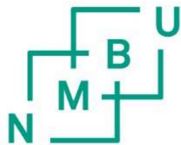


Norwegian University
of Life Sciences

Master's Thesis 2022 30 ECTS
Faculty of Biosciences

Review of viruses in raspberry production

Jakob Egger
Plant Sciences

Table of Contents

1. Abstract…………………………………………………………………..3
2. Introduction………………………………………………………………4
3. Literature review…………………………………………………………5
    3.1 General information about the genus *Rubus*……………………...5
    3.2 The raspberry fruit……………………………………………….....6
        3.2.1 Uses of the raspberry fruit………………………………......7
        3.2.2 Worldwide production of raspberry fruit…………………....8
        3.2.3 Raspberry cultivars and breeding ………………...…........8
    3.3 Important non-viral raspberry diseases……………………………..10
    3.4 Viral diseases of the raspberry shrub……………..………………...12
        3.4.1 Aphid-transmitted viruses………………………............…12
        3.4.2 Nematode-borne viruses…………………………………...15
        3.4.3 Pollen-borne/seed borne viruses…………………………...17
        3.4.4 Mite-borne viruses…………………………………………19
        3.4.5 Infection with multiple viruses (co-infection)………………..19
    3.5Diagnostic tools for virus detection…………………………………20
    3.6Management strategies……………………………………………....21
4. Material & Methods……………………………………………….…23
    4.1Bioassays…………………………………………………...….23
    4.2ELISA……………………………………………………….…….25
    4.3RT-PCR…………………………………………………………...26
5. Results……………………………………………………………….…29
    5.1Bioassays…………………………………………………….…....29
    5.2ELISA & PCR………………………………………………….…35
6. Discussion…………………………………………………..……..37
7. Conclusion……………………………………………………………....39
8. Acknowledgements………………………………............................40
9. Bibliography………………………………………………….………41
10. Appendix……………………………………………………………..46
    10.1 Tables documenting virus development …………………………46
    10.2 ELISA plate readings & bioassay photo-series…………….........52

1. Abstract

Raspberries are one of the commercially most important kinds of berry fruits. Botanically, they are members of the large and diverse genus *Rubus* and belong to the greater rose family (*Rosaceae*). The raspberry shrub shares many characteristics with roses - beside the spines and bristles also a susceptibility for many fungal, bacterial and viral pathogens. This review talks about general knowledge of raspberries: their use, challenges, worldwide production and breeding efforts. However, the main part of this review focuses on diseases of the raspberry shrub, with a special emphasis on viral agents of disease. As is the case for plant viruses in general, raspberry viruses need vector animals to gain entry into the cell where they can use the nucleic acid machinery to replicate their own genetic material (Wilson, 2014). In raspberries, the role of vector is primarily performed by aphids (for overground infection) and nematodes (for underground infection). Nevertheless, many viruses capable of attacking the raspberry plant use lesser known vectors such as mites and whiteflies or infiltrate the pollen of a host plant to be expelled, transported and ultimately proliferated by the wind.

This review also contains a smaller body of work in the form of an experimental section. There, inoculation experiments of eight virus isolates on test plants are described. Inoculation experiment are performed using traditional sap inoculation from frozen leaf material. *Nicotiana spp.* and *Chenopodium quinoa* are used as test plants. In a separate inoculation experiment, fresh fine root material from ten samples was used to test for arabis mosaic virus (ArMV) vectored by the dagger nematode genus (*Xiphenema* spp.) Bioassays are concluded by re-inoculation of suspected ArMV-diseased plants to confirm virus transmissibility.

In order to evaluate the species relationship of the virus isolates used in the bioassay, three ELISA-tests were performed to test harvested virus samples for tomato black ring virus (TBRV) and ArMV. In a separate molecular analysis, ribonucleic acid (RNA) was extracted from suspected ArMV, TBRV and beet ringspot virus (BRSV). The RNA was converted via reverse transcriptase to cDNA, amplified with polymerase chain reaction (PCR) and separated into size-specific molecular fragments using gel-electrophoresis.

Two of the studied virus isolates were identified as TBRV (Campanula isolate 2000 and Begonia isolate 1996), but the other six isolates studied could not be clearly identified. The isolate from roots of raspberry from a Xiphinema location gave test plant results indicating ArMV.

2. Introduction

This Master's thesis is in strong connection to the on-going (2020-2024) KAPPAberry project: "Healthy berries for a changing climate" -a scientific collaboration by Norway and the Czech Republic (https://www.umbr.cas.cz/en/research/scientific-projects/noberryvirus/). Aim of the project is the development of new biotechnological procedures for virus diagnostics, vector studies and safe preservation of strawberry and raspberry.

The goals of this Master's thesis are:

1) to provide theoretical background knowledge to better understand the genus *Rubus* and to provide knowledge to understand breeding efforts and cultivars in raspberry
2) to offer an overview of viruses infecting raspberries and to link them to their specific vector(s)
3) to identify raspberry-related *Nepoviruses* and to confirm and distinguish some of the harvested virus-infected plant material serologically via bioassay, enzyme-linked immunosorbent assay (ELISA) and by reverse transcriptase polymerase chain reaction (PCR)
4) to isolate *Nepovirus* from field with known nematode presence

This thesis has a heavy focus on literature review. This is explained by restricted access to the laboratory at NIBIO (Norwegian Institute of Bioeconomy Research) up until the beginning of March due to COVID-19 measures.

3. Literature review

3.1 General information about the genus *Rubus*

The European red raspberry (*Rubus idaeus*) is a berry-bearing shrub grown primarily in temperate conditions. The term *Rubus* stems from the Latin word ‘ruber’, which translates to the colour ‘red’. This is primarily, because many of the fruits associated with the species in this genus produce red-coloured fruit. Another term often used interchangeably with *Rubus*, especially in older literature, are ‘brambles’. Botanically speaking, *Rubus* are part of the larger rose family (*Rosaceae*). They possess many characteristics typical for rosaceous plants. The most notable feature of genus *Rubus* are woody stems, often termed canes, lined with spines or prickles to deter herbivores. Nearly all *Rubus* spp. are hermaphrodites – this means that the flowers of most *Rubus* species bear no distinctly male or female characteristics. One notable exception is the cloudberry (*Rubus Chamaemorous*) , a diecious forage berry mostly found in Northern Europe, Siberia and Northern Canada (Pelettier et al. 2001). The center of origin of the *Rubus* genus is the Far East (Martin et al. 2013).

Rubus is a very diverse genus and according to various sources contains from 740 until upwards of 1350 species (Hummer, 1996). It is a genus known for its taxonomic complexity and naming conventions. The lack of clear distinction-factors cause disagreement among botanists of different cultural regions. The reason for these widespread disagreements among scientific expert is the diversity of evolutionary mechanism of the genus. Rubus is also known to readily form hybrids within the genus (Sochor et al. 2022).

In terms of physical structure, Rubus species are shrubs made of herbaceous shoots that lignify into woody canes over the first growing season. Fruit is then produced on lignified canes from the previous growing season. This bearing habit has been bred out in many commercial raspberry cultivars. Raspberry shrubs have a perennial rootstock that is preserved until the plant’s death.

The European red raspberry (*Rubus idaeus*) is part of the larger genus *Rubus*. Most of the species are not appreciated for their fruits and therefore not cultivated on a large scale. However, a select few have been domesticated for their fruity berries. Nowadays, the most cultivated *Rubus* species are the European red raspberry (*Rubus idaeus*), the North American red raspberry (*Rubus idaeus,* subspecies *rugosus*) , the American black raspberry (*Rubus occidentalis*) and the blackberry (several different *Rubus* species).

Future challenges for the genus Rubus lie in climate change, which can be a trigger in the change of many abiotic and biotic growing factors. Another very demanding step in the future of commercial cultivation of Rubus is the demand by costumers for more environmentally sustainable production methods. The most pressing issue is reduction in the use of phytochemicals to reduce incidence of pathogens. (Graham & Brennan, 2018).

3.2 The raspberry fruit

In everyday use of the English language, the term raspberry is used for both the fruit and the plant itself. This colloquial use of the term 'raspberry' applies to all commercially relevant *Rubus* fruits; e.g. the term 'blackberry' is also used for both the herbaceous plant as well as the fruit itself. In most commercially available cultivars of raspberry, fruits are red in colour. Contrary to popular belief, the defining difference between raspberries and blackberries is not the colour of the mature fruit. Instead, the difference lies in whether the receptacle (also known as the stem) 'stays with' the fruit once removed from the herbaceous part of the plant. The receptacle of a fully mature raspberry stays with the raspberry plant, while a mature picked blackberry is plucked with the receptacle inside the fruit.

The raspberry fruit is a compound fruit made up of 50-150 individual fruit drupelets (most often around 100). Every individual drupelet is botanically to be understood as a berry, so the raspberry fruit in reality is a collection of about one hundred berries, bunched together as one fruit (Aprea et al. 2015). Each individual drupelet consists of an outer skin with defensive hair-like structures on the outside. The inside of a raspberry drupe consists of juicy pulp and a central seed.

The raspberry fruit is very interesting from a sensory point of view. The fruit drupelets contain high levels of volatile compounds, which are defined as relatively small and light compounds that vaporise easily in room temperature. Volatile compounds have several advantages for the raspberry fruit in nature. For instance, they attract pollinators and indicate to insects that the fruit is mature and read for consumption. Herbivorous mammals and rodents will then eat the fruit containing the mature seeds and disperse them. The main compound responsible for the characteristic taste of ripe raspberries is 4-(4-hydroxyphenyl)butan-2-one, also known as 'raspberry ketone' (Aprea et al. 2015).

Raspberries are a very nutritious food and contain many vitamin groups such as C, A, B, B1, B2, PP and E. Furthermore, the berries contain folic acid, anthocyanins, phytochemicals and ellagitannins, all of which contribute health benefits (Rommel & Wrolstad, 1993). Raspberries also contain elemental iron and potassium. They are strongly recommended by dieticians for their healthy characteristics (Bobinaitė et al., 2016).

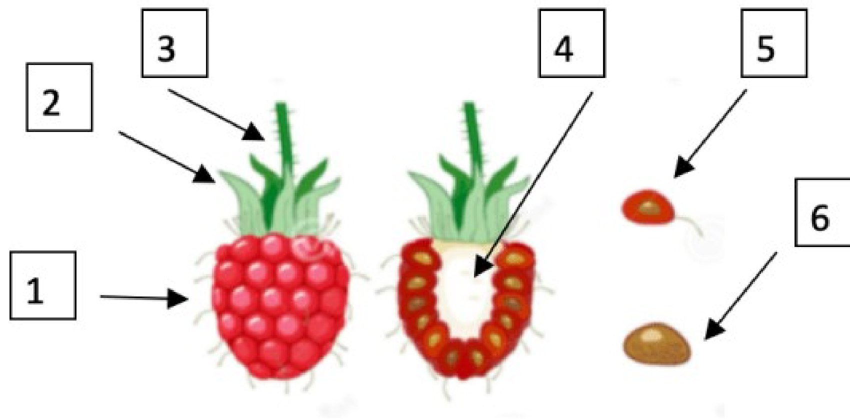


Fig. 1: the raspberry compound fruit and its components. 1) fruit aggregate, consisting of about 100 individual drupelets, 2) sepals, which function as protection for the flower 3) peduncle with which the fruit is connected to the raspberry plant, 4) floral receptacle, commonly known as the stalk, 5) drupelet and 6) seed.(italianberry, 2021)

3.2.1 Uses of the raspberry fruit

In rough general terms, harvested raspberries are either sold on the fresh market or further processed industrially. Some of the industrial products obtained from raspberries are: jams, jellies, syrup, juice, purée or raspberry fruit concentrate. Most of these products use a combination of both the naturally occurring fructose, as well as added table sugar (sucrose) for preservation. Other common methods to preserve raspberries include shock freezing or drying.

Another way of processing raspberries is in the form of alcoholic beverages. Fermentation of raspberries, which are naturally high in fructose will yield raspberry wine. However, a disadvantage of fermenting raspberries is the degradation of anthocyanins, the main colouring compound of raspberry. This can result in raspberry exhibiting a pinkish colour, often perceived by costumers as ‘unnatural’ (Rommel et al. 1990).

A much less explored property of raspberries is their contribution to human health. The compound fruit has favourable calorific and nutritional values. 100 grams of fresh raspberries only contain 52 kcal (Rao & Snyder, 2010). Also, raspberries are rich in dietary fibres and fructose. The consumption of raspberry helps in the regulation of blood sugar levels by slowing down digestion. The fibre contributes to a satiating effect. This effect can be useful in dietary programmes if consumed as a snack in between larger meals to slow down hunger. Natural oils contained in raspberries are largely of the valuable unsaturated kind (97.8% unsaturated fatty acids). Raspberries make for a good addition to a well-balanced diet, low in saturated fats

and sodium and rich in healthy lipids, fibre, potassium and antioxidant phytochemicals (Rao & Snyder 2010; Aprea et al 2015). Furthermore, dietary supplements containing raspberry ketone claim to help in burning fat and ultimately in losing weight.

Raspberry ketone also has major uses in the cosmetics and perfume industry. However, the quantities of raspberry needed to produce raspberry ketone for the fragrance and cosmetic industry far outweighs the quantities of raspberry cultivated. Thus, most of the raspberry ketone used in perfumes, dietary supplements and cosmetic products is synthetically produced (Lee, 2016). In the case of dietary supplements, claims that portray raspberry ketone as a 'wonder weapon' in helping with weight loss are often unsubstantiated and lack the needed scientific credibility. Finally, dietary supplements often contain much more than what is recommended in dietary and fragrance products. There is a severe lack of toxicology reports outlining the recommended dosage of raspberry ketones in dietary supplements (Lee, 2016).

