Leaves were checked for aphids

Aphids, collected in tube.

Fig 2. Workflow followed for RT-PCR based detection of symptomatic leaf and aphid samples.

Surveys were carried out in summer 2021, in different raspberry growing sites of Norway (in Vestland, Viken and Agder counties). Selected plantations were visually inspected and shoots from canes with leaves that had virus like symptoms or aphids were collected by the local advisory service (in Vestland and Agder), by NIBIO (in Viken) or Njøs (Vestland). A total number of ninety-five plant samples, comprising of mainly three raspberry cultivars and uncultivated ('wild') raspberry nearby, were collected in plastic bags and brought to NIBIO (Table 5). Each plant sample comprised of shoots with several leaves (with the possible exception of Agder samples). From each plant samples, leaf samples were collected for virus detection whereas, aphids were checked using stereo microscope in same plant sample (not necessarily in same leaf sample that were collected for virus detection). Aphids were found in 22 plant samples (out of 95) and were collected separately in 2 ml Eppendorf tube filled with RNA Shield/RNA Later (buffer; 100µl in each tube). Number of aphids put in each tube was based on the number of aphids present and their size. If there were only nymphs, then 3-5 nymphs were collected in one tube but if there were adults, then the number was reduced to 1-2. Some of the plant samples had greater number of aphids. In those cases, aphids were collected in 2-3 tubes. Thus, altogether there were 95 leaf samples and 31 aphid samples

collected from 95 plant samples, which were stored in -80°C in freezer until the RNA isolation proceed.

Table 5. Number of plant samples collected from different counties and cultivars.

County/ Cultivar	Vestland	Viken	Agder	**Total**
Glen Ample	10	5	3	**18**
Glen Mor	10	0	0	**10**
Veten	11	0	0	**11**
[1] Wild	26	6	7	**39**
[2] Other	11	5	1	**17**
Total	**68**	**16**	**11**	**95**

[1] "Wild" means uncultivated raspberry of unknown origin, found in the boundary vegetation of raspberry plantations. [2] "Other" implies different unreleased breeding lines of raspberry in Norway.

3.1.2 RNA extraction

3.1.2.1 RNA extraction from leaf samples

The stored frozen leaf samples were placed into a mortar containing an appropriate amount of liquid nitrogen to cover the sample. With the help of a pestle, the materials were grinded into a very fine powder with liquid nitrogen. The grinding quality is very crucial for both the quantity and the quality of the RNA. After this, 20-40 mg of the powder was collected in 2 ml Eppendorf tube, and the remaining powder placed in another tube as backup. RNA extractions were carried out using the NORGEN plant/fungi total RNA purification kit (Cat. no. 25850; Norgen biotek corp.) according to the manufactural instruction with some modifications. The concentration and purity (A260/A280) of total RNA extracted was measured using Nanodrop spectrophotometer (Thermo scientific). Finally, the purified RNA was stored at -20°C.

Positive controls for different raspberry viruses were cultured in isolated chamber in the greenhouse of Kirkejord, SKP, NMBU (Fig 3). Young and unfolded leaves were selected form each infected plant separately and RNA extraction was performed as describe above.

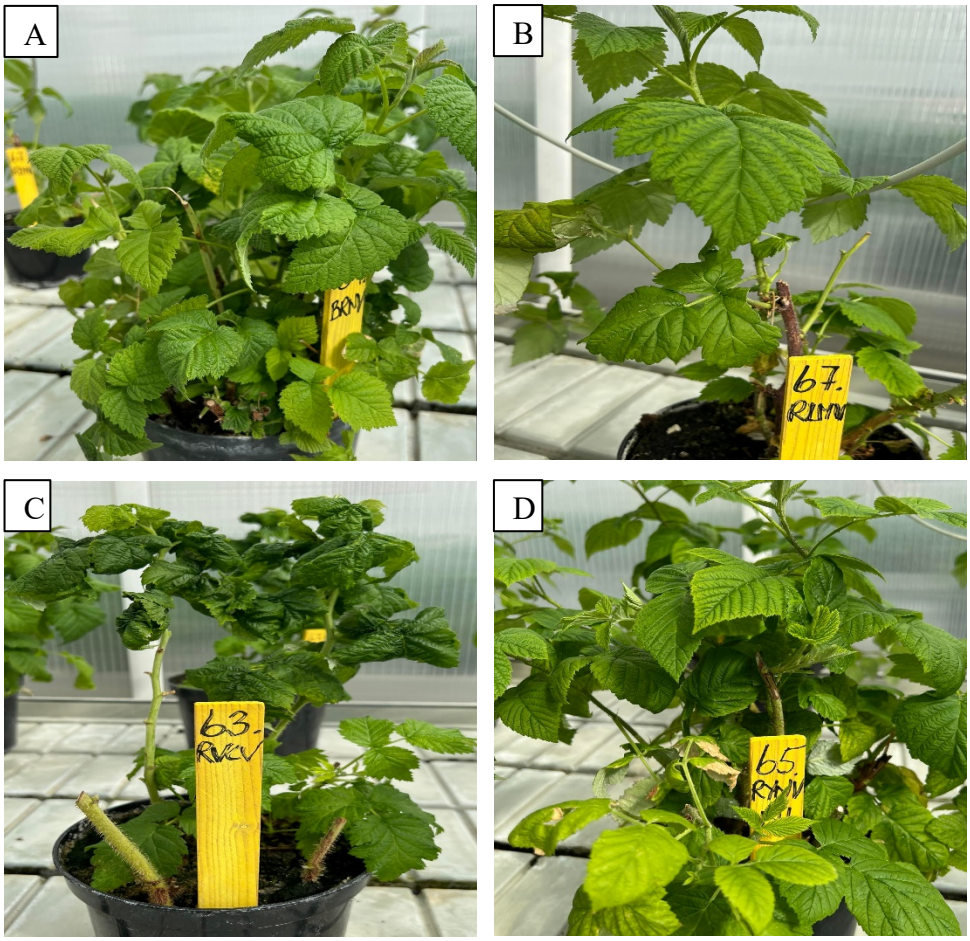


Fig 3. Raspberry plants for positive control. A. Plant infected with BRNV. B. Plant infected with RLMV. C. Plant infected with RVCV. D. Plant infected with RYNV.

3.1.2.2 RNA extraction from aphid samples

The aphid samples were taken from -80 °C freezer and were crushed inside the tube. Aphids kept in RNA shield were crushed directly using small glass rod whereas aphids kept in RNA later were separated and crushed using Trizol reagent (600 µl). After this, RNA extraction protocol provided by ZYMO research (Direct-zol RNA Miniprep) were followed. The purified RNA was stored at -20 °C.

3.1.3 C-DNA synthesis

C-DNA synthesis was performed using superscript IV (SSIV, Invitrogen). 1µL of deoxyribonucleoside triphosphates, dNTP (10 µmole each) and 1µL of random primer (concentration) were mixed with 3 µL of nuclease free water in 2 ml Eppendorf tube. After this 8 µL of extracted RNA was mixed and centrifuged slightly. The mixture was then incubated at 65°C for 5 minutes and instantly cooled on ice for 2 minutes. Now, added 1 µL of SSIV reverse transcriptase, 4 µL of SSIV buffer,1 µL of DTT and 1 µL of nuclease free water. Finally, it was

placed in a thermal cycler with the appropriate incubation cycle programmed. i.e., incubation at 25°C for 10 min followed by 42°C for 50 minute and finally 70°C for 10 min.

3.1.4 PCR amplification using virus specific primers

PCR microtubes were taken and PCR master mix was prepared by mixing 2.5µL of 10X Buffer, 1µL of 10mM dNTP, 0.2µl of Taq polymerase (Invitrogen), 17.8µL of RNase/DNase free water, 1µL of F (forward) primer and 1µL of R (reverse) primer (10mM of concentration) of the virus to be tested are added. After this, cDNA Sample (2 µL) was added in it. Beside this, cDNA templet of positive control and RNase/DNase free water (2 µL) for the negative control was also prepared on the PCR master mix. And finally performed the recommended thermal cycling condition (Table 7 to Table 11) accordance to the primer used (Table 6).

Table 6. Primers used to detect viruses with their sequence and product size.

Name	**Primer name**	**Sequence (5`-3`)**	**Product size (bp)**	**Reference**
NAD (Plant)	NAD-F	GATGCTTCTTGGGGCTTCTTGTT	181	(Menzel et al., 2002)
	NAD-R	CTCCAGTCACCAACATTGGCATAA		
COI (aphid)	LCO1490	GGTCAACAAATCATAAAGATATTGG	700	(Folmer et al., 1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		
BRNV	1153	GCGCACTGAACCCAAGTTTA	502	(McGavin and MacFarlane, 2016)
	1154	CAACATCGAATCCCTCAAGC		
RLMV	CPhF	CGAAACTTYTACGGGGAAC	470	(Tzanetakis et al., 2007)
	CPhR	CCTTTGAAYTCTTTAACATCGT		
RVCV	3649	CCAACAAAGCTGATATWCCAG	257	(Jones et al., 2019)
	3648	CCTCATCTAAGTARTCTTCCA		
RYNV (leaf)	1752	TCCAAAACCTCCCAGACCTAAAAC	350	(Jones et al., 2002)
	1753	ATAATCGCAAAAGGCAAGCCAC		
RYNV (aphid)	RYNV_F alternative	TCCAAAACCTCCCAGACCTMAAAC	350	Personal Communication, kappa-berry project
	RYNV_R alternative	TTGTTATATAATCACAAAAAGCTAACCA		

Table 7. PCR reaction components for amplification control (plant mitochondrial NADH dehydrogenase, NAD) for leaf sample.

PCR reaction components			**PCR cycle**			
Master mix	10X PCR buffer with Mgcl2	2.5 µl	Initial denaturation	95 °C	2 min	
	dNTPs (10mM)	0.5 µl				
	Forward primer (10mM)	1 µl	Denaturation	95 °C	30 sec	X35
	Reversed Primer (10mM)	1 µl				
	Taq DNA polymerase	0.2 µl	Annealing	50 °C	30 sec	
	RNase/DNase free water	17.8 µl	Extension	72 °C	45 sec	
Template: cDNA		2 µl	Final extension	72°C	7 min	
Total		25 µl	Cooling	4°C		

Table 8. PCR reaction components for amplification control (Cytochrome C oxidase subunit I, COI) for aphid sample mitochondrial from diverse metazoan invertebrates.

PCR reaction components			**PCR cycle**			
Master mix	10X PCR buffer with Mgcl2	2.5 µl	Initial denaturation	95 °C	2 min	
	dNTPs (10mM)	0.5 µl				
	Forward primer (10mM)	1 µl	Denaturation	95 °C	30 sec	X40
	Reversed Primer (10mM)	1 µl				
	Taq DNA polymerase	0.2 µl	Annealing	47 °C	60 sec	
	RNase/DNase free water	17.8 µl	Extension	72 °C	30 sec	
Template: cDNA		2 µl	Final extension	72°C	2 min	
Total		25 µl	Cooling	4°C		

Table 9. PCR reaction components for BRNV and RLMV detection.

PCR reaction components			**PCR cycle**			
Master mix	10X PCR buffer with Mgcl2	2.5 µl	Initial denaturation	94 °C	5 min	
	dNTPs (10mM)	0.5 µl				
	Forward primer (10mM)	1 µl	Denaturation	94 °C	30 sec	X35
	Reversed Primer (10mM)	1 µl	Annealing	60 °C	30 sec	
	Taq DNA polymerase	0.2 µl	Extension	72 °C	30 sec	
	RNase/DNase free water	17.8 µl	Final extension	72°C	2 min	
Template: cDNA		2 µl	Cooling	4°C		
Total		25 µl				

Table 10. PCR reaction components for RVCV and RYNV (leaf sample only) detection.

PCR reaction components			**PCR cycle**			
Master mix	10X PCR buffer with Mgcl2	2.5 µl	Initial denaturation	94 °C	5 min	
	dNTPs (10mM)	0.5 µl				
	Forward primer (10mM)	1 µl	Denaturation	94 °C	30 sec	X40
	Reversed Primer (10mM)	1 µl	Annealing	55 °C	30 sec	
	Taq DNA polymerase	0.2 µl	Extension	72 °C	30 sec	
	RNase/DNase free water	17.8 µl	Final extension	72°C	2 min	
Template: cDNA		2 µl	Cooling	4°C		
Total		25 µl				

Table 11. PCR reaction components for RYNV (from aphid sample) detection.

PCR reaction components			**PCR cycle**			
Master mix	10X PCR buffer with Mgcl2	2.5 µl	Initial denaturation	94 °C	5 min	
	dNTPs (10mM)	0.5 µl				
	Forward primer (10mM)	1 µl	Denaturation	94 °C	30 sec	X35
	Reversed Primer (10mM)	1 µl	Annealing	62 °C	30 sec	
	Taq DNA polymerase	0.2 µl	Extension	72 °C	30 sec	
	RNase/DNase free water	17.8 µl	Final extension	72°C	2 min	
Template: cDNA		2 µl				
Total		25 µl	Cooling	4°C		

3.1.5 Gel electrophoreses

After the PCR amplification, the samples were loaded in the wells of agarose gel including positive and negative controls (MQ water). The agarose gel was prepared by mixing agarose (cat.no BN50004; Bionordika) with 1 X Tris-borate-EDTA, TBE buffer (1.5 gm of agarose per 100ml of buffer). 1 liter of TBE buffer was made my dissolving 10.8 g Tris and 5.5 g boric acid in 900 ml of distilled water. After this, 4 ml of 0.5 M Na_2EDTA was added on that mixture and finally adjusted the volume to 1 liter. The agarose solution that was mixed with TBE buffer was boiled and then cooled for about 5 minutes. After this, agarose mixture was treated with ethidium bromide (1 drop for each 50ml of agarose solution). Finally, the gel was poured in the gel plate and combs were placed to prepare the wells. The 100 bp ladder was used throughout the experiment and the results were read via the Quantity One software.

3.1.6 Sangar sequencing

Aphid samples that were amplified using COI primer were sent for sangar sequencing for their identification. The nucleotide sequence obtained from sangar sequencing were edited using Snap-gene software and were Blasted in NCBI website. Furthermore, if the gel images were unclear or were observed unspecific band, those samples were also sent from sangar sequencing for further confirmation.