Raspberries have high post-harvest metabolic activities, which makes them very perishable. The berry has a short ripening period, but also a short period until the start of natural senescence (Han et al. 2004; Tezotto-Uliana et al. 2014). The complex question of shelf-life is often reduced to the time it takes for the first fungal infection to occur and spread to nearby fruit. That is why the single most important factor to lengthen shelf-life is rapid harvest and immediate storage at cool temperatures (Ghaouth et al. 1991). When packaged raspberries are stored in the refrigerator at standard temperatures of 0-4 °C, shelf-life is usually less than 5 days (Han et al. 2004), but depends strongly on the specific cultivar.

3.2.2 Worldwide production of raspberry fruit

The most recent available data on raspberry production from the year 2020 suggests a production of 822,493 tonnes (worldwide-raspberry-production, www.atlasbig.com,). Raspberries are largely grown in Europe, North America and Russia. Asia, Africa and South America either do not or only produce very small quantities of raspberries. The largest producer of raspberries is the Russian Federation with 174,000 tonnes, followed by Mexico (128,848 tonnes) and Serbia (120,058 tonnes). Other important producers of raspberry are The United States, Spain, Poland and Ukraine. The consumption of fresh raspberry has dramatically increased from the year 2000 onwards. This is especially true for the regions of North America, the British Isles and Scandinavia (Giongo et al. 2019).

Raspberry is a high value crop when sold as fresh produce and has a lot of potential to expand and intensify production in Eastern Europe, where climatic conditions for raspberries are often ideal. What is holding this production region back is lack of technical skill in raspberry growing (right choice of cultivar, correct pruning of canes) and the financial backing needed to install supporting and anchoring structures (Georgieva et al. 2020; Parausić et al. 2016).

3.2.3 Raspberry cultivars and breeding

Raspberry varieties are distinguished based on whether they are floricane or primocane. Floricane raspberries have the habit to bear fruit on canes of the first growing season, while primocane raspberry varieties bear fruit only on canes from the past growing season (Klodd & Worth, 2021; Heide and Sønsteby, 2011; Carew et al. 2001)

Primocane raspberries are also referred to as fall-bearing raspberries, harvested from late summer until the arrival of the first frost in late autumn. They are becoming increasingly important due to them stretching the season for raspberries well into late autumn, when summer-bearers do not supply any raspberry fruits anymore. Floricane raspberries are otherwise known as summer-bearing raspberries, because they produce fruit only throughout the summer months. When freshly planted, floricane raspberries do not produce fruit in the first growth year and only produce a small quantity of fruit in the second year. Full-yield can be expected starting from the third growing year (Klodd & Worth, 2021).

The lack of certified plant material (Georgieva et al. 2020) and the threat of accidental introduction of new plant viruses from contaminated rootstocks makes the outbreak of local to regional to international pathogen epidemics a constant possibility (Dolan et al. 2018). A consequence of modern agriculture is the use of genetically uniform crop plants to control the rate and behaviour or plant growth. This is a dangerous practice that often aids in the spread of many pathogens, as the presence of a practically unlimited supply of susceptible host plants makes it possible for localized infections to transform into large-scale plant epidemics (Wilson, 2014).

Breeding programmes in various growing regions share common goals and are strongly influenced by environmental conditions, as well as consumer requirements. Consumers are increasingly sceptical towards the application of pesticides in the production of small berries. This puts additional pressure on the need to breed cultivars with resistances against many types of pathogens. Essential quality characteristics focused on in breeding programmes are: yield, fruit quality and ease of pick (Graham & Brennan, 2018). This is essential, because the fragile raspberry relies on being gently hand-picked. After being picked, raspberries tend to perish very quickly. This is the reason why some raspberry breeding programmes focus on retaining firmness and thus prolonging shelf-life (Giongo et. al 2019; Jennings, 1988).

What follows is a selection of raspberry cultivars especially relevant for Norwegian raspberry cultivation.

- cv. Polka: Primocane variety with red coloured fruit and harvest in early autumn. Bears large and conical fruit. Sweet in flavour. Vigorous in growth habit and tends to have even spatial cane spread (Klodd & Worth, 2021, rhsplants.co.uk)

- cv. Tulameen: Raspberry cultivar from British Columbia, Canada. Fruits are dark pink, sweet and easy to pick. They are long and conical in shape. They need a well-drained soil as they do not deal well with water-logging (rhsplant.co.uk). Mostly sold on the fresh market.

- c.v. Glen Ample: Red in colour with harvest in mid-summer. Developed by the Scottish James Hutton Institute (www.hutton.ac.uk/). The most important cultivar in Norwegian raspberry cultivation

- cv. Glen Mor: One of the newest additions to the raspberry market bred by the James Hutton Institute in Scotland (www.hutton.ac.uk/) and released to the public in 2020. An interesting *Phytophtora*-resistant cultivar. It is a spine-free primocane variety that can be grown in soil or substrate. It has a low chilling requirement and is suitable for cultivation in a wide range of climatic conditions. Ideal for fresh consumption.

- cv. Willamette: Willamette was developed by the Oregon breeding program in the early 1940s. Fruits are red in colour and small to medium in size with harvest in mid to late summer. Willamette is the world's most widely cultivated variety of raspberry. It is a warmth-loving raspberry cultivar with high water needs.

3.3 Important non-viral raspberry diseases

Grey mould (*Botrytis cinerea*) is one of the most damaging diseases of the mature raspberry fruit, but can also attack raspberry canes. Botrytis is especially common in open-field raspberry production as rain plays an essential role in the dissemination strategy of this pathogenic fungus. Grey mould has an effective infection strategy: it infects raspberry plants when they are flowering and stays latent in the plant until fruits are ripening (Klodd & Worth, 2021). Disease only starts to develop at the point when the immature fruitlets have grown to their final size and start to take colour and soften. Thus, grey mould can only be spotted when raspberry fruits are close to maturation – at which point it is usually too late to intervene with fungicides.

Phytophthora root rot (*Phytophtora* spp.) is caused by a group of 'fungus-like' oomycetes in the pathogenic Phytophthora genus. Some of the more frequently listed causal agents of phytophthora root rot include *P. fragariae* var. *rubi P. megasperma*, *P. syringae*, *P. drechsleri*, *P. cactorum* and *P. cambiuora*. (Duncan et al. 1987). Phytophthora is a soilborne pathogen that rests in the soil in the form of an oospore. When the soil becomes water-logged for an extended period (which tends to happen frequently in heavy clay soils), motile spores are released from the zoospores into the soil and are taken up by raspberry roots. In raspberry shrubs, symptoms of phytophthora root rot are first noticed on the herbaceous fist year canes (primocanes). Symptomatic canes wilt and shoot tips die off. Damage to second year canes (floricanes) is less pronounced and most visible on the yellow to brown leaf edges (Duncan et al. 1987).

Erwinia amylovora is the causative agent of fire blight – a very destructive bacterial disease. It has received a lot of attention with regard to commercial production of pome fruit such as apple (*Malus* x *domestica*) and pear (*Pyrus communis*). Fire blight has the potential to cause large-scale problems in raspberry production. The disease kills off flowers, thereby stopping the formation of fruit. The disease also affects young canes, which in primocane varieties of raspberry means no or severely reduced fruit load and in floricane varieties means that the following growing season will yield no or little fruit (Braun & Hildebrand, 2010). Fire blight affects all of the aboveground plant parts of raspberry – buds, shoots, the bark and canes. Its

danger lies in the rapidity of disease-spread given the right set of conditions (Umiraliyeva et al. 2021).

Pseudomonas syringae is the causative agent of pseudomonas blight is also widely known - by the symptoms it causes - as bacterial necrosis. *P. syringae* is a complex taxon as it consists of strains with different biochemical and genetic characteristics, as well as different host ranges. Many of its strains are similar in disease development to fire blight. Symptoms of pseudomonas blight include: blossom blast and spur dieback, leaf and fruit lesions, development of cankers with gummosis of woody tissue, loss of scaffold limbs and decreased fruit yields (Ivanovic et al. 2012).

Crown gall is a disease symptom that is hard to pin-point to a specific disease agent. Crown galls are tumours that mostly affect members of the rose family and grapevine and in raspberries develop around the cane. Crown galls may be caused by a whole range of bacterial disease agents such as *Rhizobium rhizogenes* and *Agrobacterium tumefaciens* (Kuzmanović et al. 2015). Crown galls are classified as a type of tumour. They are detrimental to plant health, because they inhibit plant maintenance functions such as transport of water and nutrients. Crown galls are rarely fatal for an infected plant, but growth and vigour is usually reduced following the formation of tumorigenic galls (Pulawska et al. 2010). Infection and subsequent formation of crown galls is especially destructive for younger plants. (Kuzmanović et al. 2015).

Fig. 2: So called 'shepherd's hook', very typical for cane-tip dieback caused by *Erwinia amylovora* (Cornell University, accessed 5.8.2022)

Fig.3: Crown gall caused by *Agrobacterium tumefaciens* on raspberry rootstock (Pacific Northwest, Pest Management Handbooks, accessed 5.8.2022)

3.4 Viral diseases of the raspberry shrub

The wider *Rubus* genus is known to be a suitable host for over 40 viruses and viroids (Tzanetakis et al. 2007). Despite some viruses having raspberry directly in the name, raspberry viruses should mostly be seen as viruses that, among many other species, also affect raspberries. Most viruses that inflict damage on raspberry, also affect closely related species such as blackberries or hybrid *Rubus* berries like loganberry, tayberry, marionberry or boysenberry.

Similarly to other plant viruses, most viruses that infect raspberry shrubs require a biotic vector. The most common vectors to transmit viruses are aphids and nematodes. However, lesser-known insects such as mites and thrips (and in very rare cases whiteflies) can also serve as a vector.

The following sub-sections aim to list known plant viruses by linking them to their specific vector. Taxonomy of viruses is a difficult topic and there is much confusion around how plant viruses should be referred to. In many cases, there exist multiple terms for the same virus. In this list, virus nomenclature as suggested by the EPPO global database is used. EPPO is a global database maintained by the Secretariat of the European and Mediterranean Plant Protection Organization (EPPO). Their mission is to collect all pest-related data to provide knowledge about the most common crop pathogens in Europe.

3.4.1 Aphid-transmitted viruses

Aphids are the most successful vectors of raspberry viruses. The European large raspberry aphid (*Amphorophora idaei*) is the single most important aphid vector of the European red raspberry (*Rubus idaeus*) (McMenemy et al. 2009). The strong connection of virus disease with aphids as vectors is related to the biology and the feeding habit of aphids. They are so called sap-suckers – meaning that in order to get access to cellular nutrients, aphids puncture the plant cell using their stylet and then proceed to extract nutrients from cells. Aphids are able to transmit a large range of viruses, from non-persistent to semi-persistent to persistent. They can transmit non- persistent viruses when they are repeatedly probing with their stylet to check the suitability of a certain plant species as a source of nutrition for the aphid. They can, however, also transmit persistent viruses while engaging in the main-feeding process. In general, aphids do not destroy plant cells they pierce and suck on (Wilson, 2014).

Aside from their feeding habit, aphids are also very successful transmitters of plant disease due to their exceptional fecundity. Aphids are so specialized to produce large numbers of offspring, that they do not require a mating partner to proliferate (Wilson, 2014). Raspberries are grown either in open-field or under a plastic tunnel. In growth tunnels, temperature and humidity are generally higher than in open-field conditions, which are favourable conditions for the rapid multiplication of aphids.

Fig. 4: Adult European large raspberry aphid (*Amphorophora idaei*). This aphid is widespread throughout Europe and typically feeds on the underside of raspberry leaves (Blackman et al. 1977).

Raspberry yellow net virus

Raspberry yellow net virus, RYNV (Species *Raspberry yellow net virus*, Genus *Badnavirus*, Family *Caulimoviridae*) was first described in 1955 (Stace-Smith, 1955). The virus infects *Rubus* species worldwide and is one of the causal agents of raspberry vein banding mosaic. If present with other *Rubus*-viruses such as black raspberry necrosis virus and raspberry leaf mottle virus, raspberry yellow net virus can form a disease complex capable of causing leaf mosaics on raspberry (Vakic et al. 2022). The virus is bacilliform and transmitted by the large raspberry aphid, *Amphorophora idaei* in Europe and by the large raspberry aphid (*Amphorophora agathonica*) in North America. Raspberry yellow net virus is most closely related to gooseberry vein banding associated virus and Spiraea yellow leaf virus (Jones et al. 2006).

Raspberry leaf mottle virus

Raspberry leaf mottle virus, RLMV (Species *Raspberry leaf mottle virus*, Genus *Closterovirus*, Family *Closteroviridae*) was first described in 1924 under the now obsolete term 'raspberry mosaic disease' (RMD). It is transmitted by the aphid *Aphomorphora agathonica*. RLMV has been reported from both North America and Europe (Paunović & Jevremović, 2019). Raspberry leaf mottle virus forms together with raspberry latent virus (RpLV) and raspberry bushy dwarf virus (RBDV) a virus disease complex responsible for the formation of 'raspberry crumbly fruit'. This raspberry virus makes fruit underdeveloped, poorly matured and crumbly (Quito-Avila et al. 2014). Raspberry leaf mottle virus is also described as one of the members of the disease complex responsible for raspberry mosaic disease along with raspberry yellow net virus and black raspberry necrosis virus.

Raspberry vein chlorosis virus

Raspberry vein chlorosis virus, RVCV (Species *Raspberry vein chlorosis virus*, Genus Unassigned *Rhabdoviridae*, Family *Rhabdoviridae*) was first described in 1952 and is vectored by the small raspberry aphid (*Aphis idaei*) (Cadman, 1952). It closely resembles raspberry

crinkle virus and cyto-rhabdovirus alfalfa dwarf virus. Vein chlorosis virus has been reported from the UK, Canada, most European countries and New Zealand. RVCV causes stunted cane growth and reduced vigour (Jones et al 2019).

Fig. 5: Raspberry leaf naturally infected with Raspberry vein chlorosis virus (Diekmann et al. 1994)

Fig. 6: Raspberry vein chlorosis virus particles. Virions are bacilliform and rounded at one or both ends. Bar scale: 100nm (Diekmann et al. 1994)

Black raspberry necrosis virus

Black raspberry necrosis virus, BRNV (Species *Black raspberry necrosis virus*, Genus *Sadwavirus,* Family *Secoviridae*) is a virus known to infect black raspberry (*Rubus occidentalis*). BRNV causes symptoms of chlorosis, mottling and puckering on black raspberry. The virus was first described in 1955 by Stace-Smith in the Canadian Journal of Botany. The vector of black raspberry necrosis virus was identified as the large blackberry aphid (*Amphorophora rubi*) (Stace-Smith, 1955). BRNV does not infect the European red raspberry (*R. ideaeus)* (Jevremović et al. 2019). BRNV occurs wherever its natural vector aphid (*Amphorophora rubi*) is present (dpvweb.net).

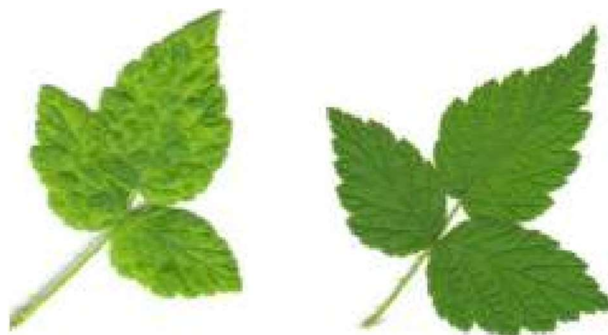


Fig. 7: Black raspberry leaf infected with Black raspberry necrosis virus. Symptoms include puckering, chlorosis and mottling. Healthy raspberry leaf for comparison (Halgren et al. 2007)

3.4.2 Nematode-transmitted viruses

As the name suggests, nematode-transmitted viruses gain entry to a host plant via soil-living roundworms, also called nematodes. However, there is also the possibility of transmission by mechanical means (Hiruki & Teakle, 1987). Nematodes were for the first time confirmed as a potential plant virus vector in 1958 when the Californian dagger nematode (*Xiphinema index*) was shown to be responsible for the transmission of Grapevine fanleaf virus (GFLV) in Californian vineyards (Hewitt et al. 1958).

There are over 35 nematode species confirmed to involved in the transmission of plant viruses; all of which are either members of the family *Longidoridae* or *Trichodoridae*. The *Longidoridae* are responsible for transmission of isometric viruses belonging to the *Nepoviruses*, while the *Trichodoridae* transmit rod-shaped viruses of the *Tobravirus* genus. In temperate regions, *Longidorus* and *Trichodorus* spp. are widespread. Nematodes have a spear-like structure with which they penetrate plant roots. They start the feeding process by transferring salivary secretions into the plant cells and then sucking content with their mouthpiece. Nepoviruses remain viable inside nematodes for up to 12 weeks, but Tobraviruses can survive for up to 2-4 years in nematodes (Wilson, 2014).

Plant roots can be distinguished into metabolically active roots (mostly root-tips) and large and old roots which fulfil more of an anchoring role. Soil-borne viruses seek entry mostly into the young metabolically active root-tips. There are several reasons why young root-tips are the preferred targets of soil-borne virus vectors:

a) they lack a protective root covering, which older roots often have (Hiruki & Teakle, 1987)
b) young, active root tips are more susceptible to infection (Hiruki & Teakle, 1987)
c) nematodes are attracted to the root exudates of young root-tips (Hiruki & Teakle, 1987)

Some raspberry growers have switched from cultivation in soil to inorganic substrates. This practice makes transmission of nematode-borne viruses very difficult. Among some other notable disease like root rot, nematode-vectored virus disease is severed reduced or prevented completely (Dolan et al. 2018).

Raspberry ringspot virus

Raspberry ringspot virus, RpRSV (Species *raspberry ringspot virus*, Genus *Nepovirus*, Family *Secoviridiae*) was first identified in 1958 as the putative causal agent of raspberry leaf curl disease (Cadman, 1958). Raspberry ringspot virus is found throughout Europe and has a broad host range. Some commercially important species include raspberry, strawberry, cherry, gooseberries, grape and red current. Raspberry ringspot virus is transmitted by members of the nematode genus *Longidorus* (Martin et. al 2013). RpRSV is subdivided into several strains, the most important ones being the Scottish strain, the English strain and the Lloyd George yellow blotch strain. The Scottish strain is transmitted by the nematode *Longidorus elongatus* (needle nematode) and the English strain is transmitted by *Longidorus macrosoma*. The larvae and the adults of *Longidorus elongatus* transmit the virus efficiently, but adults do not pass on the virus to its progeny (Cadman, 1956).

Fig. 8: Raspberry ringspot symptoms on raspberry leaves (Scottish Crop Research Institute/James Hutton Institute)

Arabis mosaic virus

Arabis mosaic virus, ArMV (Species *Arabis mosaic virus*, Genus *Nepovirus*, Family *Secoviridae*) causes yellow dwarf of raspberry. ArMV has a broad host range - some important and commonly infected crops include strawberry, hops, hemp, grapevine, peach and lettuce. The most common symptoms of ArMV are stunting and leaf flecking (dpvweb.net) However, other sources indicate a broader selection of symptoms: from complete absence of to prominent foliar symptoms, stunting, necrosis until plant death (Samuitienė et al. 2008). ArMV is vectored by the soil-borne nematode *Xiphenema diversicaudatum* . ArMV is readily transmitted mechanically (Samuitienė et al. 2008).

Tomato black ring virus

Tomato black ring virus, TBRV (Species Tomato black ring virus, Genus *Nepovirus*, Family *Secoviridae*) was first identified and described on tomato in 1946, from which the virus received its name (Smith, 1946). TBRV occurs in most of Central and Northern Europe. The virus has been reported in a few cases outside Europe, namely in Japan, India and Saudi-Arabia (cabi.org). Symptoms caused by TBRV include chlorosis, leaf malformation, stunting and formation of necrotic rings on infected tissue (Rymelska et al. 2013). TBRV has a very diverse host range. The virus has been reported from *Vitis* spp., *Prunus* spp., *Rubus* spp. ,*Ribes* spp., *Fragaria* spp. and from *Solanaceae* spp. (Harper et al. 2011). TBRV is transmitted effectively by the nematode vector *Longidorus elongatus*. Aside from nematode-vectored transmission, mechanical transmission of TBRV can be a problem in both open-field and closed-system agriculture.

Tobacco ringspot virus

Tobacco ringspot virus, TRSV (Species *Tobacco ringspot virus*, Genus *Nepovirus*, Family *Secoviridae*) was first described on smoking tobacco (*Nicotiana tabacum*) in Virginia, United States (Fromme et al. 1927). TRSV is transmitted effectively by the dagger nematode species *Xiphenema americanum* and *Xiphenema rivesi*, but also by pollen. Tobacco ringspot virus has a well-known satellite, called satellite RNA of tobacco ringspot virus (Gerlach et al. 1987).

Tomato ringspot virus

Tomato ringspot virus, ToRSV (Species *Tomato ringspot virus*, Genus *Nepovirus*, Family *Secoviridae*) was first described in 1936 by Price. The virus causes disease on a wide variety of important cultivated crop and is an especially feared pathogen in fruit trees and small fruit such as raspberry and strawberry. ToRSV is also known to infect a large number of common weeds such as the dandelion (*Taraxacum officinalis*). This constitutes a plentiful supply of fresh inoculum for nematode vectors (Sanfaçon & Fuchs, 2011).

Strawberry latent ringspot virus

Strawberry latent ringspot virus, SLRSV (Species *Strawberry latent ringspot virus*, Genus Unassigned *Secoviridae*, Family *Secoviridae*) was first reported in 1964 in Great Britain (Lister, 1964). The virus is widespread throughout Europe (Tang et al. 2013), but has also been reported from North America, North Africa and some parts of Asia (cabi.org). The virus is vectored by the nematode *Xiphenema diversicaudatum*. SLRSV attacks a wide range of hosts, many of which are commercially relevant crops. Some important examples include: strawberry, raspberry, cherry, peach, plum, celery, asparagus and even ornamentals like rose and lilies (Tang et al. 2013).

Cherry rasp leaf virus

Cherry rasp leaf virus, CRLV (Species *Cherry rasp leaf viru*s, Genus *Cheravirus*, Family *Secoviridae*) was first reported in 1942. CRLV is mostly reported from North America and is transmitted by the nematode *Xiphenema Americanum* (Bodine & Newton, 1942). CRLV resembles nepoviruses in many of its properties, but it is serologically different from them (Hansen et al. 1974). Cherry rasp leaf virus is responsible for reduced fruit production, shrub/tree vigour and reduced life expectancy Cherry rasp leaf virus has economic relevance in Rubus, stone fruit and in pome fruit, where it is connected with flat apple disease (James, 2011).

3.4.3 Pollen-transmitted viruses

In 1918, the book 'Varieties of beans susceptible to mosaic' for the first time formulated that pollination might be used by some viruses to gain entry into new host plants (Reddick, 1918). The pollen from certain virus-infected plants may be used by highly specialized viruses to gain entry into a new host plant. Pollen is mostly carried by wind, but is not restricted to that mode of transmission. Humans and wild animals as well as social pollen-collecting insects such as bees (*Apis* spp.), bumblebees (*Bombus* spp.) and many species of butterflies can aid in the distribution of pollen. Around 15-20% of all known plant viruses have at least one host species in which they are spreading via seeds or pollen The association of a plant virus via the reproductive system has many advantages for the viral parasite and the association of plant viruses with seed and/or pollen (Wilson, 2014).

Virus-spread via seed material has the added advantage of viability over several host seasons. The movement of pollen and seed-borne viruses is facilitated by seed dissemination

routes (via animals or wind), but can also be heavily favoured by handling of harvested seed by humans. For seed-transmitted viruses, the rates of successful inoculation may be relatively low. However, this is often not that crucial, because primary inoculum is established in a host plant which can then be spread further by for other vectors such as aphids (Wilson, 2014).

Members of the virus genera *Nepovirus*, *Sobemovirus*, *Ilarvirus*, *Idaeovirus* and *Potyvirus* may be transmitted by pollen. Virus transmission via pollen occurs when pollen consisting of virus particles lands and germinates on a female plant. Starting from the stigma, the virus-infected pollen germinates and spreads into the immature ovules of plants (Bhat & Rao, 2020). Subsequently, the ovules of the plant ripen and once they are fully mature, they are expelled and transported either by wind, by a plant-specific ejection mechanism or by other biotic and non-biotic mechanisms.

Raspberry bushy dwarf virus

Raspberry bushy dwarf virus (Species *raspberry bush dwarf virus* (RBDV), Genus *Idaeovirus* , Family *Unallocated ssRNA+* was first described in 1970 (Barnett & Murant, 1970). RBDV is a mostly pollen-borne virus and is closely linked to the disease 'raspberry crumbly fruit' (Quito-Avila et al. 2014). This virus has also been described as 'symptomless decline' in raspberry (Cadman & Harris, 1951). Fruit affected by the disease is underdeveloped, very crumbly and poorly matured. Diseased fruit is not marketable on the fresh fruit market. Infection with RBDV has been reported from Europe, North and South America, Australia, New Zealand and South Africa (Chamberlain et al. 2003).

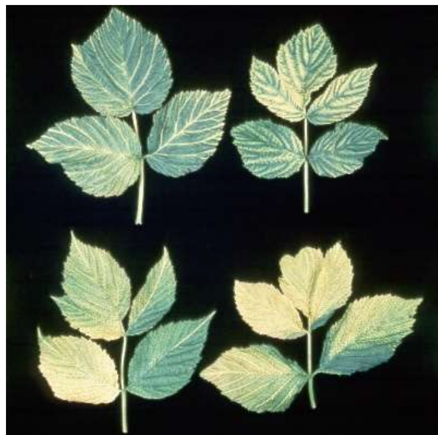


Fig. 9: Symptoms of pollen-borne raspberry bushy dwarf virus. Virus symptoms originate from veins and are clearly chlorotic. In the later stages of the virus disease, the entire leaf surface is chlorotic (James Hutton Institute).

Fig. 10: Crumbly and poorly matured raspberry drupe fruits affected by raspberry bushy dwarf virus. Fruits are crumbly and clearly unmarketable (James Hutton Institute).

3.4.4 Mite-transmitted virus

Raspberry leaf blotch emaravirus

Raspberry leaf blotch emaravirus (Species *Raspberry leaf blotch emaravirus* (RLBV), Genus *Emaravirus*, Family *Fimoviridae* was first reported from Great Britain and Serbia (Bi et al. 2012). The virus is vectored by the raspberry leaf and bud mite (*Phyllocoptes gracilis*) (McGavin et al. 2012). RLBV is common in raspberry cultivar 'Glen Ample', especially so if berries are cultivated in growth tunnels (EPPO database). Symptoms of raspberry leaf blotch emaravirus are yellow blotches on leaves and an abnormal development of leaf hairs on the abaxial (underneath) side of raspberry leaves. The more frequent occurrence of RLBV in growth tunnels is due to the temperature and air humidity levels favouring *P. gracilis* (Bi et al. 2012).