3.2 BRNV transmission study with *Ap. idaei*

3.2.1 Obtaining BRNV-free *Ap. idaei* culture

Rearing *Ap. idaei* from overwintering eggs found outdoors at first proved difficult (Appendix 1). Raspberry canes with aphid eggs had also been collected outdoors in another NIBIO project. These were provided with water and kept at 20°C and 95% humidity on 28th March 2022. After 15 days aphid colonies were observed, and they could be morphologically identified and used in the present study. Eight random aphids were tested for BRNV by RT-PCR.

Since some BRNV was detected in aphid samples, and BRNV being a semi-persistent virus, starving is a possible means to get virus-free aphids. To find out the length of starvation necessary, a small preliminary experiment was done on 15th April where, 8 adult aphids from the colony were put individually in petri dishes in a room temperature and each aphid was allowed to starve for different time. i.e., 30 min, 5 hrs., 12 hrs., and 24 hrs. The RNA was extracted immediately after respective time.

Ten adult aphids being starved for the time found in the preliminary experiment were used as founders of a virus free colony on virus-free 'Glen Ample'. They were kept in the separate culture room at NIBIO (18°C, 60% humidity and 16:8 hrs. of day: night light) on 5th May and were reared until 25th June, when the transmission experiment started. 8 random aphids were tested for BRNV before proceeding for transmission experiment to confirm that were virus free.

3.2.2 Determining starving time needed for aphids before virus acquisitions

Starving may cause aphid to settle down and begin feeding when they are introduced to virus infected leaf tissue (Gray, 2008) which can improve the efficiency of virus transmission. Thus, 8 virus-free aphids were starved for 10, 20, 30 and 40 minutes and then offered a raspberry leaf, to observe whether the aphid fed instantly or not. The feeding process was observed through a stereo microscope.

3.2.3 Virus transmission experiment

Apterous (wingless) adults and nymphs from the BRNV-free aphid culture were used in this experiment. In the transmission experiment, 80 virus free aphids (adult + nymph) were starved for 40 minutes (based on the result of preliminary experiment, 3.2.2). After that, the aphids

were allowed to feed on a BRNV infected leaf for 1 min, 5 min, 1 hr. and 24 hrs. (20 aphids in each acquisition group/ treatment). Virus (BRNV) infected leaves were used as a virus source for transmission experiment. The leaves were picked from BRNV positive plants grown in a greenhouse at NIBIO (Fig 3(A)). After the respective acquisition times, five aphids (2 adults+3 nymphs) were transferred from the BRNV-infected leaf and placed on the upper surface of a leaf of a healthy raspberry plant (Fig 4). There were four different inoculation times for each acquisition time tested, as shown in Table 12. Also, to verify whether aphids had acquired the virus, 5 aphids from each treatment were individually tested for BRNV by RT-PCR. After each inoculation time all aphids were removed from the plant and inoculated plants were maintained in the culture room of NIBIO (18°C, 60% humidity and 16:8 hrs. of light) for 30 days before processed for BRNV detection by RT-PCR, same as the processes that were followed for surveillance samples using same primer (Table 6) and same polymerase cycle (Table 9). All the 12 virus free raspberry plants were obtained from the Saga-plant ÅS, Norway.

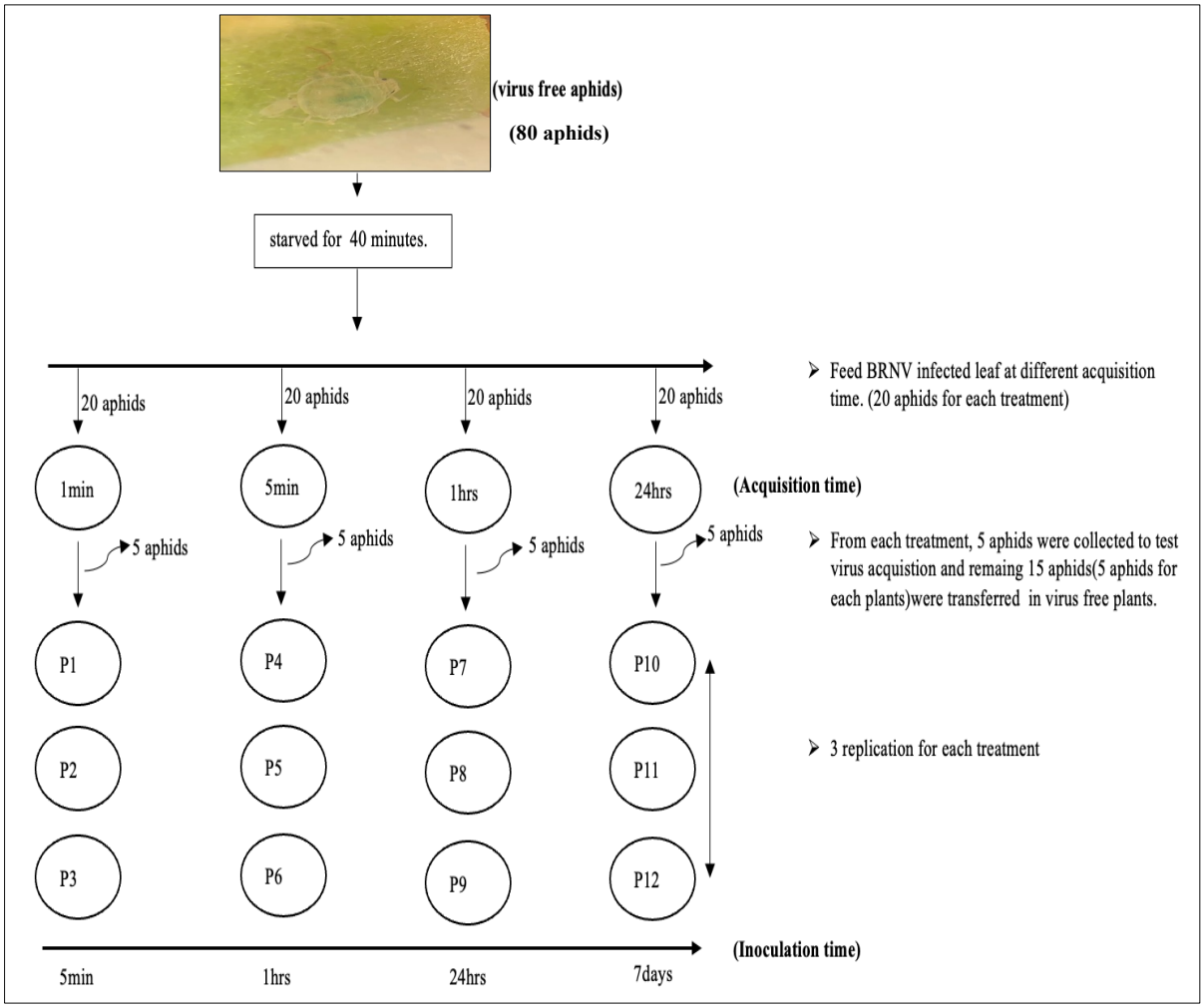


Fig 4. Transmission experiment diagram. Straight arrows pointing downward denotes aphid transfer. P1, P2, …., P12 indicates the different virus free plants.

Table 12. Experimental Treatment with different Acquisition and inoculation time.

Treatment no.	Acquisition time	Inoculation time
1	1 min	5 min
2	5 min	1 hrs
3	1 hrs	24 hrs
4	24 hrs	7 days

3.3 Statistical analysis

Statistical analyses were done using software R-studio and graphs were plotted using Microsoft Excel. Total incidence of individual viruses was compared by one way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test ($p \leq 0.05$) for mean separation.

4. Results

4.1 Surveillance result of raspberry viruses in Norway

4.1.1 Surveillance overview

Most samples that were collected in survey had some sort of virus like symptoms. Leaf yellowing, yellow leaf blotch, mosaic, leaf curling and distorted type of leaf were the most common type of symptoms that were observed during the sampling (Fig 5).

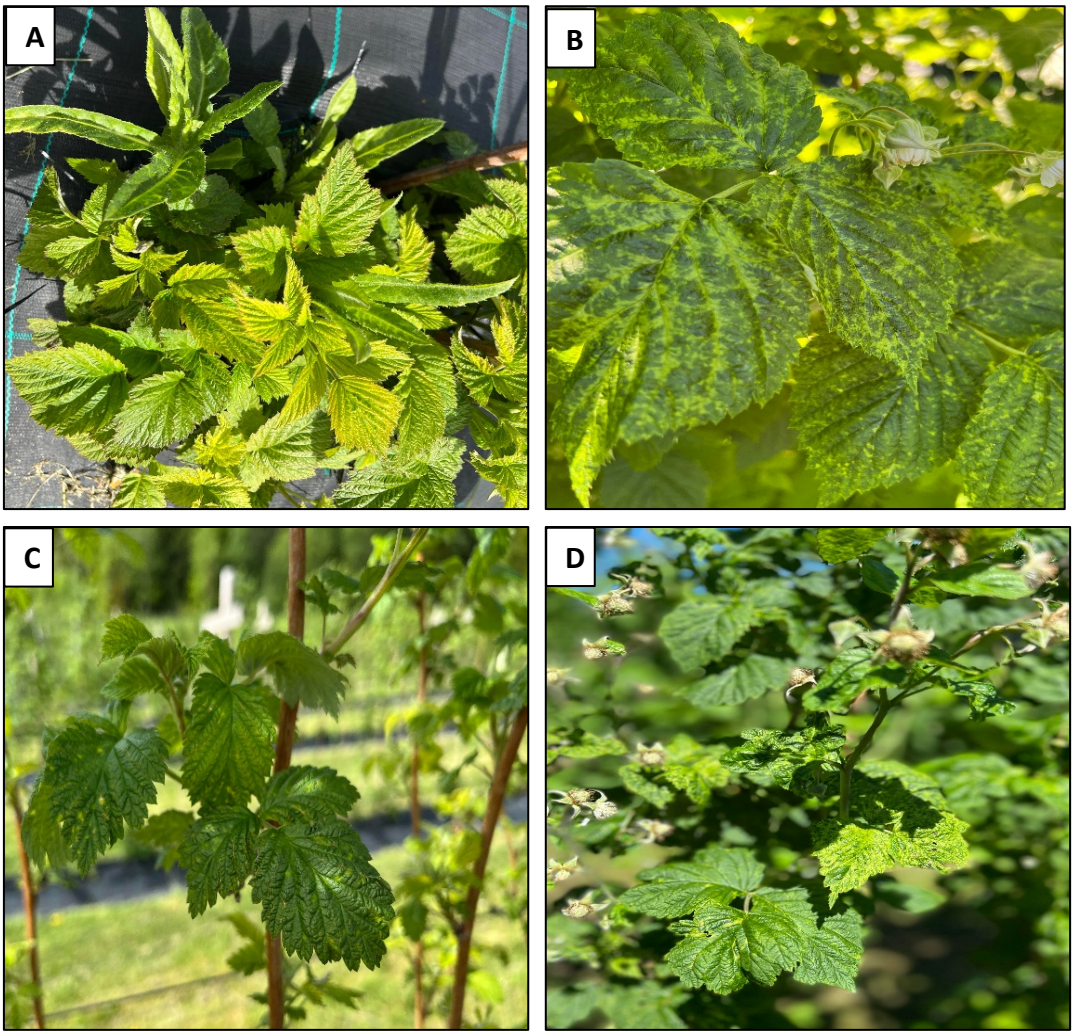


Fig 5. Plants showing virus like symptoms similar to the ones collected during the survey. A. Leaf blotch like symptoms. B. Leaf blotch with yellowing in leaf vein. C. unusual yellow spot in leaf. D. Curling of leaf.

The results of RT-PCR tests showed virus infections in 74 out of 95 tested leaf samples, whereas 21 test samples were free from all 4 viruses. BRNV, RLMV and RVCV were detected positive in 66, 21 and 6 samples whereas, RYNV was not detected in any of the sample (Table 13). Statistical analysis showed a highly significant (P=0.0011) difference between the incidences of BRNV, RLMV and RYNV. BRNV was the most prevalent virus in the samples from all three counties. Two viruses, BRNV and RVCV were detected in all three counties whereas RLMV was only detected in Vestland (Fig 6). The virus infestation according to different counties is summarized in appendix 3.

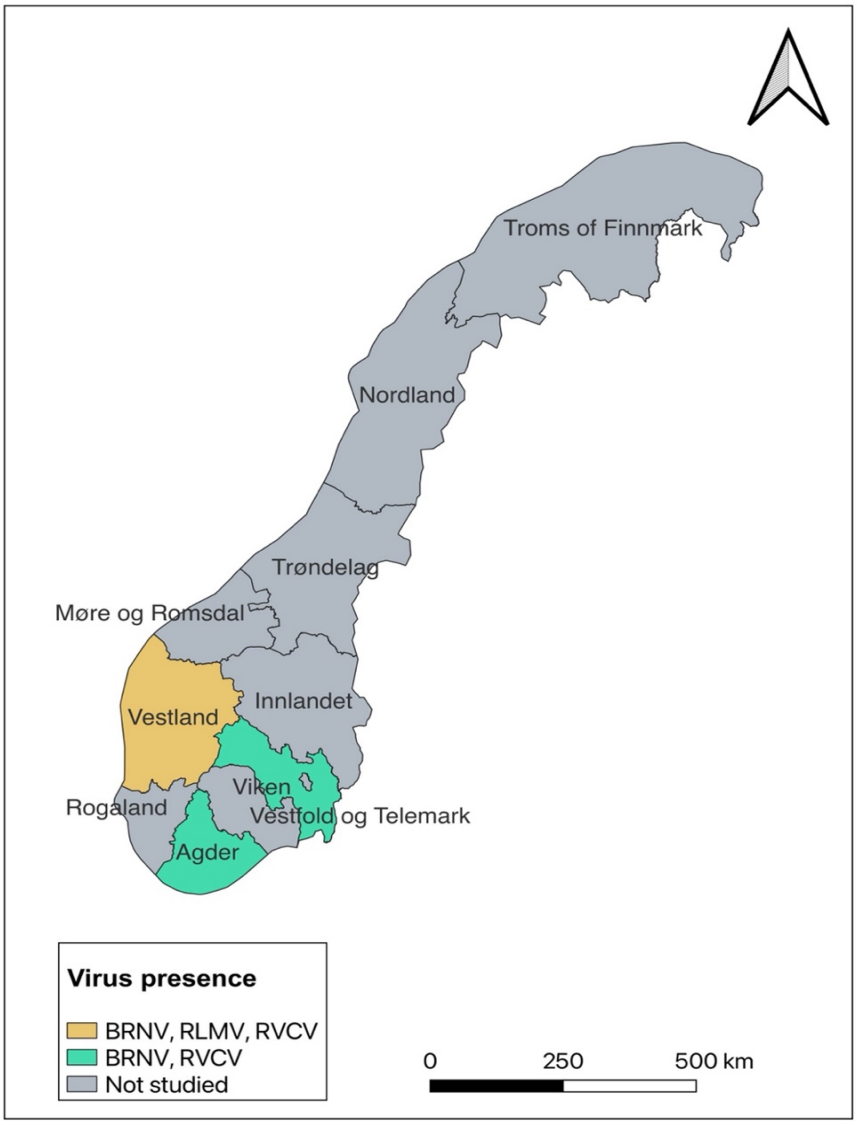


Fig 6. Map of Norway showing the information of surveillance area. Grey color in the map represents those area or counties that were not in our study area. Green color in two different counties represent the presence of 2 viruses BRNV and RVCV whereas yellow color represents the presence of 3 different virus i.e., BRNV, RLMV and RVCV.