Fig. 11: Raspberry leaf blotch emaravirus (RLBV) symptoms on red raspberry, cv. 'Willamette' (Paunović et al. 2020)

3.4.5 Infection with multiple viruses (co-infection)

It is common that more than a single virus can manipulate and attack a plant simultaneously. A recent study on virus co-infection from Serbia (Paunovic et al. 2020) tested commercial raspberry leaf samples for the four most important virus diseases in that region: raspberry leaf blotch virus (RLBV), raspberry leaf mottle virus (RLMV), black raspberry necrosis virus (BRNV) and Rubus yellow net virus (RYNV). Of seventy-four total collected samples from the main raspberry cultivation areas in Serbia, 68% were infected with two, 20% with three (RLBV, RYNV & BRNV) and 12% (RLBV, RYNV, BRNV & RLMV) with four virus diseases simultaneously. In this study, an especially prevalent combination of viruses is RLBV and RYNV, which occurred in 44% of tested samples.

Martin et al. 2013 showed the complex nature of co-infection in the case of Blackberry yellow vein disease (BYVD). This disease is characterised by yellowing and feathering of blackberry, especially in the Southern USA. Symptoms were initially attributed to TRSV, but further bioassays in blackberry revealed that the virus is typically asymptomatic in blackberry. Finally, the new virus was revealed to be a new type of virus vectored by whiteflies. This new

virus causes asymptomatic infection in most cultivars of blackberry, but if other viruses are present in the plant, they can act synergistically with the whitefly-vectored virus. Martin et al. 2013 also observed that in the case of his newly discovered virus, the types of other viruses are not as important as their sheer number.

3.5 Diagnostic tools for virus detection

The oldest and most traditional method of identifying virus is through the use of bioassays. This is the systematic inoculation of test plants from virus-infected plant material. Expertise is required to distinguish viruses based on their morphology. However, many plant viruses cannot be distinguished by morphologically assessing differences in disease symptoms.

The two most successfully established and routinely used laboratory methods to detect plant viruses are ELISA and RT-PCR. Both of these methods share some common characteristics such as a high degree of reproducibility and relatively low costs (Boonham et al. 2014).

Enzyme-linked immunosorbent assay (ELISA) is a powerful serological method to detect and quantify substances such as proteins, peptides, hormones and antibodies. The concept of the enzyme-immunosorbent assay was first published by in 1977 for the detection of Plum pox virus/sharkavirus ArMV (Clark & Adams, 1972). This was a complete breakthrough in virus diagnostics and lead to the introduction of a new era in phytodiagnostics. While at first limited to research facilities, ELISA has become one of, if not the most used tool for detection of virus in practical areas such as in plant breeding programmes, quarantine-testing and certification-programmes. Enzyme-linked immunosorbent assay is adapted to high-throughput testing, because it is a relatively simple and fast. Also, results can easily be interpreted (Booham et al. 2014).

ELISA can be used to detect a large range of viruses. The technique has become the most popular and most standardized method to test agricultural crops in large quantities for presence of various plant viruses (Boonham et al. 2014)

In general, ELISA includes the following steps:

1) Add coating
2) Add samples
3) Add conjugate
4) Add substrate
5) Measure signal

The working principle behind ELISA is a highly specific interaction between the used antibody and the antigen of interest in the plant tissue. A 96 or 384-well plate (8x12 and 16x24 respectively) is coated with virus-specific igG-antibodies. A washing step is performed. Carefully crushed plant material is suspended in sample buffer solution and plated into the wells. To have credible reference points, at least one negative control (sterile water) and positive control (plant material known to be infected by the virus of interest) need to be included in the well-plate. Another washing is performed. Enzyme-labelled antibodies (also

known as conjugates) are plated and after another washing is performed. Substrate buffer is added to the plates. A change in colour can be observed for wells where the specific virus has successfully bound to igG-antibodies.

Reverse transcriptase (RT) PCR is an invaluable amplification technique, because it is a precise, sensitive and flexible way to amplify nucleic acid, while the reagents involved are relatively low-cost. Another important advantage is the possibility of scaling-up the reaction (Udvardi et al 2008). Reverse transcriptase polymerase chain reaction is a three-step process and consists of a denaturation-, an annealing- and an elongation step. The exact temperatures at which these steps are performed vary and need to be determined with experience (Freeman et al. 1999). The number of run cycles depends on the efficiency of the reaction, but also on the amount of target present.

It is good practice to also perform a control RT-PCR in which the reverse-transcriptase is omitted. The control is useful, because the very common problem of PCR product contamination can be detected (Freeman et al. 1999). The last step in RT-PCR is the detection and the quantification of amplification product. There exist two broad categories of PCR detection: the traditional 'end-point-measurement' and 'real time' monitoring of product formation. The difference between the two approaches is the point in time when measurements are performed. With traditional 'end-point-measurements', PCR products are measured at the end of amplification. Real-time PCR (not to be confused with RT-PCR), monitors the PCR reaction while it is happening inside the thermos cycler (Freeman et al. 1999).

The measurement of end-point products can be achieved via different methods. The most common of those are fluorescent-intercalating dyes which are then read by a fluorescent reader (Boonham et al. 2014). Real-time monitoring offers several advantages - for instance improved quantification. Errors in sample manipulation for end-point quantification are minimized and more information about PCR is obtained from data points for each run cycle in the reaction (Freeman et al. 1999).

3.6 Management strategies

Generally, it is very difficult if not impossible for a virus-infected plant to fully recover. There exist only a few incidences where virus-diseased plants recover from disease and freedom of disease should not be confused with absence of symptoms. Management of virus disease is based on prevention of infection. This means the implementation of prophylactic measures to keep the spread and efficient transmission of viruses to a minimum. To obtain a situation of relative freedom from virus disease, multiple management tools need to be developed and strictly followed (Wilson, 2014).

The fight to stop establishment of viruses makes it necessary to have a strong background of the biology of all parties relevant for viruses - the pathogen, the host, possible vectors and environmental factors. The possible management strategies to limit the spread of viruses are divided by Wilson, 2014 into:

1) Strategies to reduce the virus inoculum sources
2) Strategies to reduce virus spread

3) Strategies to enhance host resistance to virus or vector

The single most important measure to reduce virus-incidence in agricultural production is the use of virus-free planting material (Martin et al. 2013). In order to attack the virus-transmitting vectors, the spraying of insecticides and fungicides is common, but may have reduced efficacy due to acquired resistance by the pathogens. Also, consumers are generally opposed to the use of environmentally damaging substances and may react by choosing to purchase an environmentally friendly product over on that is not (Dolan et al. 2018).

4. Material & Methods

4.1. Bioassays

Bioassay of raspberry-related isolates on test plants:

Seven viral samples preserved at -80°C were selected based on their relatedness to raspberry-infecting viruses. The viral isolates studied were:

1) Campanula isolate 2008
2) Raspberry isolate 2008
3) Peperomia isolate 2000
4) Begonia isolate 1996
5) Aubretia isolate 1996
6) Soil isolate 2009
7) Soil isolate 2007

Nicotiana test plants were seeded in January 2022, selected for size and three weeks later transplanted into more spacious growing racks. They were transplanted once more in the first week of March. Four different species of Tobacco test plants (*N. benthamiana, N. tabacum* cv. 'Xanthi', *N. occidentalis*-P1 and *N. clevelandii*), as well as *Chenopodium quinoa* were potted from growing trays into individual pots. All five species of test plants were used for isolate 1) and 2), but for isolates 3), 4), 5), 6), 7) test plant *N. clevelandii* was omitted. Thus, a total of thirty test plants was used for sap inoculation.

Sap inoculation: Inoculation of virus isolates was performed via traditional sap inoculation by following a slightly adapted procedure from Blystad, PLV 321 Lab 3; virus detection. Hands were disinfected using soap and 10% sodium phosphate dodecahydrate ($Na_3PO_4 \cdot 12H_2O$). The largest three leaves by area were identified and marked by tearing off a small portion of leaf tip. Carborundum (silicon carbide) was applied on selected leaves.

In a mortar, 1 gram of frozen leaf material was mixed with 5 mL of 0.03M phosphate buffer (1/4 of the buffer). The leaf sample is thoroughly crushed until the mixture is homogenised. There should be no more large clumps of leaf material at this point. The mixture is diluted with the remaining phosphate buffer so that a final dilution of 1 part plant material: 20 phosphate buffer is achieved. The virus inoculation solution is mixed one more time using the pistil.

A Q-tip is dipped into the inoculation solution and is gently rubbed onto the surface of the test plant leaves marked with carborundum. When large leaves were inoculated (e.g. *N. tabacum*) the Q-tip was refreshed several times per leaf. Once all marked leaves were inoculated, the inoculation solution and the carborundum was washed off under flowing tap water. Inoculated plants were labelled with date, virus isolate and test plant species. Test plants were then transferred to controlled climate (greenhouse).

Growing conditions in the greenhouse were 20 °C with 65% relative humidity, a day period from 6 am – 22 pm and a night period of 22 pm – 6 am. When natural sunlight was absent,

artificial full spectrum light was supplemented. Fertigation was supplied via drip-system. On particularly hot days, additional water was given via hand-held watering hose.

Fig. 12: Inoculation mixture of 20 mL 0.05% phosphate buffer (pH 7) and 1 g of virus-infected leaf material

Fig. 13: *T. clevelandii* test plant with carborundum (silicon carbide) applied on inoculated leaves

Fig. 14: Growth-setup in greenhouse with fertigation tubes

Bio-assay of raspberry root samples on test plants

Ten *C. quinoa* and ten *N. benthamiana* test plants were cultivated and selected. Via sap inoculation, ten raspberry roots samples from soils with known nematode presence were inoculated onto healthy test plants. The procedure was identical to the one described in '**Bio-assay of freeze-preserved raspberry isolates on test plants'** with two exceptions. The first difference was that instead of frozen leaf material, fresh fine roots were used. Root material required much more effort to grind with mortal and pistil. The second difference was that

instead of three leaves, five leaves were inoculated per test plant. Growing conditions were also the same as outlined in '**Bio-assay of freeze-preserved raspberry isolates on test plants**'

4.2. ELISA

Enzyme-linked immunosorbent assay (ELISA) to detect TBRV in raspberry-related isolates

To detect TBRV in the seven raspberry-related isolates, coating, conjugate and positive controls were ordered from the Swiss Bioreba AG and from the German DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

Coating solution was prepared in a small graduated glass cylinder by pipetting 5 µL of TBRV-specific antibodies into 5 mL of coating buffer (dilution 1:1'000). Four columns of a 96-well plate (12x8) were coated by pipetting 100 µL of coating solution into each of the 32 wells. The plate was incubated in a refrigerator for 24 hours at 4 °C. The plate was washed with 10x washing buffer using a microplate washer.

Sample solution was prepared by placing 0.5 g of suspected TBRV-leaf material in a Bioreba AG ELISA-bag and adding 5 mL of sample buffer. Leaf samples were then mechanically crushed by using a custom-made sample-homogeniser.

The samples used were:

1) Begonia isolate 1996
2) Aubretia isolate 1996
3) Soil isolate 2009
4) Raspberry isolate 2008
5) Campanula isolate 2008
6) Soil isolate 2007
7) Raspberry isolate 2022

Table 1: Position of raspberry-related isolates in ELISA detecting for TBRV.

	1	2	3	4
A	Neg.	Neg.	7	7
B	Pos.	Pos.	1	1
C	1	1	2	2
D	2	2	3	3
E	3	3	4	4
F	4	4	5	5
G	5	5	6	6
H	6	6	7	7

100 µL of sample solution were pipetted into each of the wells as shown in table 1. Sterilised water was used as a negative control. For the ELISA-reagents bought from DSMZ,

a positive control was supplied and for ELISA-reagents bought from Bioreba AG, a homogenised TBRV-positive *C. quinoa* leaf was used.

The 96-well plate was incubated for 24 hours in a refrigerator at 4°C. The wells were then washed with 10x washing buffer using a microplate washer. This step was repeated another time to fully remove all green particles.

Conjugate solution was prepared by pipetting 5 µL of conjugate into 5 mL of sample buffer. The well plate is incubated in a refrigerator for 24 hours at 4°C. A last washing was performed with 10x washing buffer using a microplate washer. A substrate tablet was put in a boron glass bottle with 40 mL of substrate buffer. The bottle is then put to a dark place in a cabinet to dissolve for approx. one hour. 100µL of substrate buffer is then pipetted into each of the 32 wells. The well plate is then incubated for an hour at room temperature in a dark drawer or cabinet. Colour intensity was measured using an ELISA-microplate-reader.

Enzyme-linked immunosorbent assay (ELISA) to detect ArMV in raspberry-soil isolates

To detect ArMV-infection, an ELISA was performed in the seven leaf samples listed in '**Enzyme-linked immunosorbent assay (ELISA) to detect TBRV in raspberry-related isolates**'. The procedure followed is identical to the one described in the aforementioned section. ELISA reagents to detect ArMV virions were purchased from Bioreba AG.

4.3. RT-PCR

RNA-extraction: A mortar with pistil was pre-chilled with liquid nitrogen (-196°C). Virus-infected leaf material was submerged in the liquid and carefully crushed. Leaf material was finely pulverised until the colour changed to light green, almost resembling white. RNA was then extracted using the 'STRN250-Spectrum™ Plant total RNA Kit by Sigma-Aldrich.

100 µg of leaf material was lysed and unwanted cellular debris was separated from RNA. RNA was bound using a binding column and three washing steps were performed to purify RNA.

Conversion of RNA into cDNA: The conversion stop of RNA to cDNA is following Thermo-Fischer's protocol available at : https://tools.thermofisher.com/content/sfs/manuals/mmlv_rt_man.pdf

RNA was converted via reverse transcriptase into cDNA. To facilitate this, Moloney murine leukaemia virus (M-MLV) reverse transcriptase was used to synthesize a complementary DNA strand from RNA. The protocol followed was: https://tools.thermofisher.com/content/sfs/manuals/mmlv_rt_man.pdf.

Amplification of nucleic acid: Nucleic acid was amplified using the Bio-Rad T100 Thermal cycler. The protocol for Nepo-B and BRSV-primers was as follows:

- Denaturation at 94°C for 5 minutes
- 94°C for 30 seconds
- Annealing at 48°C for 30 seconds

- Extension at 72°C for 30 seconds
- Repeat for 34 cycles
- 72°C for 30 seconds
- 4°C forever

The PCR-protocol for ArMV-primers was slightly different from the one used for Nepo-B and BRSV-primers in that annealing temperature was 50°C for 30 seconds.

Agarose gel : Agarose gel-electrophoresis is a common tool used in many applications in molecular biology. It can be used to visualise PCR-products. There are two ingredients needed to make the agarose-gel: running buffer (either TAE buffer or TBE buffer) and agarose powder. The difference between the two buffer solutions lies in their composition: TAE buffer contains Tris, boric acid and EDTA, while TBE contains Tris base, glacial acetic acid and EDTA (www.goldbio.com, accessed 05.09.2022). Quantity of buffer should be chosen according to the area of gel tray needed to be plated. To make later mixing of buffer and agarose powder simple and risk-free, it is of utmost importance that buffer solution is added to either an Erlenmeyer-flask or a boron glass bottle (without lid). The flask should be filled to no more than two fifths (i.e. a 500 mL Erlenmeyer-flask should not be used to mix more than 200 mL of gel).

To the selected amount of buffer solution, between 0.6 and 3% molecular-science-grade agarose powder was weighted out in precision scales and carefully poured into the flask or bottle. Agarose powder was poured straight into the buffer, not to touch the adjacent glass wall. Higher percentages of agarose powder should be used if target nucleic acid fragment sizes are large (1,000 – 20,000 bp). Conversely, lower percentages of agarose powder should be used for smaller target nucleic acid fragment sizes (200-1,000 bp) (University of Leicester, https://www.youtube.com/watch?v=wXiiTW3pflM&t=152s, accessed 05.09.2022). The percentage of agarose powder that works best for visualization of PCR-products needs to be determined empirically.

The flask was swirled and put in a microwave oven. It was heated for 30 seconds and removed from the microwave (with heat protection gloves). The solution was checked to see if fully dissolved, given another few swirls and put back in the microwave. This process was repeated until the gel solution had been dissolved completely. Slightly tilting the flask and holding it towards a light source can be helpful to check if solution has been dissolved properly. This should take about 3-5 minutes on average.

Once fully dissolved, the flask is removed from the microwave (with heat protection gloves) and the gel is cooled by running tap water along the outside walls. When the flask is cooled to a point where it is tolerable to touch without gloves, 1 µL of staining dye per 10 mL of buffer is added using a micropipette.

A gel rack of appropriate dimensions was prepared by putting a comb with desired number of wells into the last slot of the rack. The agarose gel rack was put into a gel caster. The flask was swirled to thoroughly mix the staining dye with the gel and carefully poured into the prepared gel rack. Lastly, a disposable micropipette tip is used to move air bubbles that have formed around the wells. The gel was set for approx. 20 minutes to solidify.

Gel electrophoresis:

When the gel had solidified, the comb was carefully removed from the gel. The gel was transferred with its rack into an electrophoresis chamber. The electrophoresis chamber was filled with running buffer to the indicated mark. The gel should be covered by a thin layer of running buffer.

A strip of parafilm was cut and 2 µL of 6x loading dye was pipetted onto the waxy side of the parafilm strip. This was repeated for 24 samples (3x6 PCR-product samples, 3x1 negative control, 3x1 positive control for a total of 24 samples). Twenty-four drops of loading dye were pipetted about a thumb-breadth from another. Samples were then dyed by pipetting 10 microliters of PCR-product onto a drop of loading dye on the parafilm strip. The mixture was pipetted up and down to mix well.

Before loading, it was important to release all air from the pipette tip so that when the tip was inserted in a fresh well, the content could be released smoothly without any air disturbing the well. When all wells were loaded, the lid of the electrophoresis chamber was closed and the power cords were attached to the power source. It was made sure that the anode was connected to the – pole and the catode was connected to the + pole. The gel was run for 50 minutes at 100V.

The same procedure as described in 'Polymerase chain reaction to detect TBRV in raspberry-related isolates' was used to first extract, then convert from RNA to cDNA and then to amplify the nucleic acid in raspberry isolate 2022.

5. Results

Out of a total of 32 sap inoculations onto healthy test plants, 21 resulted in local and or systematic lesions (table 2). There was a large variance when it came to the speed of lesion development. The first local virus lesions appeared as early as day 3 post inoculation for campanula isolate 2008, while Aubretia isolate 1996 only caused lesion on *N. benthamiana* after 14 DPI (Appendix 10.1). In general, virus lesions were first noticeable in the form of local lesions as chlorotic yellow spots. These spots gradually became more numerous on inoculated leaves, until they appeared on other non-inoculated leaves through systematic disease spread. There was only a single case where virus lesions stayed confined to a local infection: Campanula isolate 2008 on N. tabacum cv. 'Xanthi'. In all other cases where virus inoculation was successful, local lesions spread to other plant parts through systematic spread. The most common symptom of the studied virus isolates on test plants was chlorosis. Less common were symptoms such as leaf and tip necrosis, stream necrosis, stunting and mosaics.

5.1. Bioassays

A complete and detailed documentation of virus symptoms can be found in the Appendix under 10.1 'tables documenting virus development' and under 10.2 'ELISA plate readings & bioassay photo-series'.

Table 2: Overview of virus isolates on test plants

	C. quinoa	*N. tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1	*N. clevelandii*
Campanula isolate 2008	+/+	**-/-**	+/+	+/+	+/+
Raspberry isolate 2008	+/+	+/+	**-/-**	+/+	+/+
Peperomia isolate 2000	**-/-**	**-/-**	**-/-**	**-/-**	**n.t.**
Begonia isolate 1996	+/+	**-/-**	**-/-**	**-/-**	**n.t.**
Aubretia isolate 1996	**-/-**	**-/-**	+/+	+/+	**n.t.**
Soil isolate 2009	+/+	+/+	+/+	+/+	**n.t.**
Soil isolate 2007	+/+	+/+	+/+	+/+	**n.t.**
Raspberry isolate 2022	+/+	**n.t.**	+/+	**n.t.**	**n.t.**

N.B.: The sign in front of the slash (/) indicates presence (+) or absence (-) of local lesions and the sign after the slash indicates systematic infection. The abbreviation n.t. stands for 'not tested'.

Description of lesion formation:

Campanula isolate 2008

C. quinoa: Overall growth not inhibited. Infected leaves develop numerous yellow spots that become dark brown and ultimately necrotic and fall off. Strong systematic spread of disease.

N. tabacum cv. 'Xanthi': Overall growth not inhibited, development of local lesions on inoculated leaves. However, no systemic infection and after infected leaves were harvested for further ELISA analysis, no systemic lesions formed on leaves that were not inoculated.

N. benthamiana: Strongly stunted stature. Necrotic virus infections. At 21 DPI, the shoot tips become fully necrotic and the plant's growth came to a halt.

N. occidentalis P1: Necrotic lesions on inoculated leaves. However, no systematic spread of virus disease. Overall, the plant is not infected systematically and only leaves directly adjacent to infected areas are showing viral disease spread.

N. clevelandii: It is unclear whether there is infection with a type of virus or not. From 10 DPI, symptoms become clearer. Symptoms observed are vein clearing of leaves and small brown-ish spots. What speaks most in favour of viral infection, are the curled-up leaf tips.

Fig. 15: *N. benthamiana* test plant inoculated with Campanula isolate 2008

Fig. 16: *C. quinoa* test plant inoculated with Campanula isolate 2008

Raspberry isolate 2008

C. quinoa: Strongly stunted stature, viral spots on the first third of infected older leaves. Younger leaves are fully infected. Shoots and young leaves tend to be very misshapen or even necrotic.

N. tabacum cv. 'Xanthi': Overall growth not inhibited, no stunting. Development of numerous yellow to white-ish lesions on both inoculated and non-inoculated leaves stemming from systemic infection.

N. benthamiana: Inoculated leaves show no signs of infection and no systematic infection.

N. occidentalis P1: Formation of sunken-in brown necrotic spots that gradually spread towards the whole of the inoculated leaves. Yellowing of the inner leaf rosette and inconsistent necrosis of systematically infected leaves.

N. clevelandii: No stunting. Necrotic lesions on inoculated leaves. It is difficult to identify systematic infection in this plant. At the last week of symptom observation, leaf-tips began to curl and very small brownish spots formed on leaves. This indicated systemic virus infection.

Fig. 17: *N. tabacum* cv. 'Xanthi' inoculated with Raspberry isolate 2008

Fig. 18: *N. occidentalis* P1 inoculated with Raspberry isolate 2008

Peperomia isolate 2000

*C. quino*a: No local or systematic symtoms

N. tabacum cv. 'Xanthi': No local or systematic symptoms

N. benthamiana: No local or systematic symptoms

N. occidentalis P1: No local or systematic symptoms

Begonia isolate 1996

C. quinoa: After seven DPI development of yellow viral spots. At 8 DPI there are first symptoms of systematic infection. Strong leaf necrosis resulting in partial or complete detachment from the stem. Also necrotic lesions on the stem.

N. tabacum cv. 'Xanthi': No local or systemic symptoms

N. benthamiana: No local or systematic symptoms

N. occidentalis P1: No local or systematic symptoms

Fig. 19 & 20: *C. quinoa* inoculated with Begonia isolate 1996

Aubretia isolate 1996

C. quinoa: No local or systematic symptoms

N. tabacum 'Xanthi': No local or systematic symptoms

N. benthamiana: No local or systematic symptoms for the first two weeks of growth. At 14 DPI, systematic lesions of vein clearing and later complete leaf chlorosis appear. Some leaves detach from the stem.

N. occidentalis P1: Yellowing of leaf rosette at eight DPI. Partial leaf necrosis on inoculated leaves.

Fig. 21: *N. benthamiana* inoculated with Aubretia isolate 1996

Fig. 22: *N. occidentalis* P1 inoculated with Begonia isolate 1996

Soil isolate 2009

C. quinoa: First lesions after six days post inoculation. The virus infection becomes systematic after nine days post inoculation.

N. tabacum cv. 'Xanthi': After four days post inoculation development of brownish half-circles. Brownish lesions quickly become necrotic. After ten days from the point of infection, the virus isolate has spread systematically.

N. benthamiana: First local lesions after eight days post inoculation. However, just two days later the virus infection becomes systematic. Light vein clearing close to leaf boundaries.

N. occidentalis P1: After four days post inoculation development of viral lesions. After eight days post inoculation, the lesions have become systematic. Inoculated leaves are completely necrotic and systematically infected leaves are necrotic along the leaf veins and close to the petioles.

Fig. 23: *C. quinoa* inoculated with Soil isolate 2009

Fig. 24: *N. occidentalis* P1 inoculated with Soil isolate 2009

Soil isolate 2007

C. quinoa: Strong local lesions at three DPI. Viral lesions are yellow-brownish in colour. At five DPI the entire inoculated leaf becomes necrotic with symptoms of systematic infections - young shoots become wilted and also show viral spots. However, at 14 DPI there are signs of recovery. Newly grown leaves are symptomless or only marginally infected. The stalk is scared by necrotic lesions

N. tabacum 'Xanthi': At four DPI there are the first symptoms of viral infection on the inoculated leaves. At five DPI, lesions have become very clear and at 6 DPI, lesions start to appear on non-inoculated leaves.

N. benthamiana: After eight DPI there are the first signs of infection. Interestingly, the first lesions are not local, but systematic. Young leaves are rugged and slightly chlorotic if

compared to older leaves. On nine DPI, there appear local symptoms of infection and chlorotic leaf spots.

N. occidentalis P1: No development of symptoms until one week after inoculation. At 8 DPI there are local lesions, but there is systematic infection. Local lesions become clear after nine days from the point of infection. Inoculated leaves are fully necrotic and systematically infected leaves have a mixture of necrotic and chlorotic spots.

Fig. 25: *N. tabacum* cv. 'Xanthi' inoculated with Soil isolate 2007

Fig. 26: *N. occidentalis* P1 inoculated with Soil isolate 2007

Bioassay of raspberry root samples

Two out of ten raspberry root samples caused virus symptoms on both *C. quinoa* and *N. benthamiana* test plants. *C. quinoa* test plants showed lesions after 7 DPI while *N. benthamiana* only showed local lesions after 13 DPI. The other 16 test plants (8 each of C. quinoa and N. benthamiana) stayed free of symptoms.

C. quinoa: The first signs of infection came from systematically infected shoots and young leaves. Inoculated leaves caught up in lesion development days after the first signs of systematic virus disease. Leaf near shoot-tips were strongly deformed. Yellow viral spots turned more and more yellow until they become light brown and necrotic. Infected leaves were curled-up.

N. benthamiana: Vein clearing and very rugged leaf surface. Leaves near shoot-tips were strongly deformed.

Fig. 27: *C. quinoa* inoculated with raspberry isolate 2022

Fig. 28: *N. benthamiana* inoculated with raspberry isolate 2022

A re-inoculation experiment to confirm whether the viruses were transmissible was performed on four C. quinoa test plants. Inoculation was performed via sap inoculation. All four test plants showed local and systematic lesions.