Table 13. List of the 95 plant samples and the results of testing a leaf from each of them for BRNV, RLMV, RVCV and RYNV (RYNV not shown, as no samples with this was detected).

Sample n.o.	County	Cultivar	Detected virus (es)		
			BRNV	RLMV	RVCV
1	Agder	Wild	-	-	-
2	Agder	Wild	+	-	-
3	Agder	Glen Ample	+	-	-
4	Vestland	Wild	-	-	-
5	Vestland	Glen Ample	-	-	-
6	Vestland	Veten	+	+	-
7	Vestland	Wild	+	-	-
8	Vestland	Wild	+	+	-
9	Vestland	Wild	+	+	-
10	Vestland	Other	+	-	-
11	Vestland	Other	+	-	-
12	Vestland	Other	+	-	-
13	Viken	Other	-	-	-
14	Viken	Other	-	-	-
15	Viken	Other	+	-	-
16	Agder	Other	+	-	-
17	Vestland	Glen Mor	+	-	-
18	Vestland	Wild	+	+	-
19	Vestland	Wild	+	-	-
20	Vestland	Wild	+	+	-
21	Vestland	Glen Ample	+	-	-
22	Vestland	Glen Mor	+	+	-
23	Vestland	Glen Mor	+	-	-
24	Vestland	Glen Mor	+	-	-
25	Vestland	Glen Mor	+	-	-
26	Vestland	Wild	+	+	-
27	Vestland	Wild	+	+	-
28	Vestland	Wild	+	-	-
29	Vestland	Wild	+	-	-
30	Vestland	Wild	+	+	-
31 *	Agder	Glen Ample	+	-	-
32 *	Agder	Glen Ample	+	-	+
33 *	Viken	Other	-	-	+
34 *	Vestland	Wild	+	-	-
35 *	Vestland	Wild	+	+	-
36 *	Vestland	Wild	+	+	-
37 *	Vestland	Wild	+	-	-
38 *	Vestland	Veten	+	-	-
39 *	Vestland	Veten	+	-	-
40 *	Vestland	Veten	+	+	-
41 *	Vestland	Veten	-	+	-
42 *	Vestland	Veten	-	-	-
43 *	Agder	Wild	-	-	-
44 *	Agder	Wild	+	-	-
45 *	Agder	Wild	+	-	-
46 *	Vestland	Other	+	-	-
47 *	Vestland	Other	-	-	-
48 *	Vestland	Other	-	-	-
49 *	Vestland	Other	+	+	-

50 *	Vestland	Other	-	+	-
51 *	Vestland	Veten	+	+	-
52 *	Vestland	Wild	+	-	-
53	Viken	Wild	+	-	-
54	Viken	Wild	+	-	-
55	Viken	Wild	+	-	-
56	Viken	Wild	+	-	-
57	Viken	Wild	+	-	-
58	Viken	Wild	+	-	-
59	Viken	Glen Ample	+	-	-
60	Viken	Glen Ample	+	-	-
61	Vestland	Glen Ample	+	-	-
62	Vestland	Glen Ample	+	-	-
63	Vestland	Glen Ample	+	-	-
64	Vestland	Glen Ample	+	-	-
65	Vestland	Glen Mor	-	-	-
66	Vestland	Wild	+	-	-
67	Vestland	Wild	+	-	-
68	Vestland	Glen Ample	+	-	-
69	Vestland	Glen Ample	+	-	-
70	Vestland	Glen Ample	+	-	-
71	Vestland	Glen Ample	+	-	-
72	Vestland	Glen Mor	-	-	+
73	Vestland	Wild	-	-	+
74	Viken	Other	-	-	-
75	Agder	Wild	-	-	-
76	Agder	Wild	-	-	-
77	Vestland	Other	-	-	-
78	Vestland	Other	-	-	+
79	Vestland	Other	-	-	-
80	Vestland	Wild	-	-	-
81	Vestland	Wild	-	-	+
82	Vestland	Veten	+	-	-
83	Vestland	Veten	+	-	-
84	Vestland	Veten	+	+	-
85	Vestland	Veten	+	-	-
86	Vestland	Wild	+	+	-
87	Vestland	Wild	+	+	-
88	Vestland	Wild	+	+	-
89	Vestland	Wild	-	+	-
90	Viken	Glen Ample	-	-	-
91	Viken	Glen Ample	+	-	-
92	VIken	Glen Ample	-	-	-
93	Vestland	Glen Mor	-	-	-
94	Vestland	Glen Mor	-	-	-
95	Vestland	Glen Mor	-	-	-
No of RT-PCR positive samples of each virus / Total number of infected samples (%)			66/74 89% [a]	21/74 28% [b]	6/74 8% [c]

* Leaf Samples from which aphids were collected. 29 aphid samples denoted by "*" were picked from the specific leaf samples whereas 2 samples were picked randomly. "+" indicate the virus detection by RT-PCR whereas, "-" indicate the absence of virus.

The proportion of mixed infection and dominant combination of viruses were analyzed (Table 14). Out of 21 samples which were infected by RLMV, 18 were in mixed infection with BRNV (i.e., 94.7% on total mixed infection with two or more viruses and 24.3% on total virus infection). Only one sample was coinfected with BRNV and RVCV. There was no coinfection between RLMV and RVCV. Similarly, co-infection with 3 viruses were also absent. Furthermore, out of 3 viruses, largest number of single virus samples was infected with BRNV (47 out of 74 infected samples) followed by RVCV and RLMV.

Table 14. Presence and incidence of detected viruses in 74 infected raspberry samples.

Detected virus(es)	**No of positive sample**	**Percentage of positive samples in total number of single or mixed infected sample (%).**	**Percentage of positive samples in total number of infected samples (%).**
BRNV	47	85.5	63.5
RLMV	3	5.5	4.1
RVCV	5	9.1	6.8
One virus detected in total	**55**	**100**	**74.3**
BRNV+RLMV	18	94.7	24.3
BRNV+RVCV	1	5.3	1.4
RLMV+RVCV	0	0	0
Co-infection with 2 viruses(total)	**19**	**100**	**25.7**
Co-infection with 3 viruses (BRNV+RLMV+RVCV)	**0**	**0**	**0**

4.1.2 Virus infection and cultivars

All three cultivated cultivars (Glen Ample, Glen Mor and Veten), wild species and “others” were infected with at least one of the mentioned viruses (Fig 7). Veten samples had the highest infection percentage (91%; 10 out of 11 samples), followed by samples of uncultivated raspberry, wild (85%; 33 out of 39 samples), and Glen Ample (83%, 15 out of 18 samples). Glen Mor had 60% virus infection (6 out of 10 samples) and other cultivars accounts 59% of infection (10 out of 17 samples).

Virus infestation within the cultivars were analyzed and BRNV was the dominant virus in all cultivars. RVCV was not detected in Veten cultivar whereas, RLMV was not detected in Glen Ample (Fig 8).

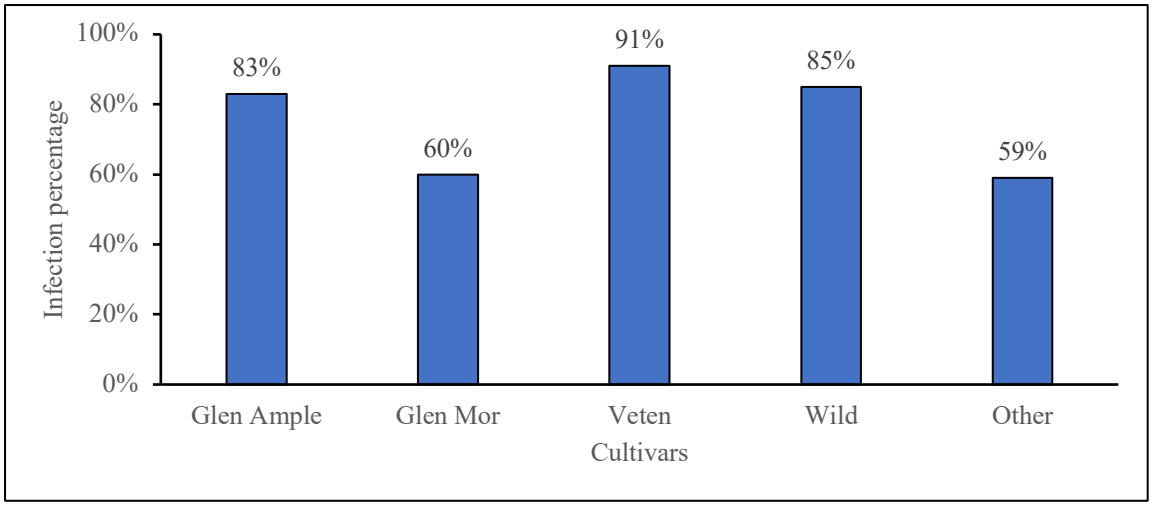


Fig 7. Average virus infection percentage of different cultivars. Veten cultivar has the highest infection percentage.

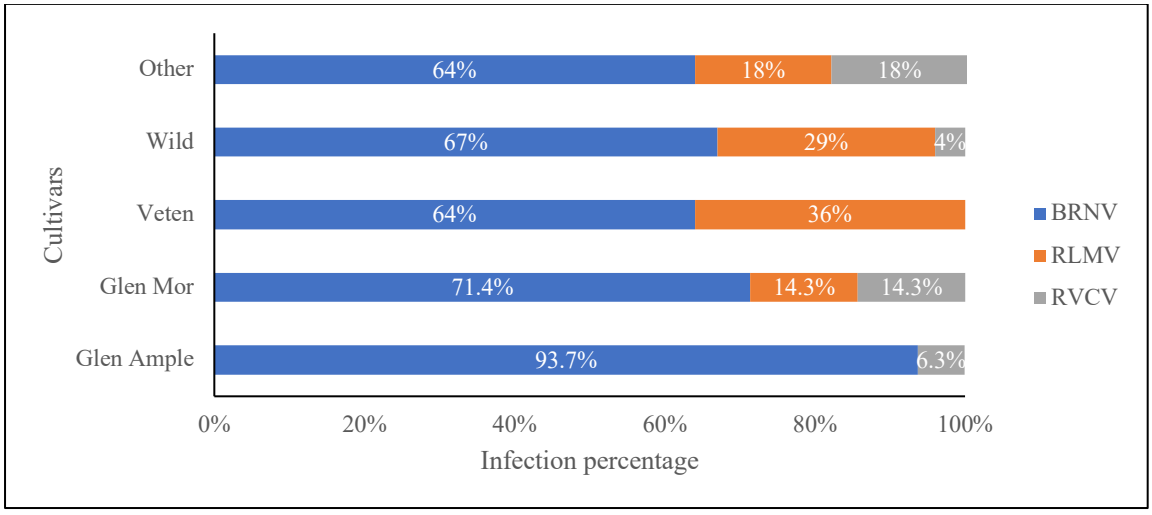


Fig 8. Infection percentage of different viruses within the cultivars. BRNV was dominant in all cultivars.

4.1.3 Correlation of virus infection between leaf and aphid sample

The overview of different virus infestation in aphid species according to county and cultivar is summarized in appendix 4, which reveals that 13 out of 31 aphid samples were found to contain at least one of the viruses studied. Only two viruses i.e., BRNV and RLMV were detected in those infected samples. The correlation between virus infection between leaf and aphid sample is summarized in Fig 9, where about 41% the sample of BRNV and about 83% of sample of RLMV was found to be correlated with virus infection.

All infected aphid samples were amplified using COI primer, sent for Sanger sequencing and the results from blast run revels that both *Ap. idaei* and *Am. idaei* were positive for BRNV and RLMV (Table 15).

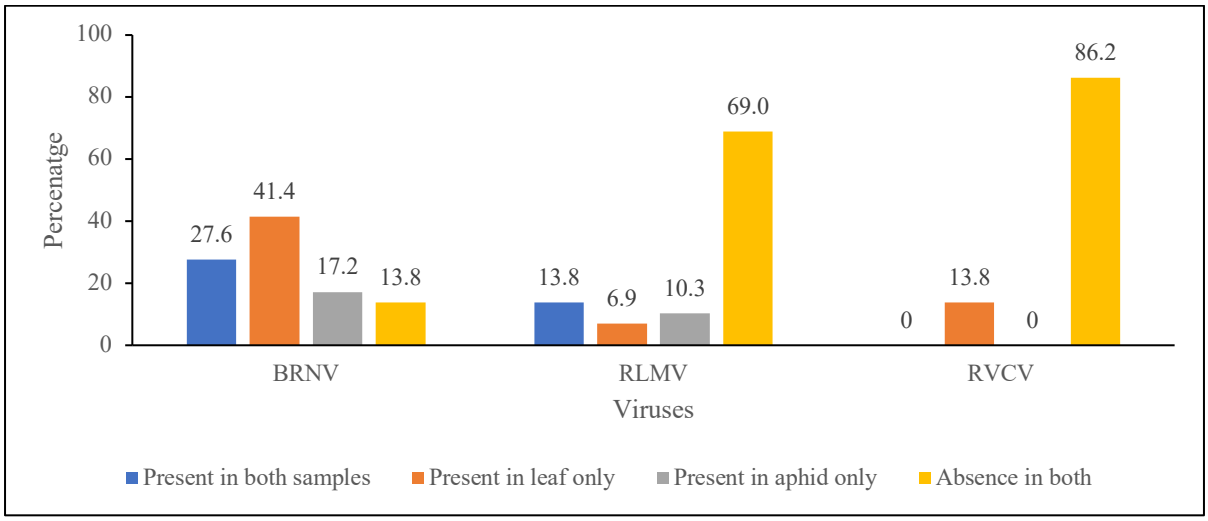


Fig 9. Virus presence in aphids and the leaf, sampled from the same plant samples, sorted by four possibilities i.e., virus present in both samples, virus present in leaf sample only, virus present in aphid sample only, and no virus in either sample.