5.2. ELISA & RT-PCR

Nanodrop measurements confirmed the high quality of RNA extracted from Campanula isolate 2008, Raspberry isolate 2008 and Raspberry isolate 2022. Measurements were in the optimum range of 2-2.2 for Raspberry isolate 2022 and Raspberry isolate 2008. The measurement for Campanula isolate 2008 was only slightly outside the ideal range.

Begonia isolate 1996 was positive for TBRV in the ELISA performed with DSMZ reagents, but only weakly positive with the Bioreba AG reagents. However, ELISA performed with Bioreba AG reagents identified Soil isolate 2009, Aubretia isolate 1996 and Raspberry isolate 2008 as positive for TBRV. Bioreba AG reagents gave no clear results regarding ArMV infection of plant samples. The test gave weak positives for Begonia isolate 1996, Aubretia isolate 1996 and Raspberry isolate 2022.

RT-PCR performed using Nepo B primers (forward: TCTGGITTTGCYTTRACRGT, reverse: CTTRTCACTVCCATCRGTAA) identified Campanula isolate 2008 as TBRV and RT-PCR performed with BRSV primers (forward: TGCGTACACTCCAACACCAAT, reverse: TGGCTGCTTGACTTCTGTTGA) also identified Campanula isolate as TBRV. RT-PCR performed using ArMV primers (forward: TTGGCCCAGATATAGCGTAAAAAT, reverse: CAGCGGATTGGGAGTTCGT) gave no positive results for any of the three tested isolates.

Table 3: Nanodrop measurements to check for quality of extracted RNA. Optimum range for 260/280 is 2-2.2				
	Nucleic acid (ng/µL)	A 260	A 280	260/280
Campanula isolate 2008	929	23,236	10,821	2.38
Raspberry isolate 2008	451	11,289	5,249	2.19
Raspberry isolate 2022	562	14,068	6,722	2.09

Table 4: Combined ELISA and RT-PCR results						
	TBRV-ELISA (DSMZ)	TBRV-ELISA (Bioreba AG)	ArMV-ELISA (Bioreba AG)	RT-PCR Nepo B primers	RT-PCR Nepo B primers	RT-PCR ArMV primers
Begonia isolate 1996	+	?	?	n.t.	n.t.	n.t.
Aubretia isolate 1996	-	+	?	n.t.	n.t.	n.t.
Soil isolate 2009	-	+	-	n.t.	n.t.	n.t.
Raspberry isolate 2008	-	+	-	-	-	-
Campanula isolate 2008	-	-	-	+	+	-
Soil isolate 2007	-	-	-	n.t.	n.t.	n.t.
Raspberry isolate 2022	-	-	?	-	-	-

6. Discussion

There is a need for more research in the field of plant viruses and even more so in the field of small berries. Various types of viruses (but most importantly those transmitted by nematodes and aphids) pose a serious threat to raspberry production. This is especially bothersome for raspberries sold on the fresh market, as even minimal irregularities in the shape of a raspberry fruit can make the difference in being bought or not. It can seem like an uphill battle to keep raspberries free from viruses. Aphids are very good at replicating and hard to control and nematodes are soil-living and thus very hard to control as well. Furthermore, the lack of virus-free raspberry stocks (Martin et al. 2013; Georgieva et al. 2020) makes it almost impossible to keep virus pathologies out of a productive raspberry orchard. The role of investments made to fund scientific work on virus-free rootstocks cannot be understated. In the mid to long run, these research efforts will guarantee continued success in the cultivation of raspberries. Another important message is the furthering of researching into prophylactic control measures such as weedy host plant eradication and soil heat treatments to combat nematode infestation. Research dedicated to exploring chemicals to reduce the population of vectors is less ecologically-minded, but is nevertheless an important weapon in the arsenal of raspberry farmers.

This review of the properties of the raspberry plant in the wider genus *Rubus* has a strong focus on virus diseases in raspberries and made an effort to order the most relevant viruses of raspberry according to the type of vector involved in transmission. In several cases, this distinction was difficult to make as several viruses use multiple modes of transmission. Here, an objective assessment on the basis of scientific literature was made and the virus was ordered according to the most prevalent vector. Generally speaking, raspberry viruses that transmit via aphids and nematodes are far more numerous than those spread by pollen or mites. This knowledge could be used as a decision making tool when deciding which group of vectors to focus on in treating a specific raspberry virus.

The website ‘description of plant viruses’ (dpv.net) describes ArMV as causing yellow dwarf in raspberries. This combination of stunting and chlorotic viral spots was observed in raspberry isolate 2022. This lead to the suspicion that this isolate was ArMV, but unfortunately ELISA-test by Bioreba AG did not support this hypothesis. The same site describes lesions caused by tomato blackring virus on *C. quinoa* as chlorotic and necrotic lesions. Those symptoms were also observed in Begonia isolate 1996 inoculated on *C. quinoa*. Dpv.net describes ‘Local chlorotic or necrotic spots or rings’ for TBR infection on *N. tabacum* cv. ‘Xanthi’. This was not verified in the bioassay as *N. tabacum* cv. ‘Xanthi’ stayed symptom-free in the inoculation experiment performed with Begonia isolate 1996.

There were strong interactions between the virus isolates and the test plants used for inoculation. Some of the tested virus isolates produced symptoms very quickly, while others caused fewer lesions at the start of symptom-onset, but become more destructive at a later point. Also, while certain combinations of virus isolate and test plant only cause local infections, most become systematic at some point.

Several of the studied virus isolates were found to be of known raspberry virus species - Begonia isolate 1996 and Campanula isolate 2008 were identified as TBRV. The virus isolate

obtained from raspberry root samples – Isolate 2022 – was identified as belonging to ArMV via bioassay, but unfortunately ELISA did not confirm this hypothesis. However, it needs to be mentioned that TBRV and ArMV ELISA-reagents supplied by Bioreba AG have produced some doubtful results. There is a clear need for comparison of ELISA-results with other suppliers of ELISA-reagents to double-check the validity of test results. Several of the studied varus isolates gave only negative or contradiciting results for the three used analysis tools (Peperomia isolate 2000, soil isolates 2007 and soil isolate 2009). These isolates will need further study to determine their exact genetic relationship.

ELISA performed with reagents supplied by Bioreba AG suggested that Begonia isolate 1996 and Aubretia isolate 1996 were both positive for TBRV and ArMV. Moreover, RT-PCR identified Aubretia isolate 1996 as positive for Nepo B and BRSV-primers. This phenomenon is known as co-infection (further explained in section '3.4.5 infection with multiple raspberry viruses', page 19-20). Tomato blackring virus and beet ringspot virus are closely related, but distinct members of subgroup B of the genus *Nepovirus*. Intraspecies recombination is very common between TBRV and BRSV (Fowkes et al. 2022). This paper also mentions that two different commercially available ELISA test kits gave differing results for isolates tested for TBRV and BRSV. In other words, the two used ELISA test kits gave incorrect, but complementary results. The best currently available solution is the use of a combination of diagnostic tools (bioassay, ELISA and molecular tools) and double and triple check for best accuracy.

Significant contamination in the made results for RT-PCR performed with ArMV and BRSV primers (for raspberry isolate 2008 and raspberry isolate 2022) unusable. Further molecular analysis via reverse transcriptase PCR need to be done for a more complete picture. This was unfortunately not possible as time in the laboratory was limited due to COVID-19 restrictions.

7. Conclusion

The study of plant viruses is a very complex field in phytopathology. What makes categorization of viruses especially difficult are unclear naming conventions and a difficult to establish field of plant virus taxonomy. This review used naming conventions from the EPPO Global Database for consistency.

This review provides a condensed source of information about the most important characteristics of the genus *Rubus*. It also summarises the most relevant viruses in raspberry (and other important *Rubus* species such as blackberry) production and categorizes them according to their most relevant vector. This can help in practical plant virus management, since the most effective option to fight an acute virus outbreak is to fight the specific vector.

The study of viruses is a scientific field in rapid evolution. Taxonomic conventions are not completely established and new knowledge about genetic relationships of viruses is constantly being generated. This adds to the challenge of clearly identifying a virus isolate on a species level.

Some of the studied virus isolates could be attributed to a specific species (Campanula isolate 2000 and Begonia isolate 1996 as TBRV), while others are doubtful (Aubretia isolate 1996, Soil isolate 2009, Raspberry isolate 2008 might be TBRV and Raspberry isolate 2022 might be ArMV). They require more testing with different ELISA reagents and more RT-PCR to clarify their status. No positive results were recorded for Soil isolate 2007.

In order to obtain a more detailed understanind of raspberry viruses, a study containing a complete picture of raspberry root, leaf, cane and pollen samples should be conducted. Since ELISA-testing can be unreliable and provide incorrect information, it is heavily suggested that further studies use multiple diagnostic tools for identification and categorisation of raspberry viruses.

8. Acknowledgements

I want to thank my main supervisor Prof. Dag-Ragnar Blystad at the Norwegian Institute of Bioeconomy Research (NIBIO) for the inoculation experiment of raspberry-related viruses into different *Nicotiana* species and *Chenopodium quinoa*. I also want to thank him for the revision of my work and the useful and feedback along the way of compiling this master's thesis. Also, I want to thank co-supervisor Zhibo Hamborg (also at NIBIO) for the help provided in the laboratory work at the virus laboratory at NIBIO. Furthermore, I want to extend my thanks to engineer at NIBIO Carl-Henrik Lensjø Alvin, who has given me background knowledge and showed me how to conduct RNA-extraction, conversion of RNA to cDNA, PCR and gel-electrophoresis.

I want to thank my family for their help and encouragement. First off, my ever-caring mother Waltraud and my two amazing sisters Lisa and Maria Magdalena for their supportive words and encouragements in the months of putting together this work. Thanks also go to my (bro)ther-in-law Darmiel for assembling the photo-series of test plants in the appendix. All of my family also deserve many thanks for looking through my applications for job positions, internships and possibly further PhD-positions.

Also, I want to express my gratitude towards the supervisor of my bachelor's degree, Prof. Sanja Barić at the Free University of Bozen/Bolzano. It is mostly her who sparked my interest for plant pathology and disease management during my studies for the bachelor of agri-environmental sciences.

Last but certainly not least, I would like to be thankful towards the members of my beloved chess club 'Arci Scacchi' of Bolzano in South Tyrol, Northern Italy. I cannot wait to capture some pieces and push pawns down the board with them after my return to Italy following the conclusion of my Master's studies.

9. Bibliography

1) Arabis mosaic virus Available at: https://www.dpvweb.net/dpv/showdpv/?dpvno=333 (Accessed: 8.5.2022)

2) Barnett, O. & Murant, A. (1970). Host range, properties and purification of raspberry bushy dwarf virus. Annals of applied Biology, 65 (3): 435-449.
3) Bhat, A. I. & Rao, G. P. (2020). Virus transmission through pollen. In Characterization of Plant Viruses, pp. 61-64: Springer.

4) Bi, Y., Artola, K., Kurokura, T., Hytönen, T. & Valkonen, J. (2012). First report of Raspberry leaf blotch virus in raspberries in Finland. Plant Disease, 96 (8): 1231-1231
5) Braun, P. & Hildebrand, P. (2006). Epidemiology of fire blight of floricane fruiting
6) Blackman, R., Eastop, V. & Hills, M. (1977). Morphological and cytological separation of Amphorophora Buckton (Homoptera: Aphididae) feeding on European raspberry and blackberry (Rubus spp.). Bulletin of Entomological Research, 67 (2): 285-296.
7) Blystad, PLV 321 Lab 3; virus detection

8) Bobinaitė, R., Viškelis, P. & Venskutonis, P. R. (2016). Chemical composition of raspberry (Rubus spp.) cultivars. Nutritional composition of fruit cultivars: 713-731.
9) red raspberry caused by Erwinia amylovora. Canadian journal of plant pathology, 28 (1): 95-99.
10) Bodine, E. & Newton, J. (1942). The rasp leaf of Cherry. Phytopathology, 32 (4).
11) Budzyńska, D., Hasiów-Jaroszewska, B. & Elena, S. F. (2021). Genetic variability and evolutionary dynamics of tomato black ring virus population. Plant Pathology, 70 (6): 1521-1531. DELETE
12) Cadman, C. (1952). Studies in Rubus virus diseases II. Three types of vein chlorosis of raspberries. Annals of Applied Biology, 39 (1): 61-68.
13) Carew, J. G., Gillespie, T., White, J., Wainwright, H., Brennan, R. & Battey, N. H. (2000). The control of the annual growth cycle in raspberry. *The Journal of Horticultural Science and Biotechnology*, 75 (5): 495-503.
14) Chamberlain, C., Kraus, J., Kohnen, P., Finn, C. & Martin, R. (2003). First report of Raspberry bushy dwarf virus in Rubus multibracteatus from China. Plant Disease, 87 (5): 603-603.
15) Clark, M., Adams, A. & Barbara, D. (1976). The detection of plant viruses by enzyme-linked immunosorbent assay (ELISA). X International Symposium on Fruit Tree Virus Diseases 67.

16) Diekmann, M., Frison, E. A. & Putter, T. (1994). FAO/IPGRI Technical guidelines for the safe movement of small fruit germplasm, vol. 13: Bioversity International.
17) Dolan, A., MacFarlane, S. & Jennings, S. N. (2018). Pathogens in Raspberry and Other Rubus spp. In *Raspberry*, pp. 41-61: Springer.
18) Duncan, J., KENNEDY, D. M. & SEEMULLER, E. (1987). Identities and pathogenicities of Phytophthora spp. causing root rot of red raspberry. Plant Pathology, 36 (3): 276-289.
19) Fowkes, A., Adams, I. P., Jones, R. A., Fox, A., McGreig, S. & Boonham, N. (2021). Historical and recent tomato black ring virus and beet ringspot virus isolate genomes reveal interspecies recombination and plant health regulation inconsistencies. *Plant Pathology*.