Table 15. Distribution of aphid species in the 13 aphid samples that tested positive for at least one virus.

Aphid species	**No of infected samples**
Ap. idaei	5 (4=only BRNV, 1=BRNV+RLMV)
Am. idaei	6 (1=only RLMV, 5=BRNV+RLMV)
*N.A. (*Binodoxys similis)*	1
Bad sequence	1
Grand Total	**13**

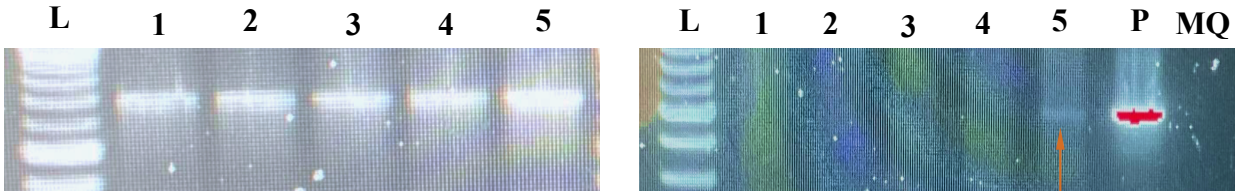
* Not available, Blast run revels the sequence of *Binodoxys smilis*

4.2 Virus transmission result

4.2.1 Preliminary test result

BRNV infected *Ap. idaei* lose its virulence after at least five hours of starvation (Fig 10). Aphids that were starved for 5 hrs., 6hrs, 12 hrs. and 24 hrs. were detected negative whereas, only those aphids that were starved for 30 minutes were tested positive with BRNV (Fig 10(B)).

The result was further confirmed by amplification control (Fig 10(A)) where all the aphids were positive with COI primers (internal control).



A B

Fig 10. A. Amplification control using Co I primers. B. BRNV test of aphids that were starved for different time. Only aphids that were starved for 30 minutes was positive. Different number i.e.,1, 2, 3, 4 and 5 represent 24 hrs., 12 hrs., 6 hrs., 5 hrs. and 30 min respectively. Letter L, P and MQ represent 100 bp ladder, BRNV positive control and water.

In the second preliminary test which was done to know the starvation time of aphids, reveals that the 40 minutes of starvation was sufficient for aphids before transferring them into a virulence plant material for acquisition (Table 16).

Table 16. Different starvation time for aphid with their feeding.

Treatment	Starvation Time	Observation (feeding time)
1	10 min	High movement
2	20 min	High movement
3	30 min	Feed within 10 second
4	40 min	Instantly feed.

4.2.2 Transmission result

Altogether, 5 aphids that were separated from each treatment after allowing feeding for different times (1min, 5min, 1hrs and 24hrs) were tested for BRNV infection and found positive result on 1hrs and 24 hrs. of feeding time/acquisition (Fig 11). To confirm the transmission role of *Ap. idaei*, all the inoculated raspberry plants was tested for BRNV after 30 days of aphid inoculation and none were positive for BRNV (Fig 12).

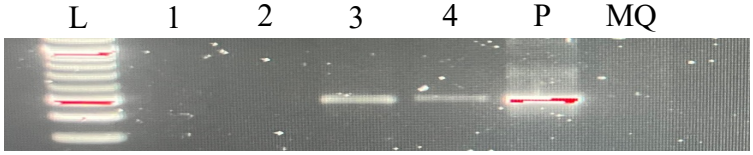


Fig 11. RT-PCR result of aphids after feeding the BRNV infected leaf. Different number i.e.,1,2,3 and 4 represent 1 min, 5 min ,1hrs and 24 hrs. of acquisition time respectively. Letter L, P and MQ represent 100 bp ladder, BRNV positive control and water respectively.

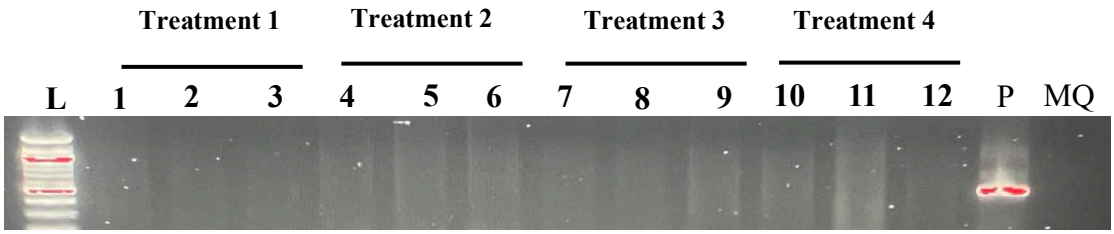


Fig 12. RT-PCR result of 12 raspberry plants that were inoculated with *Ap. idaei* (after feeding with BRNV infected leaf). Different number i.e.,1,2,3…12 represent different raspberry plants. Treatment 1, 2 3 and 4 represent the raspberry plants which were inoculated with aphids for 5 min, 1 hr., 24 hrs. and 7 days respectively after different acquisition time. Letter L, P and MQ represent 100 bp ladder, BRNV positive control and water respectively.

5. Discussion

5.1 Surveillance of aphid borne raspberry viruses in Norway

Raspberry can be grown in different part of Norway from southern part of Agder to Nordland (65º N) in north but the main growing area for raspberries in Norway is in the West, in the fjord district of Sogn og Fjordane of Vestland (Perasović, 2013). As a result, most of the sample accounts from Vestland county in our study (68 out of 95). Furthermore, time, budget and manpower limited the sampling size from other two counties. Different peoples were involved in sampling. As a result, some of the samples maynot have sepecific symtoms that were also considered in this study.

The result of this study which was obtained by nucleic acid-based virus detection method (RT-PCR) showed the presence of 3 aphid borne raspberry viruses i.e., BRNV, RLMV and RVCV with highly significant difference of incidence in selected counties of Norway (Agder, Viken and Vestland). BRNV incidence was quite confined to Sogn og Fjordane / Vestland area in surveillance result of NIBIO, 2018/2019 (NIBIO, unpublished). However, our result showed the increasing infestation of BRNV in all selected counties. This indicates the spread of viruses. This might be due to virus acquisition and transmission times of aphid vector (*Am. idaei*) which is 15-30 minutes and 2 minutes respectively for BRNV (Stace-Smith, 1955a) which is quite fast as compared to other two viruses. The same, *Am. idaei* is responsible to transmit both RLMV and RYNV. The acquisition and transmission time of RLMV is 30 minutes and 60 minutes respectively (McMenemy et al., 2009) and that of RYNV is 1 hr. and 4 hrs. respectively (Stace-Smith, 1955a). In addition to this, natural aphid migration, the viruses are also spread by passive aphid movement due to wind and rainfall (Converse, 1987). In our result, RLMV was detected only in Vestland (Fig 6) which revels that the RLMV may be confined only in this region. However, it should be noted that sample size of Vestland was high (69 out of 95) as compared to Agder (11 out of 95) and Viken (16 out of 95). Most cultivars of red raspberries infected with BRNV exhibits no visible symptoms (Stace-Smith, 1955b; Jones & Jennings 1977) however in our case, almost 50% of the sample were infected with BRNV alone. This may indicate the presence of different viral strain of BRNV in Norway. So, further study on BRNV and its viral strain should be done. As, BRNV and RLMV both got transferred by the same aphid vector i.e., *Am. idaei* (Martin et al., 2013), their mixed infestation was seen in greater amount in our study (18 out of 21 RLMV infected sample). Converse (1987) also mentioned that the mixed infection between BRNV and RLMV was quite common in Europe

in his book. RVCV was detected in a few samples in all three counties (1 in Agder, 4 in Vestland and 1 in Viken). It is transmitted by *Ap. idaei* and the acquisition and inoculation access periods of *Ap. idaei* is 7 and 30 days, respectively (Jordovic, 1976) which is quite a long period of time. RVCV is transmitted in a persistent way and can remain in its vectors for long period of time, probably for the life of the insect. (Cadman, 1952a; Jordovic, 1963; Nault, 1997*).* Therefore, disease caused by RVCV infection might be quite serious if not controlled. RYNV, which is also one of the important aphids borne virus and can cause raspberry mosaic disease combining with BRNV and RLMV was not detected in our study. During RT-PCR of RYNV, we had obtained some unspecific band in aphid species at the beginning. The PCR products of those samples were sent for Sanger sequencing, and the sequences were blasted in NCBI. The result turned out that the obtained sequences were highly identical (98.5%) to18s ribosomal RNA gene of aphid species. So, alternative primers which are mentioned in table 6 were used for RYNV detection on aphid samples. Due to a small amount of samples in the present study, the absence of RYNV cannot be used to claim the total absence of this virus in Norway. Thus, Sampling of more raspberry plantation in following years is recommended. Similarly, 21 out of 95 symptomatic samples received were tested negative for all 4 aphid borne viruses in the study. Apart from some of the symptomless plant samples that were considered in our sampling, it is a possibility that these symptoms might be caused by other viruses or virus complexes which was not the part of this surveillance (i.e., raspberry bushy dwarf virus (RBDV), raspberry leaf blotch virus (RLBV)). Moreover, these virus-like symptoms may be caused by other plant pathogenic organisms such as phytoplasma or by nutrient deficiency or by the application of pesticides (Wilson, 2014).

Three different cultivated raspberry cultivars in addition to wild raspberry, were sampled in this study and average infestation percentage of all cultivars were more then 50%. The result further demonstrates that Veten, which has been the main cultivar in Norway for more than 30 years, has highest virus infection percentage (10 out of 11 samples, 91%). Veten cultivar has been replaced by *Am. idaei* resistant cultivar Glen Ample during last 20 years in Norway (Heiberg et al., 2002). This cultivar change may have shifted the aphid populations and resulted in presenting of more competitive aphids that can cause more damage towards Veten cultivar. The resistance largely depends on two single major genes (A1 or A10) or having multiple minor genes that control aphid numbers and subsequently the spread of the viruses that they transmit (Jones, 1986). Until now, 5 biotypes (Biotype: 1, 2, 3, 4 and X) of *Am. idaei* have been discovered in Europe (Briggs, 1965; McMenemy et al., 2009) where they found that the Glen

Ample having A1 gene was resistant to aphid biotype 1 and 3. However, Jones et al. (2000) found that the *Am. idaei* resistance conferred by A1 gene has now been widely overcome by other biotypes of this aphid resulting in a large increase in the incidence of the *Am. idaei* transmitted viruses. Furthermore, they also observed *Am. idaei* biotypes were able to overcome gene A10, a resistance gene of raspberry, which has resistance for all biotypes of *Am idaei*. Similar observation was seen in our study where, both Glen Ample with resistance gene A1, and Glen Mor cultivar (which was cultivated in 2020) with resistance gene A10, have been tested to be virus infected and aphids were also observed in the field. Although both BRNV and RLMV got transmitted by same aphid vector (*Am. idaei),* Glen Ample samples were infected with BRNV and RVCV but not by RLMV which may be due to the limited sample size because both BRNV which was heavily infested and RLMV which was absent in our study were transmitted by same vector (Converse et al., 1987). RVCV was not detected in cultivar Veten which may indicates the resistance of RVCV or *Ap. idaei* in this cultivar but the resistance result on our finding may be due to the smaller sampling size (11 out of 95 samples) and possibly infected samples may have escaped during our sampling.

In our result, we found 13 out of 31 aphid samples were infected with BRNV or RLMV or both. Neither RVCV nor RYNV was detected in aphid samples in our study. Virus presence in 22 leaf samples were compared with the virus presence in the aphid that were picked from the same plants and found that around 42% of BRNV and 83% of RLMV are correlated (i.e., either virus present or absence in both aphid and leaf). This correlation seems understandable since both the viruses are transmitted by common vector, *Am. idaei.* Similarly, 41.4% BRNV infection was observed in leaf samples but not in aphid samples. Stace-Smith (1955b) studied that BRNV may be lost after 3 hrs. of starvation. In our case, aphids may have loss the viruses during the sample collection step (separated from the leaf and collected in the sample tubes). Another interesting observation was revealed in some cases in our study: aphid was infected, but the leaf was free from virus even though both aphid and leaf belong to same plant. This may be due to the infected aphid may have landed in the leaf just before we collected the sample or may be fallen from nearby plant samples. Sangar Sequencing result, amplified by COI primers were blast run in NCBI data base to barcode the 13 infected aphid samples. Two species of aphid i.e., *Ap. idaei* and *Am. rubi* were revealed which was quite surprising. *Am. rubi* is host specific to black raspberry whereas, *Am. idaei* is specific to red raspberry and both species doesn't have any alternative host (Converse et al., 1987). Similarity, in some samples, blast run revels the 100% identification with both *Am. rubi*, as well as *Am. idaei* which made

complex in the molecular differentiation between *Am. rubi* and *Am. idaei.* As, all the samples in our study were collected from red raspberries *Am idaei* must be the aphids that was infecting our samples. (Personal communication, Zhibo Hamborg). The molecular analysis between these two aphids should be done to gain proper knowledge on their identification.

5.2 *Aphis idaei* and its role in BRNV transmission

Although, *Ap. idaei* was considered the vector for RVCV, for the first time, we have detected BRNV and RLMV in *Ap. idaei* by RT-PCR. To verify the result, one simple BRNV transmission test was conducted. Only BRNV was considered in this experiment due to the limitation of time. BRNV transmission results verify the acquisition of BRNV in *Ap. idaei*. but it couldn't confirm the transmission of BRNV into healthy raspberry plants. Although acquisition of BRNV virus is the new finding but its failure in transmission confirms the previous work of Cadman & Hill (1947). In our study, we found that *Ap. idaei* needed at least 1 hrs. of acquisition time to acquire BRNV and need at least 5 hrs. of starvation to loss the virus from their body. We had detected the virus in *Ap. idaei* at 30 min of starvation which may indicate that the virus may have entered the aphid's body but escaped as excrement with honeydew. Gray & Gildow (2003) detect viral particle, *luteovirus*, in honeydew of its aphid vector. Though, the raspberry virus is different from *luteovirus* but the study of honeydew composition of virulence *Ap. idaei* could give better explanation of our finding on virus acquisition and loss. The feeding behavior of aphid were studied in different literature. The normal behavior of aphids is to make short probes by penetrating its stylet into the plant tissues to recognize the plant as a host before settling and penetrating the stylet into the phloem for feeding (Klingauf, 1988). Virus transmission is successful, if the virus acquired in the aphid gets entered the salivary gland and salivation during the probing or feeding and carries the virus particles into the host plant (Wilson, 2012). In our study, virus might have failed to return in salivary gland of *Ap. idaei* which is crucial for transmission. More detail study on virus/vector association is necessary. Furthermore, Stace-Smith (1961) observed that the densely packed colonies of *Ap. idaei* appeared in the spring were the more efficient vectors than that of dispersed summer form of the aphid. They also mentioned that the number of aphids also have impact on virus transmission. As this experiment were conducted in summer with dispersed form of *Ap. idaei* and only 5 aphids were released per plant, vector efficiency might get decreased in our study.

6. Conclusion

This study reports the occurrence of 3 different aphid borne viruses (BRNV, RLMV and RVCV) on selected counties of Norway. Based on the result presented, BRNV was the most prevalent and widely distributed viruses in all the symptomatic plant samples. All the RLMV positive sample were detected from Vestland county only, which does suggest that this part of Norway may have been a source of RLMV. The obtained result showed the highest proportion of mixed infection (BRNV and RLMV) in raspberry samples which leads the raspberry mosaic symptoms in the plants. Similarly, Glen Ample and Glen Mor which has the resistance gene A1 and A10 respectively were infected with aphids and were detected virus infection which may suggest the breakdown of aphid resistance in these cultivars. This knowledge of prevalence viruses, risk areas, outbreaks of contamination and the cultivar response on viruses or vector can be used to guide farmers on the management tools to be used and the raspberry varieties to be chosen.

Ap. idaei and *Am. idaei* both aphid species were identified in the sampling and observed some degree of associated on transmission of different viruses. This is the first report on the detection of BRNV and RLMV viruses in *Ap. idaei*. BRNV transmission experiment on *Ap. idaei* revels that the acquisition time of this aphid is at least 1 hr. and can loss the virus in at least 5 hrs. of starvation but the transmission was not proved. Further research on biology and its transmission role on all aphids borne viruses is recommended.

7. References

Alford, D. V. (2007). *Pests of fruit crops: a color handbook.* Elsevier, 441 pp.

Allen, J. & Raffle, S. (2000). Raspberries under cover. *The Grower*, *135* (5), 12-4.

Amari, K., Huang, C. & Heinlein, M. (2021). Potential Impact of Global Warming on virus propagation in infected plants and agricultural productivity [Perspective]. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.649768.

Barnett, O. W. & Murant, A. F. (1970). Host range, properties, and purification of raspberry bushy dwarf virus. *Annals of Applied Biology,* 65 (3), 435–449.

Baumann, G. (1982). Elimination of a heat-stable raspberry virus by combining heat treatment and meristem culture. *Acta Horticulturae,* 129: 11-12.

Berger, P. H. & Ferris R. S. (1989). *Mechanism of arthropod transmission of plant viruses. Implications for the spread of disease*, pp. 40-84. In Jeger, M. S. [ed.], *Spatial components of plant disease epidemics.* Prentice-Hall, Englewood Cliffs, NJ.

Bobinaitė, R., Viškelis, P. & Venskutonis, P. R. (2016). Chemical Composition of Raspberry (*Rubus* spp.) Cultivars: Nutritional Composition of Fruit Cultivars. *Academic Press*, 713–731. https://doi.org/10.1016/B978-0-12-408117-8.00029-5

Bojkovska, K., Joshevska, F., Tosheva, E., & Momirceski, J. (2021). Global raspberries market trends and their impact on the Macedonian raspberries market. *International Journal of Research and Review*, *8* (2), 362-369.

Borowiak-Sobkowiak, B. (2005). Bionomy and ecology of *Aphis idaei* vd Goot on raspberry. *Aphids Hemipt. Insects*, 11, 5-16.

Bøthun, M. & Heiberg, N. (2004). Satsing på økologisk bringebær (Production of organic raspberries in Norway). *Norsk Frukt og bær,* 7 (4): 16-18.

Briggs, J. B. (1965). The distribution, abundance, and genetic relationships of four strains of Rubus aphid (*Amphorophora rubi* (Kalt.)) in relation to raspberry breeding. *Journal of Horticultural Science*, 40, 109 – 117.

Butter, N. S. (2018). *Insect Vectors and Plant Pathogens*. CRC Press, 496 pp. https://doi.org/10.1201/9780429503641

Cadman, C. H. (1951). Studies in *Rubus* virus diseases. I. A latent virus of Norfolk Giant raspberry. *Annals of Applied Biology*, 38: 801-811. https://doi.org/10.1111/j.1744-7348.1951.tb07851.x

Cadman, C. H. (1952a). Studies *in Rubus* virus diseases. II. Three types of vein chlorosis of raspberries. *Annals of Applied Biology*, 39: 61-68. https://doi.org/10.1111/j.1744-7348.1952.tb00998.x

Cadman, C. H. (1952b). Studies in Rubus virus diseases. III. A vein-banding disease of raspberries. *Annals of Applied Biology*, 39, 69–77. https://doi.org/10.1111/j.1744-7348.1952.tb00999.x

Cadman, C. H. (1961). Raspberry viruses and virus diseases in Britain. *Horticultural Research*, 1: 47-61.

Cadman, C. H. & Fisken, A. G. (1958). Susceptibility of raspberry varieties to infection by aphid-borne viruses. *Journal of Horticultural Science,* 33: 13-20.

Cadman, C. H. & Hill, A. R. (1947). Aphid vectors of European raspberry viruses. *Nature* (London) 160: 837-838.

Cadman, C. H. & Stace-Smith, R. (1970). *Raspberry vein chlorosis*. p. 119-120. In: Frazier N. W, [ed.], *Virus diseases of small fruits and grapevines*. University of California, Division of Agricultural Sciences, Berkeley, 290 pp.

Chambers, J. (1954). Heat Therapy of Virus-infected Raspberries. *Nature*, 173 (4404), 595-596. https://doi.org/10.1038/173595a0

Cheong, E., Jeon, A., Kang, J., Mock, R., Kinard, G. & Li, R. (2014). In vitro elimination of Black raspberry necrosis virus from black raspberry (*Rubus occidentalis*)-Short Communication. *Horticultural Science*, 41 (2), 95-99.

Cieślińska, M. (2021). First Report of Raspberry Leaf Mottle Virus Infecting Raspberry in Poland. *Plant Disease*, 105 (3), 714-714.

Converse, R. H. (1963). Influence of heat-labile components of raspberry mosaic virus complex on growth and yield of red raspberries. *Phytopathology*, *53* (11), 1251.

Converse, R. H. (1966). Effect of heat treatment on the raspberry mosaic complex in 'Latham' red raspberry. *Phytopathology*, 56: 556-559.

Converse, R. H. (1987). *Virus diseases of small fruits* (No. 631). US Department of Agriculture, Agricultural Research Service, 254 pp.

Converse, R. H., Stace-Smith, R. & Cadman, C. H. (1970). *Raspberry mosaic,* p. 111-119. In: Frazier N. W, [ed.], *Virus diseases of small fruits and grapevines.* University of California, Division of Agricultural Sciences, Berkeley, 290 pp.

Converse, R. H., Stace-Smith, R. & Jones A.T. (1987). Raspberry mosaic, p. 168–175. In: Converse, R.H. [ed.], *Virus Diseases of Small Plants,* Agriculture Handbook, Washington, D.C: USDA, ARS.

Dicker, G. H. L. (1941). The biology of the Rubus aphides. *Journal of Pomology and Horticultural Science*, 18 (1), 1-33

Dietzgen, R. G., Mann, K. S., & Johnson, K. N. (2016). Plant virus–insect vector interactions: current and potential future research directions. *Viruses*, *8* (11), 303. https://doi.org/10.3390/v8110303

FAO. (2022). FAOSTAT statistical database. Rome: Food and Agriculture Organisation of the United Nations. http://www.fao.org/faostat/en/#data/QC

Folmer, O., Hoeh, W. R., Black, M. B., & Vrijenhoek, R. C. (1994). Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology*, *3* (5), 294-299.

Freeman, J. A., & Stace-Smith, R. (1970). Effects of raspberry mosaic viruses on yield and growth of red raspberries. *Canadian Journal of Plant Science, 50 (5), 521–527.* https://doi.org/10.4141/cjps70-099

Gray, S. & Gildow, F. E. (2003). Luteovirus-aphid interactions. *Annual review of phytopathology*, *41*, 539.

Grigorov, S.P. (1965). Listnite vschki i borbata s tiach. Zemizdat, sofia, 54-56

Halgren, A., Tzanetakis, I. E. & Martin, R. R. (2007). Identification, characterization, and detection of black raspberry necrosis virus. *Phytopathology,* 97 (1), 44–50. https://doi.org/10.1094/PHYTO-97-0044

Heiberg, N., Standal, R. and Måge, F. (2002). Evaluation of red raspberry cultivars in Norway. *Acta Hortic,* 585, 199-202. https://doi.org/10.17660/ActaHortic.2002.585.31

Jennings, D. L. (1988). Raspberries and Blackberries: Their Breeding, Diseases and Growth. *Academic Press,* London, UK. 230 pp.

Jeong, J.-J., Ju, H.-J., & Noh, J. (2014). A Review of detection methods for the plant viruses. *Research in Plant Disease*: *Korean Society of Plant Pathology.* https://doi.org/10.5423/rpd.2014.20.3.173

Jones, A. T. (1976). Purification of aphid-borne viruses directly from raspberry. *Scottish Horticultural Research Institute*, 73-74.

Jones, A. T. (1986). Advances in the study, detection and control of viruses and virus diseases of Rubus, with particular reference to the United Kingdom. *Crop Research*, 26, 127-171.

Jones, A. T. & Jennings, D. L. (1980a). Genetic control of the reactions of raspberry to black raspberry necrosis, raspberry leaf mottle and raspberry leaf spot viruses. *Annals of Applied Biology,* 96 (1), 59–65. https://doi.org/10.1111/j.1744-7348.1980.tb04769.x

Jones, A. T. & Jennings, D. L. (1980b). Disease and pest resistance. *Scottish Horticultural Research Institute,* Annual Report, 52-53.

Jones, A. T. & Murant, A. F. (1972). Some properties of a mechanically transmissible virus widespread in raspberry *(Rubus idaeus)* in Scotland. *Plant Pathology*, 21: 166-170. https://doi.org/10.1111/j.1365-3059.1972.tb01753.x

Jones, A. T. & Roberts, I. M. (1976). Ultrastructural changes and small bacilliform particles associated with infection by rubus yellow net virus. *Annals of Applied Biology*, 84 (3), 305-310. https://doi.org/10.1111/j.1744-7348.1976.tb01773.x

Jones, A. T., & Roberts, I. M. (1977). *Rubus* host range, symptomatology, and ultrastructural effects of a British isolate of black raspberry necrosis virus. *Annals of Applied Biology,* 86 (3), 381–387. https://doi.org/10.1111/j.1744-7348.1977.tb01853.x

Jones, A. T., McGavin, W. J., Geering, A. D. W. & Lockhart, B. E. L. (2002). Identification of rubus yellow net virus as a distinct badnavirus and its detection by PCR in Rubus species and in aphids. *Annals of Applied Biology*, *141* (1), 1-10.

Jones, A.T., McGavin, W. J. & Birch, A.N.E. (2000). Effectiveness of resistance genes to the large raspberry aphid, Amphorophora idaei Börner, in different raspberry (Rubus *idaeus* L.) genotypes and under different environmental conditions. *Annals of Applied Biology,* 136, 107 – 113. https://doi.org/10.1111/j.1744-7348.2000.tb00014.x

Jones, S., McGavin, W., & MacFarlane, S. (2019). The complete sequences of two divergent variants of the rhabdovirus raspberry vein chlorosis virus and the design of improved primers for virus detection. *Virus research*, *265*, 162-165.

Jordovic, M. (1963). Investigation of the economically most important virus diseases of raspberries in Yugoslavia. *Archiv za Poljoprivredne Nauke*, 16: 3-27.

Kennedy, J. S., Day, M. F. & Eastop, V. F. (1962). A conspectus of aphids as vectors of plant viruses. *Commonwealth Institute Entomology*, London, 5-99

Klingauf, F.A. (1988). *Feeding, adaptation and excretion*. In: Minks, A. K. & Harrewijn P. [ed.], World Crop Pests. *Aphids. Their Biology, Natural Enemies and Control,* Elsevier, 209-253.

Lightle, D. & Lee, J. (2014). Raspberry viruses affect the behavior and performance *of Amphorophora agathonicain* single and mixed infections. *Entomologia Experimentalis et Applicata,* 151 (1), 57–64. https://doi.org/10.1111/eea.12170.

Mabberley, D. J. (2017). *Mabberley's plant-book: a portable dictionary of plants, their classification and uses*. Cambridge university press. 996 pp. https://doi.org/10.1017/9781316335581

Martin, R. R., Macfarlane, S., Sabanadzovic, S., Quito, D., Poudel, B., & Tzanetakis, I. E. (2013). Viruses and Virus Diseases of Rubus. *Plant Disease*, *97* (2), 168-182. https://doi.org/10.1094/pdis-04-12-0362-fe

Matthews, R. E. F., & Hull, R. (2002). *Matthews' plant virology*. Gulf professional publishing. 983 pp.

McGavin, W. J. & MacFarlane, S. A. (2016). The complete nucleotide sequence of pelargonium leaf curl virus. *Archives of virology*, *161* (5), 1415-1417.

McGavin, W. J. & MacFarlane, S.A. (2010). Sequence similarities between Raspberry leaf mottle virus, Raspberry leaf spot virus and the closterovirus Raspberry mottle virus. *Ann. Appl. Biol*, *156*, 439–448.

McGavin, W.J., McMenemy, L.S. & MacFarlane, S.A. (2010). The complete sequence of a UK strain of Black raspberry necrosis virus. *Journal of General Virology,* 93: 430- 437.

McMenemy, L. S., Mitchell, C., & Johnson, S. N. (2009). Biology of the European large raspberry aphid (*Amphorophora idaei):* its role in virus transmission and resistance breakdown in red raspberry. *Agricultural and Forest Entomology,* 11 (1), 61–71. https://doi.org/10.1111/j.1461-9563.2008.00409.x

Mellor, F.C & Stace-Smith, R. (1979). A heat-stable black raspberry necrosis virus. *Acta Horticulturae*, 95: 71-75.