20) Freeman, W. M., Walker, S. J. & Vrana, K. E. (1999). Quantitative RT-PCR: pitfalls and potential. Biotechniques, 26 (1): 112-125.
21) Georgieva, M., Kondakova, V. & Yancheva, S. (2020). A comparative study on raspberry cultivars in micropropagation. *Bulgarian Journal of Agricultural Science*, 26 (3): 527-532.
22) Gerlach, W. L., Llewellyn, D. & Haseloff, J. (1987). Construction of a plant disease resistance gene from the satellite RNA of tobacco ringspot virus. Nature, 328 (6133): 802-805.
23) Giongo, L., Ajelli, M., Poncetta, P., Ramos-García, M., Sambo, P. & Farneti, B. (2019). Raspberry texture mechanical profiling during fruit ripening and storage. *Postharvest Biology and Technology*, 149: 177-186.
24) Graham, J. & Brennan, R. (2018). Raspberry: breeding, challenges and advances: Springer.
25) Han, C., Zhao, Y., Leonard, S. & Traber, M. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria× ananassa) and raspberries (Rubus ideaus). Postharvest biology and Technology, 33 (1): 67-78.
26) Hansen, A. J., Nyland, G., McElroy, F. & Stace-Smith, R. (1974). Cherry rasp leaf disease in North America. Phytopathology, 64: 721-727.
27) Halgren, A., Tzanetakis, I. E. & Martin, R. R. (2007). Identification, characterization, and detection of Black raspberry necrosis virus. *Phytopathology*, 97 (1): 44-50.
28) Hewitt, W.B. (1958), The probable home of Pierce's disease virus. *Plant Disease Reporter*, 211-215.
29) Hiruki, C. & Teakle, D. S. (1987). Soil-borne viruses of plants. In Current topics in vector research, pp. 177-215: Springer.
30) https://tools.thermofisher.com/content/sfs/manuals/mmlv_rt_man.pdf
31) Isogai, M., Yoshida, T., Nakanowatari, C. & Yoshikawa, N. (2014). Penetration of pollen tubes with accumulated Raspberry bushy dwarf virus into stigmas is involved in initial infection of maternal tissue and horizontal transmission. *Virology*, 452: 247-253.
32) Institute of plant molecular biology. Healthy berries in a changing climate: development of new biotechnological procedures for virus diagnostics, vector studies, elimination and safe preservation of strawberry and raspberry. Available at: https://www.umbr.cas.cz/en/research/scientific-projects/noberryvirus/ (accessed: 5.15.2022)
33) James, D. (2011). Cherry rasp leaf virus. Virus and Virus-Like Diseases of Pome and Stone Fruits. APS Press, St. Paul, MN, USA: 137-141.
34) Jevremović, D., Leposavić, A. & Paunović, S. A. (2019). Molecular and biological characterization of Black raspberry necrosis virus on red raspberry in Serbia. Scientific-Experts Conference of Agriculture and Food Industry: Springer.
35) 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.
36) Jones A., A. Murant, D. Jennings and G. Wood.Annals of Applied Biology 1982 Vol. 100 Issue 1 Pages 135-147
37) Jones A, W. J. McGavin, A. Geering and B. Lockhart. Annals of applied biology 2002 Vol. 141 Issue 1 Pages 1-10
38) Kader, A. A. & Watkins, C. B. (2000). Modified atmosphere packaging—toward 2000 and beyond. *HortTechnology*, 10 (3): 483-486.
39) Kroemer, T. Choosing Between TAE buffer and TBE Buffer for Agarose Gel Electrophoresis Available at:

https://www.goldbio.com/blog/post?slug=ChoosingBetween-TAE-and-TBE+Buffer-Agarose-Gel-Electrophoresis (Accessed: 5.12.2022)

40) Kuzmanović, N., Prokić, A., Ivanović, M., Zlatković, N., Gašić, K. & Obradović, A. (2015). Genetic diversity of tumorigenic bacteria associated with crown gall disease of raspberry in Serbia. European journal of plant pathology, 142 (4): 701-713.
41) Lee, J. (2016). Further research on the biological activities and the safety of raspberry ketone is needed. NFS journal, 2: 15-18.
42) Lister, R. (1964). Strawberry latent ringspot: a new nematode-borne virus. Annals of Applied Biology, 54 (2): 167-176.
43) Lister, R. & Murant, A. (1967). Seed-transmission of nematode-borne viruses. Annals of Applied Biology, 59 (1): 49-62.
44) Making an agarose gel - Universit of Leicester. Available at: https://www.youtube.com/watch?v=wXiiTW3pflM&t=152s (Accessed: 8.5.2022)
45) Mannozzi, C., Cecchini, J. P., Tylewicz, U., Siroli, L., Patrignani, F., Lanciotti, R., Rocculi, P., Dalla Rosa, M. & Romani, S. (2017). Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life. *LWT-Food Science and Technology*, 85: 440-444.
46) 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.
47) McMenemy, L. S., Hartley, S. E., MacFarlane, S. A., Karley, A. J., Shepherd, T. & Johnson, S. N. (2012). Raspberry viruses manipulate the behaviour of their insect vectors. *Entomologia Experimentalis et Applicata*, 144 (1): 56-68.
48) 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.
49) McGavin, W. J., Mitchell, C., Cock, P. J., Wright, K. M. & MacFarlane, S. A. (2012). Raspberry leaf blotch virus, a putative new member of the genus Emaravirus, encodes a novel genomic RNA. Journal of General Virology, 93 (2): 430-437.
50) M-MLV Reverse Transcriptase (2015) Available at: https://www.thermofisher.com (Accessed: 8.5.2022)
51) Paunović, S. A. & Jevremović, D. (2020). High proportion of mixed virus infections in raspberry plantations in Serbia. Pesticidi i fitomedicina, 35 (1): 27-37.
52) Paunovic, S. A. & Jevremovic, D. (2020). The first detection of raspberry leaf mottle virus in Serbia. Journal of Plant Pathology, 102 (3): 925-925.
53) Parausić, V. & Simeunović, I. (2016). Market Analysis of Serbias Raspberry Sector and Cluster Development Initiatives. *Economics of Agriculture*, 63 (4): 1417-1431.
54) Pelletier, L., Brown, A., Otrysko, B. & McNeil, J. N. (2001). Entomophily of the cloudberry (Rubus chamaemorus). *Entomologia Experimentalis et Applicata*, 101 (3): 219-224.
55) Pritts M. Raspberries: Fire blight Available at: (https://blogs.cornell.edu/berrytool/raspberries/raspberries-fire-blight (Accessed: 4.3.2022)
56) Pulawska, J. (2010). Crown gall of stone fruits and nuts, economic significance and diversity of its causal agents: Tumorigenic Agrobacterium spp. Journal of Plant Pathology: S87-S98.
57) Quito-Avila, D. F., Jelkmann, W., Tzanetakis, I. E., Keller, K. & Martin, R. R. (2011). Complete sequence and genetic characterization of Raspberry latent virus, a novel member of the family Reoviridae. *Virus Research*, 155 (2): 397-405.

58) Quito-Avila, D.F., Lightle D. and Martin R.R.Effect of Raspberry bushy dwarf virus, Raspberry leaf mottle virus, and Raspberry latent virus on plant growth and fruit crumbliness in 'Meeker'red raspberry.Plant disease 2014 Vol. 98 Issue 2 Pages 176-183
59) Rao, A. V. & Snyder, D. M. (2010). Raspberries and human health: a review. Journal of Agricultural and Food Chemistry, 58 (7): 3871-3883.
60) Raspberry (Rubus spp.)-Crown and Cane Gall. Available at: https://pnwhandbooks.org/plantdisease/host-disease/raspberry-rubus-spp-crown-cane-gall (Accessed: 4.3.2022)
61) Raspberry Polka - primocane https://www.rhsplants.co.uk/plants/_/raspberry-polka-pbr/classid.2000011104/
62) Reddick, D. (1918). Varieties of beans susceptible to mosaic.
63) Rommel, A. & Wrolstad, R. E. (1993). Influence of acid and base hydrolysis on the phenolic composition of red raspberry juice. *Journal of Agricultural and Food Chemistry*, 41 (8): 1237-1241.
64) Rommel, A., Heatherbell, D. & Wrolstad, R. (1990). Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *Journal of Food Science*, 55 (4): 1011-1017.
65) Rubus breeding. Available at: https://www.hutton.ac.uk/ (Accessed: 5.5.2022)
66) Rymelska, N., Borodynko, N., Pospieszny, H. & Hasiów-Jaroszewska, B. (2013). Analysis of the biological and molecular variability of the Polish isolates of Tomato black ring virus (TBRV). Virus Genes, 47 (2): 338-346.
67) Samuitienė, M., Navalinskienė, M. & Jackevičienė, E. (2008). Arabis mosaic virus on ornamental plants. biologija, 54 (4).
68) Sanfaçon, H. & Fuchs, M. (2011). Tomato ringspot virus. Virus and virus-like diseases of pome and stone fruits, 41: 48.
69) Smith, K. M. (1946). Tomato black-ring: a new virus disease. Parasitology, 37 (3-4): 126-130.
70) Sochor, M., Hroneš, M. & Manning, J. (2022). Guide to the genus Rubus L.(Rosaceae) in South Africa–Disentangling a taxonomic Gordian knot with the help of ploidy and reproductive data. South African Journal of Botany, 147: 511-567.
71) Stace-Smith, R. (1955). Studies on Rubus Virus Diseases in British Columbia: II. Black raspberry necrosis.Canadian Journal of Botany, 33 (4): 314-322.
72) Tang, J., Ward, L. I. & Clover, G. R. (2013). The diversity of strawberry latent ringspot virus in New Zealand. Plant disease, 97 (5): 662-667.
73) Tezotto-Uliana, J. V., Fargoni, G. P., Geerdink, G. M. & Kluge, R. A. (2014). Chitosan applications pre-or postharvest prolong raspberry shelf-life quality. Postharvest Biology and Technology, 91: 72-77.
74) Tomato black ring virus. Available at: https://dpvweb.net/dpv/showdpv/?dpvno=38 (Accessed: 8.5.2022)

75) Thodra. (2021) Raspberry: Good harvest and post-harvest practices.Available at: www.italianberry.it (Accessed: 3.20.2022)

76) Tzortzakis, N. G. (2007). Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative food science & emerging technologies*, 8 (1): 111-116.

77) Tzanetakis, I., Postman, J. & Martin, R. (2007). First report of Blackberry chlorotic ringspot virus in Rubus sp. in the United States. *Plant Disease*, 91 (4): 463-463.

78) Udvardi, M. K., Czechowski, T. & Scheible, W.-R. (2008). Eleven golden rules of quantitative RT-PCR. The Plant Cell, 20 (7): 1736-1737.

79) Umiraliyeva, Z. Z., Kopzhassarov, B. K., Jaimurzina, A. A., Niyazbekov, Z. B., Issenova, G. Z., Tursunova, A. K. & Berganayeva, G. E. (2021). Epidemiology of Fire Blight in Fruit Crops in Kazakhstan. AGRIVITA, Journal of Agricultural Science, 43 (2).

80) Vakic, M., Stainton, D., Delic, D. & Tzanetakis, I. E. (2022). Characterization of the first Rubus yellow net virus genome from blackberry.

81) Wilson, C. R. (2014). *Applied plant virology*: CABI.ISBN: 987-1-84593-991-5

10. Appendix

10.1 Tables documenting virus development

The following tables show detailed results of virus inoculation onto test plants. For a period of approximately three weeks, development of virus symptom development was measured nearly every day.

Explanations of signs used to describe virus disease symptoms:

- The + sign indicates presence of local lesions.
- The ++ sign indicates presence of strong and numerous local lesions
- The – sign indicates absence of lesions.
- The ? mark indicates that presence of lesions is not clear. The following days will clarify if symptoms are present or not.

The abbreviation 'syst.' indicates systematic infection. This means that local lesions have spread from the point of inoculation to other plant parts.

Table I: Development of local and systematic symptoms in Peperomia isolate 2000 at progressive stages.

Peperomia isolate 2000	*C. quinoa*	*N.tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1
1 DPI	-	-	-	-
2 DPI	-	-	-	-
4 DPI	-	-	-	-
5 DPI	-	-	-	-
6 DPI	-	-	-	-
7 DPI	-	-	-	-
8 DPI	-	-	-	-
9 DPI	-	-	-	-
10 DPI	-	-	-	-
11 DPI	-	-	-	-
12 DPI	-	-	-	-
13 DPI	-	-	-	-
14 DPI	-	-	-	-
16 DPI	-	-	-	-
17 DPI	-	-	-	-
18 DPI	-	-	-	-
19 DPI	-	-	-	-
20 DPI	-	-	-	-

Table II: Development of local and systematic symptoms in Begonia isolate 1996 at progressive stages.				
Begonia isolate 1996	*C. quinoa*	*N. tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1
1 DPI	-	-	-	-
2 DPI	-	-	-	-
4 DPI	-	-	-	-
5 DPI	-	-	-	-
6 DPI	-	-	-	-
7 DPI	-	-	-	-
8 DPI	+	-	-	-
9 DPI	+/syst	-	-	-
10 DPI	++/syst	-	-	-
11 DPI	++/syst	-	-	-
12 DPI	++/syst	-	-	-
13 DPI	++/syst	-	-	-
14 DPI	++/syst	-	-	-
15 DPI	++/syst	-	-	-
16 DPI	++/syst	-	-	-
17 DPI	++/syst	-	-	-
18 DPI	++/syst	-	-	-
19 DPI	++/syst	-	-	-
20 DPI	++/syst	-	-	-

Table III: Development of local and systematic symptoms in Aubretia isolate 1996 at progressive stages.				
Aubretia isolate 1996	*C. quinoa*	*N. tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1
1 DPI	-	-	-	-
2 DPI	-	-	-	-
4 DPI	-	-	-	-
5 DPI	-	-	-	-
6 DPI	-	-	-	-
7 DPI	-	-	-	-
8 DPI	-	-	-	+/syst
9 DPI	-	-	-	++/syst
10 DPI	-	-	-	++/syst
11 DPI	-	-	-	++/syst
12 DPI	-	-	-	++/syst
13 DPI	-	-	-	++/syst
14 DPI	-	-	syst	++/syst

16 DPI	-	-	syst	++/syst
17 DPI	-	-	++/syst	++/syst
18 DPI	-	-	++/syst	++/syst
19 DPI	-	-	++/syst	++/syst
20 DPI	-	-	++/syst	++/syst

Table IV: Development of local and systematic symptoms in Soil isolate 2009 at progressive stages.