Menzel, W., Jelkmann, W., & Maiss, E. (2002). Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. *Journal of Virological Methods*, *99* (1-2), 81-92. https://doi.org/10.1016/S0166-0934(01)00381-0

Namba, R. (1962). Aphid transmission of plant viruses from the epidermis and sub epidermis tissue: *Myzus persicae* (Sulzer)-cucumber mosaic virus. *Virology,* 16: 267-271

Nault, L. R. (1997). Arthropod Transmission of Plant Viruses: A New Synthesis. *Annals of the Entomological Society of America*, 90 (5), 521–541.

Oliveira, P. B., Oliveira, C. M., Lopes-da-Fonseca, L. & Monteiro, A. A. (1996). Off-season production of primocane-fruiting red raspberry using summer pruning and polyethylene tunnels. *HortScience*, *31* (5), 805-807. https://doi.org/10.21273/HORTSCI.31.5.805

Perasović, I. (2013). Red raspberry *(Rubus Idaeus L.)* cultivars in a Nordic climate: morphological traits and berry quality (Master's thesis, Norwegian University of Life Sciences, Ås).

Rautapää, J. (1967). The bionomics of the raspberry aphids *Aphis idaei* vd Goot and *Amphorophora rubi* (Kalt.) (Hom., Aphididae). *Annales Agriculturae Fenniae*, 127-144.

Sastry, K. S., & Zitter, T. A. (2014). *Plant virus and viroid diseases in the tropics:* Vol (2): *epidemiology and management*. Dordrecht: Springer 489 pp.

Stace-Smith, R & Lo, E. (1973). Morphology of bacilliform particles associated with raspberry vein chlorosis virus. *Canadian Journal of Botany,* 51: 1343-1345.

Stace-Smith, R. (1955a). Studies on rubus virus diseases in British Columbia: I. Rubus yellow net. *Canadian Journal of Botany,* 33(3), 269-274.

Stace-Smith, R. (1955b). Studies on Rubus virus diseases in British Columbia. II. Black raspberry necrosis. *Canadian Journal of Botany*, 33: 314-322.

Stace-Smith, R. (1956). Studies on *Rubus* virus diseases in British Columbia.III. Separation of components of raspberry mosaic. *Canadian Journal of Botany*, 34: 435-442.

Stace-Smith, R. (1960). Studies on Rubus virus diseases in British Columbia. VI. Varietal susceptibility to aphid infestation in relation to virus acquisition. *Canadian Journal of Botany*, 38: 283-285

Stace-Smith, R. (1961). Studies on *Rubus* virus diseases in British Columbia. VII. Raspberry vein chlorosis. *Canadian Journal of Botany*, 39: 559-565.

Stace-Smith, R. & Jones, A. T. (1978). USDA Agriculture Handbook No. 631 (USDA, Washington). 175.

Stace-Smith, R. & Leung, E. (1976). Small bacilliform particles associated with Rubus yellow net virus. *Proceedings of the American Phytopathological Society,* 3:320.

Sylvester, E. S. (1958). *Aphid transmission of plant viruses,* pp. 195-200. In: Becker, E. C. [ed.], Proceedings, 10th International Congress Entomology, Montreal, vol. 3.

Tan, J. L., Trandem, N., Fránová, J., Hamborg, Z., Blystad, D. R. & Zemek, R. (2022). Known and Potential Invertebrate Vectors of Raspberry Viruses. *Viruses*, 14 (3), 571. https://doi.org/10.3390/v14030571

Tzanetakis, I. E., Halgren, A., Mosier, N. & Martin, R. R. (2007). Identification and characterization of Raspberry mottle virus, a novel member of the Closteroviridae. *VirusResearch*, 127 (1), 26-33.

Uleberg, E., Sønsteby, A., Jaakola, L. & Martinussen, I. (2016). Climatic effects on production and quality of berries – a review from Norway. *Acta Horticulturae,* (1117), 259–262. https://doi.org/10.17660/ActaHortic.2016.1117.41

Vidaver, A. K. & Lambrecht, P. A. (2004). Bacteria as plant pathogens. *The plant health Instructor*, 10. http://doi.org/10.1094/PHI-I-2004-0809-01

Watson, M. A. & Roberts, F. M. (1939). A comparative study of the transmission of Hyocyamus virus 3, potato virus Y and cucumber virus 1 by the vectors Myzus persicae (Sulz), M. circumftexus (Buckton), and Macrosiphumgu (Koch). Proc. R. Soc. Lond. *Biol. Sci.* 127: 543-576. https://doi.org/10.1098/rspb.1939.0039

Wilson, C.R. (2014). *Applied plant virology*. CABi. 177 pp

8. Appendix

Appendix 1. Personal experience and observation on rearing *Aphis idaei* from overwintering eggs.

For aphid culture, I collected different raspberry canes (wild or cultivated) form different field of Ås, Norway on 28th January. I found aphids eggs in some of the raspberry canes. They were shiny black in color and were usually lies around the nodes of raspberry cane as shown in Fig.13(A). Those canes (with aphid eggs) were provided with water as shown in Fig.13(B) and were kept in room temperature. Unfortunately, they couldn't hatch, and eggs became shrink and dried. I collected the aphid eggs in different time frame (i.e., on 17th February 29th February and on 7th March) and reared as same process but unfortunately, they became shrink and dried out in 7-8 days in every cases. On 15th March, I again collected the raspberry canes with aphid eggs and for the first time, one eggs was hatched on 22nd February. After this, more eggs were hatched on following days. but it was very difficult to make nymph alive. After the eggs were hatched, they were transferred to virus free raspberry cultivar "Stiora". This cultivar has a lot of hairs that used to protect the aphid's nymph to reach the base of leaf/stem and suck sap (Fig.13(F)). From my observation, I found the nymphs were very sensitive and mostly prefer flower bud because they used to survive quite nicely on the flower buds. (These flower buds were developed in the collected raspberry canes which were placed in the water) (Fig. 13(E)).

There was one plant sauna project in NIBIO, where the raspberry canes that were collected from different field nearby NIBIO were treated with hot water (around 35-40 °C.) to kill the mites. In April 12, I was informed about the project and observed a lot of aphids (*Ap. Idaei*) on the control treatment of that experiments. Here, I observed the aphid colony were denser around flower buds of raspberry canes. I also found that plant materials (raspberry canes) used in that experiment lacks hairs (Fig 13(E)). I took 10 fundatrix (adult female which has developed from an overwintering fertilized egg) (Fig 13(C)) from there and cultured them on 2nd year raspberry canes.

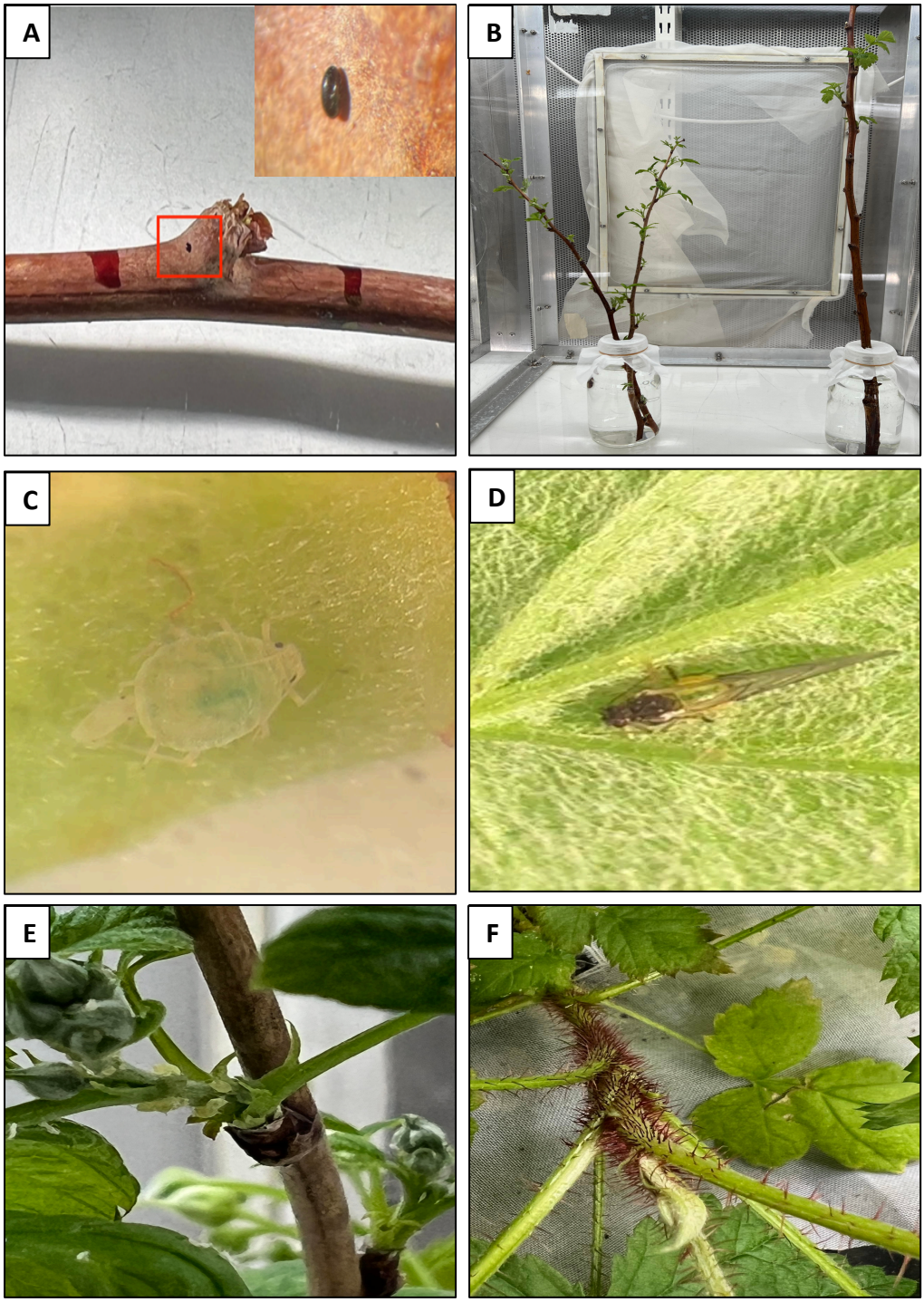
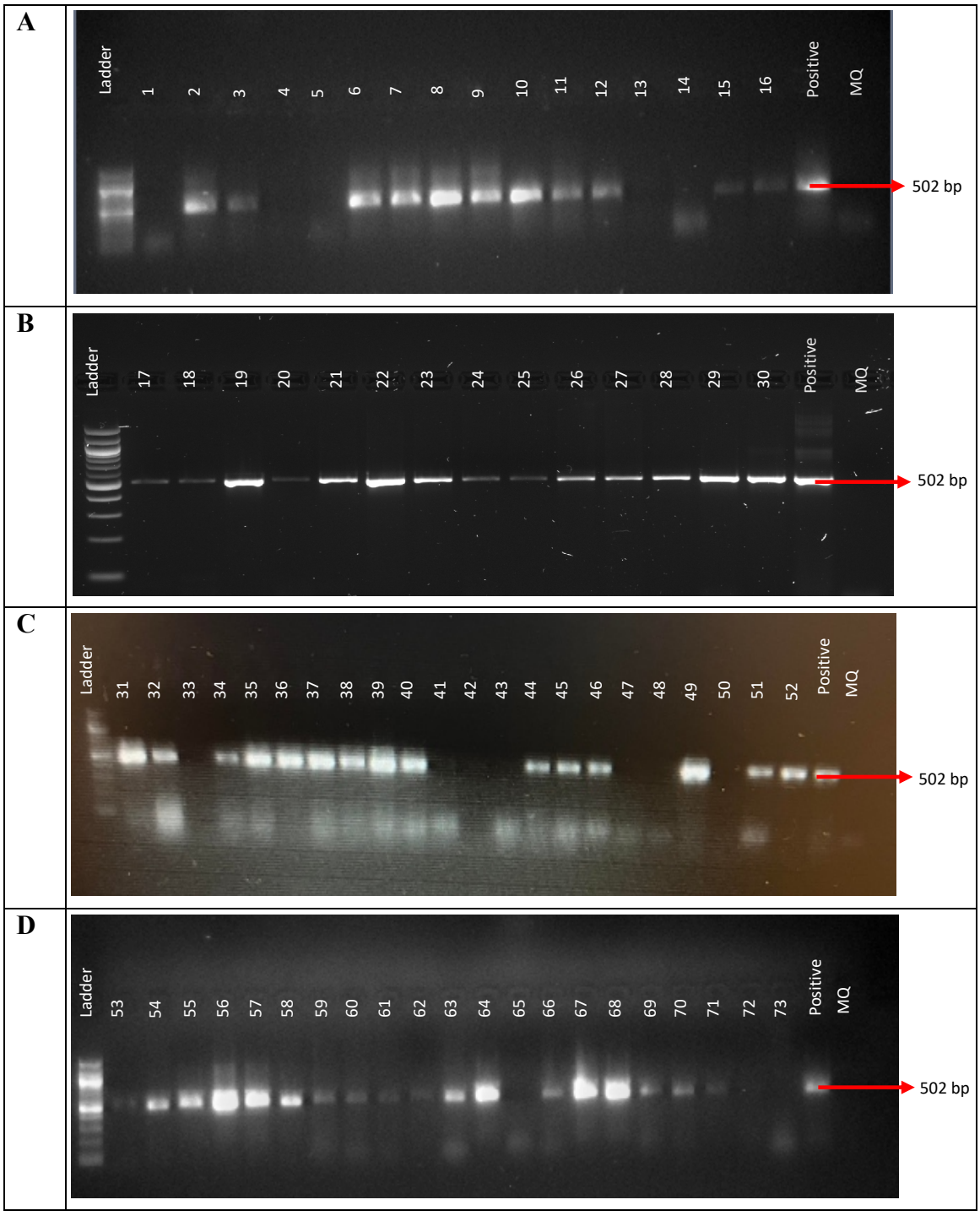


Fig 13. A. Egg of *Aphis idaei*. B. Canes containing raspberry eggs that were dipped in the water. C. Fully matured *Ap. idaei*. D. Winged form of *Ap. idaei*. E. Aphid colonized heavily around flower buds. F. "Stiora" cultivar with heavy hairs.