Soil isolate 2009	*C. quinoa*	*N. tab.* cv. 'Xanthi'	*N. benthamiana.*	*N. occidentalis.* P1
1 DPI	-	-	-	-
2 DPI	-	-	-	-
4 DPI	-		-	-
5 DPI	-	+	-	+
6 DPI	+	+	-	+
7 DPI	-	++	-	++
8 DPI	++	++	+	++/syst
9 DPI	+/syst	++	+	++/syst
10 DPI	++/syst	++/syst	++/syst	++/syst
11 DPI	++/syst	++/syst	++/syst	++/syst
12 DPI	++/syst	++/syst	++/syst	++/syst
13 DPI	++/syst	++/syst	++/syst	++/syst
14 DPI	++/syst	++/syst	++/syst	++/syst
15 DPI	++/syst	++/syst	++/syst	++/syst
16 DPI	++/syst	++/syst	++/syst	++/syst
17 DPI	++/syst	++/syst	++/syst	++/syst
18 DPI	++/syst	++/syst	++/syst	++/syst
19 DPI	++/syst	++/syst	++/syst	++/syst
20 DPI	++/syst	++/syst	++/syst	++/syst

Table V: Development of local and systematic symptoms in Raspberry isolate 2009 at progressive stages.

Raspberry isolate 2008	*C. quinoa*	*N. tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1	*N. clevelandii*
3 DPI	-	?	-	-	-
6 DPI	++	++/syst?	-	?	?
7 DPI	++/syst	++/syst	-	?	?
8 DPI	++/syst	++/syst	-	+/syst	-
9 DPI	++/syst	++/syst	-	++/syst	-
10 DPI	++/syst	++/syst	-	++/syst	-
12 DPI	++/syst	++/syst	-	++/syst	-

15 DPI	++/syst	++/syst	-	++/syst	-
16 DPI	++/syst	++/syst	-	++/syst	+
17 DPI	++/syst	++/syst	-	++/syst	++
20 DPI	++/syst	++/syst	-	++/syst	++
21 DPI	++/syst	++/syst	-	++/syst	++
22 DPI	++/syst	++/syst	-	++/syst	++
23 DPI	++/syst	++/syst	-	++/syst	++/syst
24 DPI	++/syst	++/syst	-	++/syst	++/syst

Table VI: Development of local and systematic symptoms in Campanula isolate 2008 at progressive stages.

Campanula isolate 2008	*C. quinoa*	*N. tabacum* cv. 'Xanthi'	*N. benthamiana*	*N. occidentalis* P1	*N. clevelandii*
3 DPI	++	-	-	-	+?
6 DPI	++	++	+?	++	++
7 DPI	++/syst?	++	+?	++	++
8 DPI	++/syst?	++	+?/syst	++	++
9 DPI	++/syst	-	+/syst	++	++
10 DPI	++/syst	-	++/syst	++	++
12 DPI	++/syst	-	++/syst	++/syst	++/syst
15 DPI	++/syst	-	++/syst	++/syst	++/syst
16 DPI	++/syst	-	++/syst	++/syst	++/syst
17 DPI	++/syst	-	++/syst	++/syst	++/syst
20 DPI	++/syst	-	++/syst	++/syst	++/syst
21 DPI	++/syst	-	++/syst	++/syst	++/syst
22 DPI	++/syst	-	++/syst	++/syst	++/syst
23 DPI	++/syst	-	++/syst	++/syst	++/syst
24 DPI	++/syst	-	++/syst	++/syst	++/syst

Table VII: Development of local and systematic symptoms in Soil isolate 2007 at progressive stages.

Soil isolate 2007	C. quinoa	Nic. tab. 'Xanthi'	Nic. benth.	Nic. occ. P1
1 DPI	-	-	-	-
2 DPI	-	-	-	-
4 DPI	+	-	-	-
5 DPI	+	+?	-	-
6 DPI	++	++	-	-
7 DPI	++	++/syst	-	+/syst
8 DPI	++/syst	++/syst	syst	+/syst
9 DPI	++/syst	++/syst	++/syst	++/syst
10 DPI	++/syst	++/syst	++/syst	++/syst
11 DPI	++/syst	++/syst	++/syst	++/syst

12 DPI	++/syst	++/syst	++/syst	++/syst
13 DPI	++/syst	++/syst	++/syst	++/syst
14 DPI	++/syst	++/syst	++/syst	++/syst
15 DPI	++/syst	++/syst	++/syst	++/syst
16 DPI	++/syst	++/syst	++/syst	++/syst
17 DPI	++/syst	++/syst	++/syst	++/syst
18 DPI	++/syst	++/syst	++/syst	++/syst
19 DPI	++/syst	++/syst	++/syst	++/syst
20 DPI	++/syst	++/syst	++/syst	++/syst

Table VIII: Overlook of virus-presence from ten raspberry root samples from soil with known nematode presence. Odd-numbered test plants are *C. quinoa* and even-numbered test plants are *N. benthamiana*. 1-10 DPI.

from 1 to 10 DPI.

root sample	**test plant**	1 DPI	2 DPI	3 DPI	4 DPI	5 DPI	6 DPI	7 DPI	8 DPI	9 DPI	10 DPI
1	1	-	-	-?	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
2	3	-	-	-	-	-	-	syst	+/syst	+/syst	++/syst
	4	-	-	-	-	-	-	-	-	-	-
3	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
4	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
5	9	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-
6	11	-	-	-	-	-	-	-	+/syst	+/syst	++/syst
	12	-	-	-	-	-	-	-	-	-	-
7	13	-	-	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-	-	-
8	15	-	-	-	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-	-	-	-
9	17	-	-	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-	-	-
10	19	-	-	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-	-	-

Table IX: Overlook of virus-presence from ten raspberry root samples from soil with known nematode presence. Odd-numbered test plants are *C. quinoa* and even-numbered test plants are *N. benthamiana*. 10-20 DPI.

from 1 to 10 DPI.

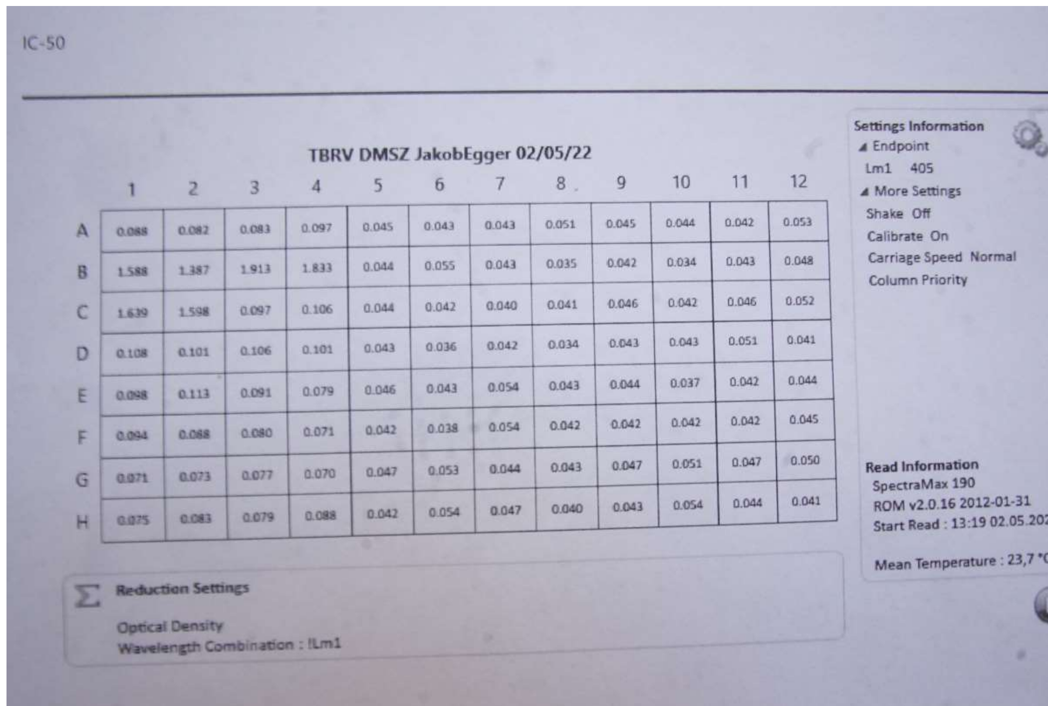
root sample	Test plant	11 DPI	12 DPI	13 DPI	14 DPI	16 DPI	17 DPI	18 DPI	19 DPI	20 DPI
1	1	-	-	-	-	-	-	-	-	+/syst ?
	2	-	-	-	-	-	-	-	-	+/syst ?
2	3	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst
	4	-	-	syst	syst	++/syst	++/syst	++/syst	++/syst	++/syst
3	5	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-
4	7	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-
5	9	-	-	-	-	-	-	-	-	+/syst
	10	-	-	-	-	-	-	-	-	+
6	11	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst	++/syst
	12	-	-	syst	syst	++/syst	++/syst	++/syst	++/syst	++/syst
7	13	-	-	-	-	-	-	-	+	++/syst
	14	-	-	-	-	-	-	-	-	+
8	15	-	-	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-	-	-
9	17	-	-	-	-	-	-	-	-	+
	18	-	-	-	-	-	-	-	-	+
10	19	-	-	-	-	-	-	-	-	++/syst
	20	-	-	-	-	-	-	-	-	++/syst

Table X: Re-inoculation assay to confirm infectivity of raspberry isolate 2022

Raspberry isolate 2022, re-inoculation	test plant 1	test plant 2	test plant 3	test plant 4
1 DPI	-	-	-	-
2 DPI	-	-	+	-
3 DPI	-	-	-	-
4 DPI	+	+	+	-
5 DPI	++/syst	++/syst	++/syst	++/syst
6 DPI	++/syst	++/syst	++/syst	++/syst
7 DPI	++/syst	++/syst	++/syst	++/syst
8 DPI	++/syst	++/syst	++/syst	++/syst
9 DPI	++/syst	++/syst	++/syst	++/syst
10 DPI	++/syst	++/syst	++/syst	++/syst

11 DPI	++/syst	++/syst	++/syst	++/syst

10.2 ELISA plate readings & bioassay photo-series



IC-50

TBRV DMSZ JakobEgger 02/05/22

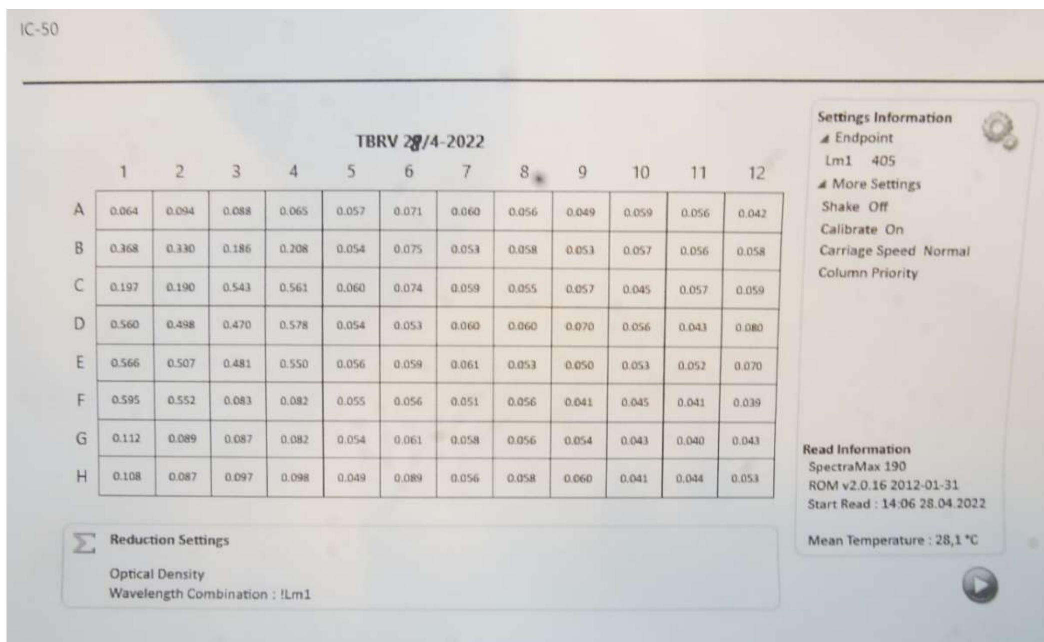
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.088	0.082	0.083	0.097	0.045	0.043	0.043	0.051	0.045	0.044	0.042	0.053
B	1.588	1.387	1.913	1.833	0.044	0.055	0.043	0.035	0.042	0.034	0.043	0.048
C	1.639	1.598	0.097	0.106	0.044