Appendix 2. Gel image obtained by RT-PCR detection.



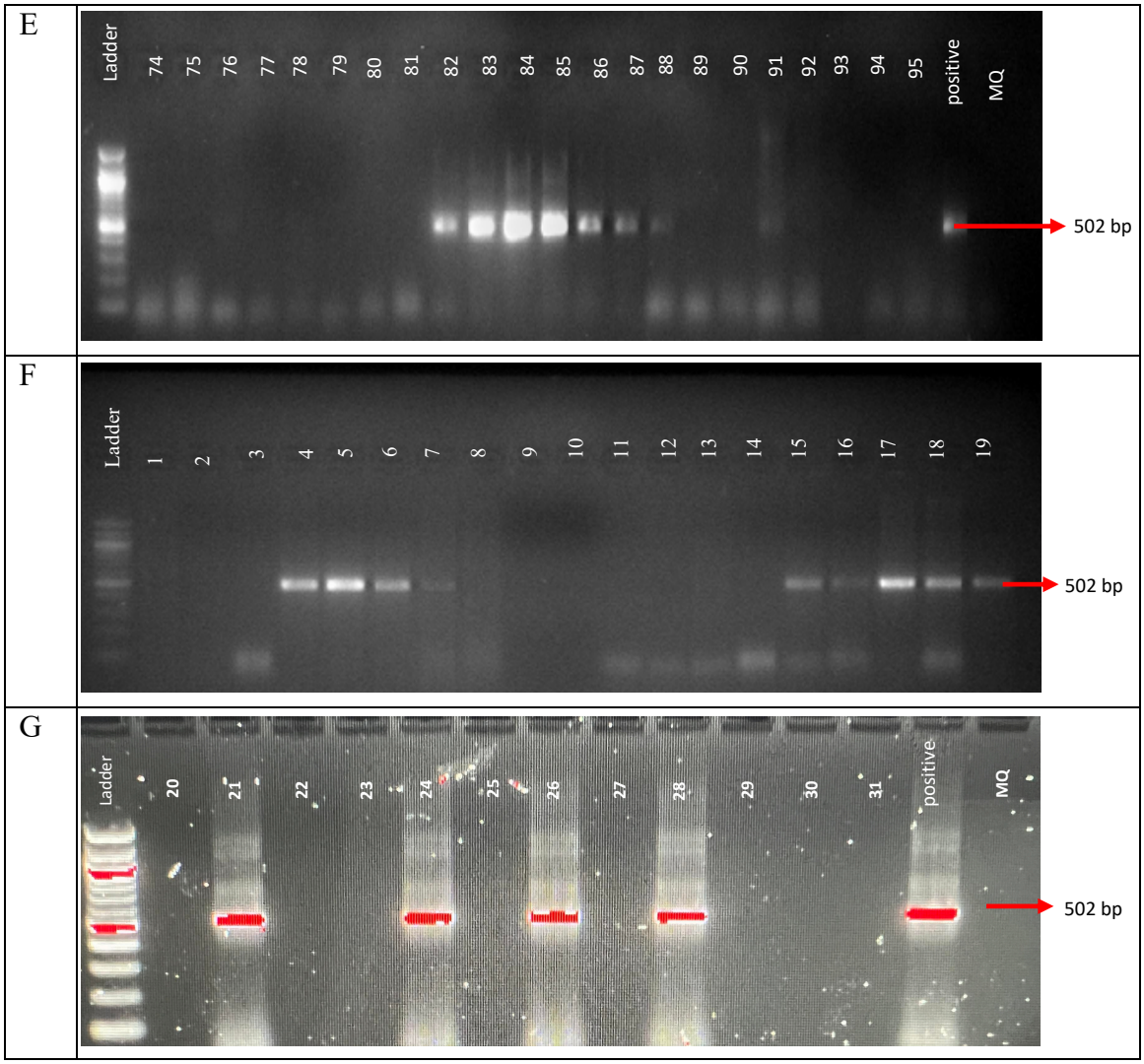
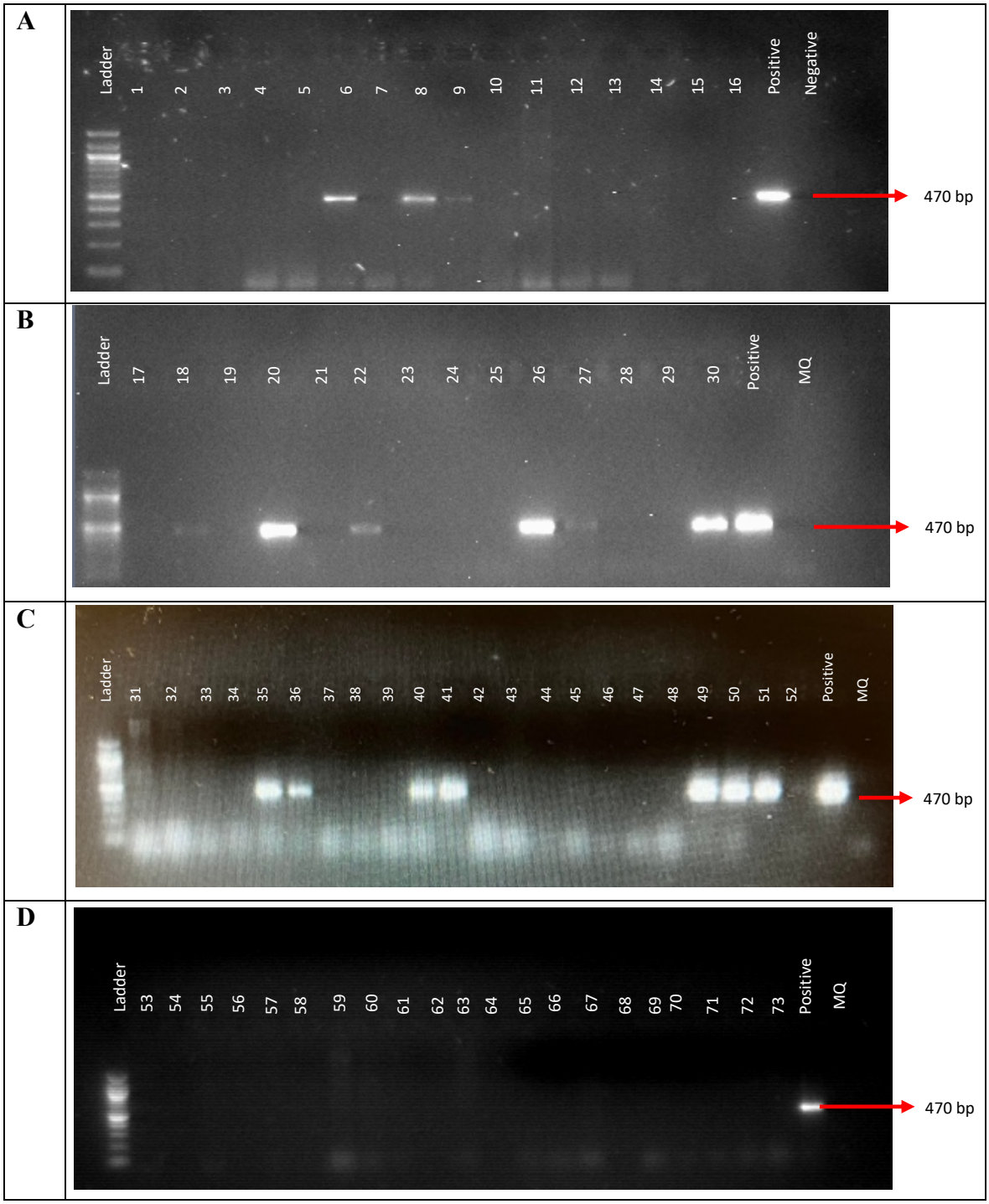


Fig 14. RT-PCR result for black raspberry necrosis virus (BRNV). A, B, C, D and E are the Gel image of 95 plant samples. F and G are the gel image of 31 aphid samples. Ladder used was 100bp and positive and MQ represent BRNV positive control and negative control (water) respectively.


A
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Positive
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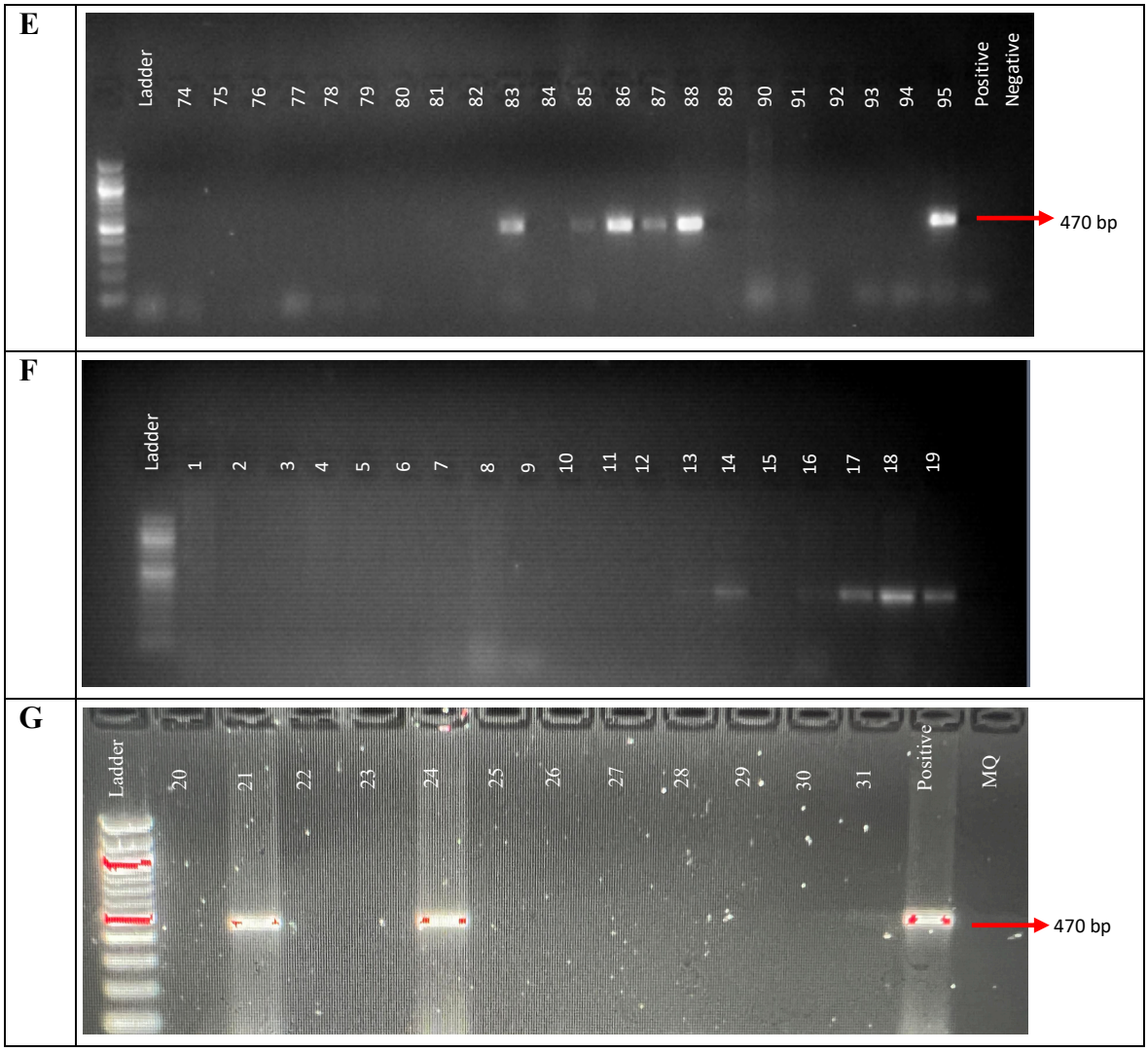
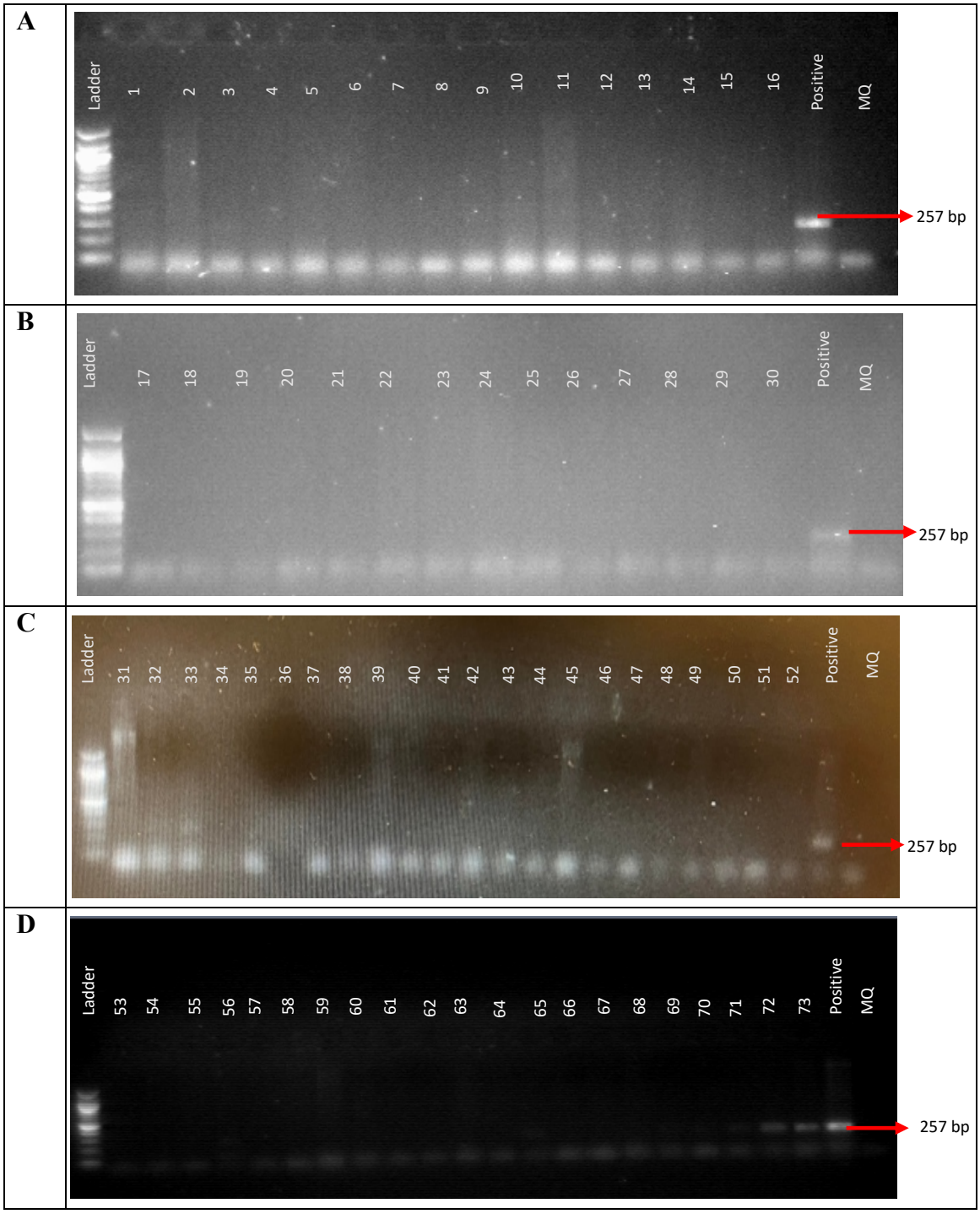


Fig 15. RT-PCR result for raspberry leaf mottle virus (RLMV). A, B, C, D and E are the Gel image of 95 plant samples. F and G are the gel image of 31 aphid samples. Ladder used was 100bp and positive and MQ represent RLMV positive control and negative control (water) respectively.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Positive
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C
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Positive
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Positive
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257 bp

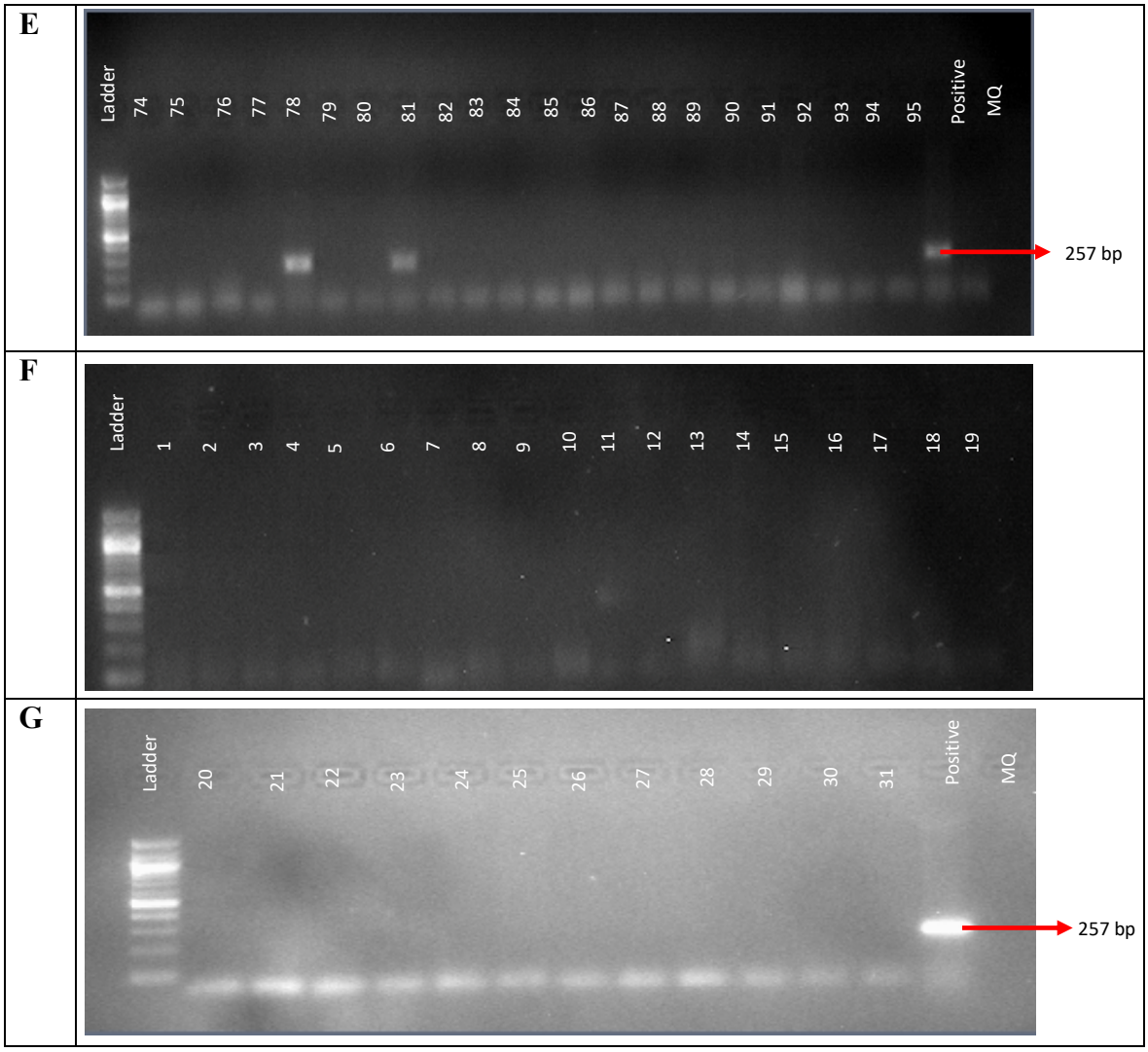
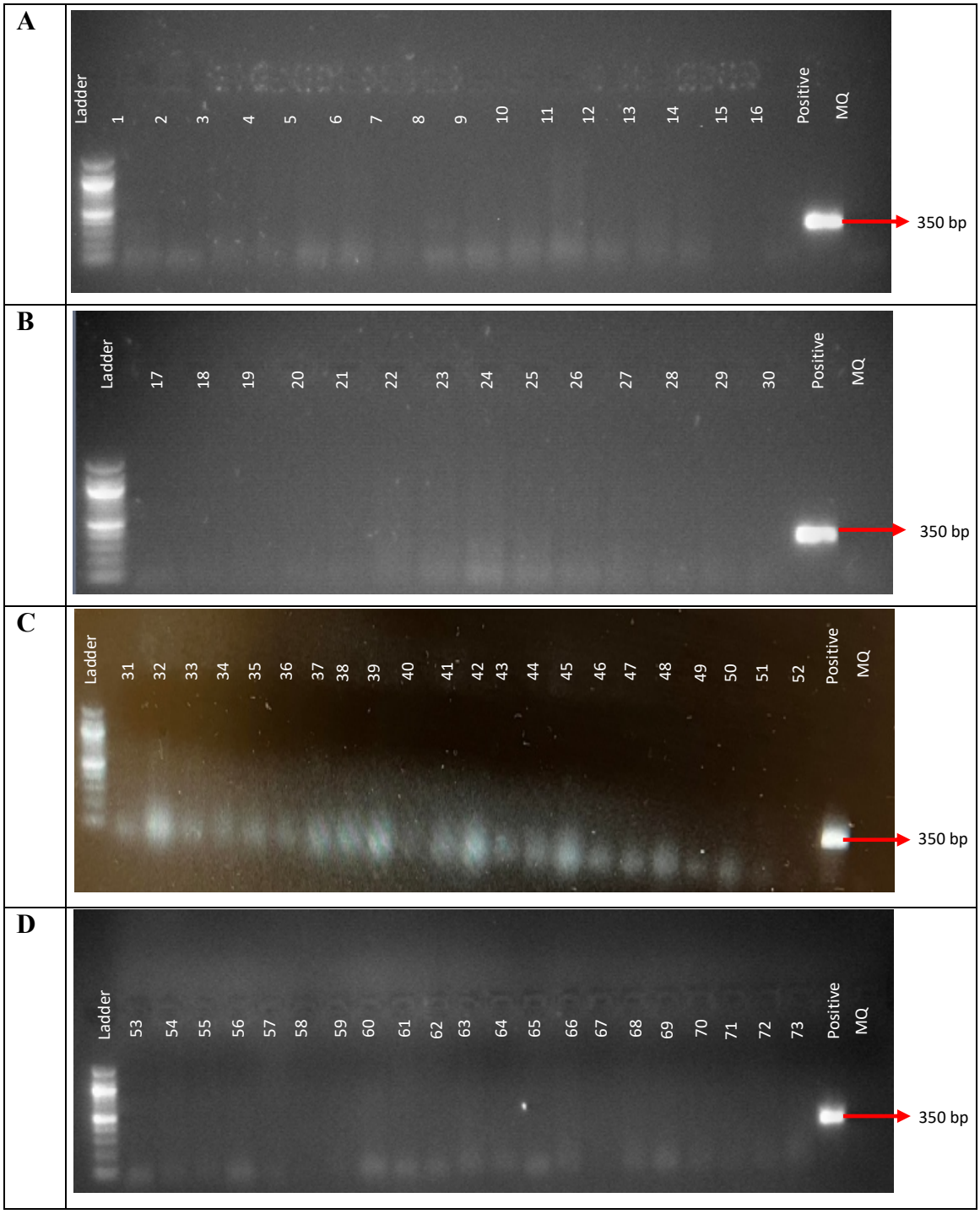


Fig 16. RT-PCR result for raspberry vein chlorosis virus (RVCV). A, B, C, D and E are the Gel image of 95 plant samples. F and G are the gel image of 31 aphid samples. Ladder used was 100bp and positive and MQ represent RVCV positive control and negative control (water) respectively.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Positive
MQ
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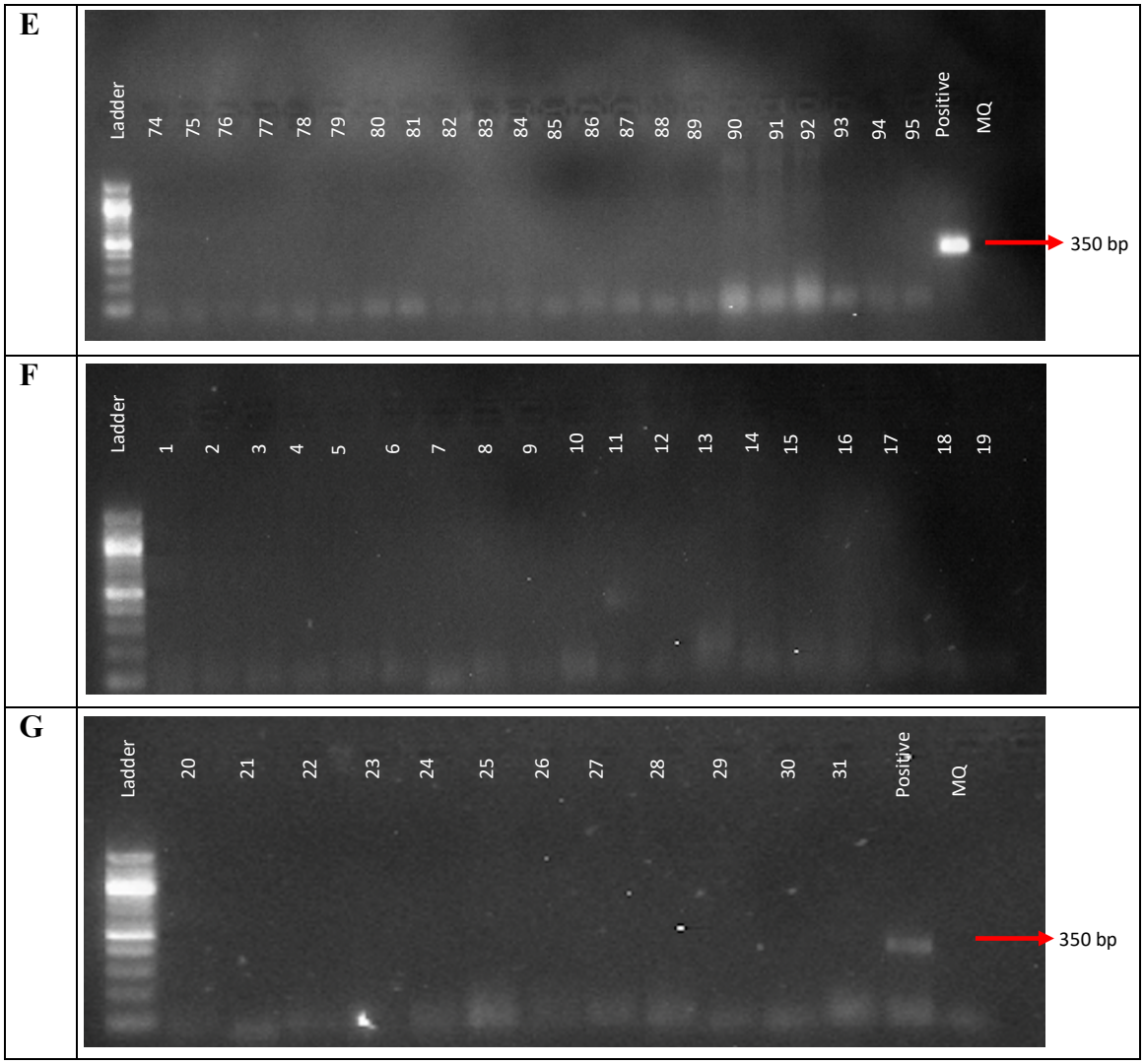

Fig 17. RT-PCR result for rubus yellow net virus (RYNV). A, B, C, D and E are the Gel image of 95 plant samples. F and G are the gel image of 31 aphid samples. Ladder used was 100bp and positive and MQ represent RYNV positive control and negative control (water) respectively.

Appendix 3. Overview of virus infestation in leaf samples from different counties and cultivars

County/ Cultivars.	BRNV.	RLMV.	RVCV.
Agder	**8**	**0**	**1**
Glen Ampel	3	0	1
Other	2	0	0
Wild	3	0	0
Vestland	**49**	**21**	**4**
Glen Ampel	9	0	0
Glen Mor	5	1	1
Other	5	2	1
Veten	9	5	0
Wild	21	13	2
Viken	**9**	**0**	**1**
Glen Ampel	3	0	0
Other	0	0	1
Wild	6	0	0
Grand Total	**66**	**21**	**6**

Appendix 4. Overview of virus infestation in aphid samples from different counties and cultivars

County/ cultivars	BRNV	RLMV
Agder	**5**	**0**
Glen Ampel	2	0
Wild	3	0
Vestland	**7**	**7**
Other	0	0
Veten	7	7
Wild	0	0
Grand Total	**12**	**7**

Norges miljø- og biovitenskapelig universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway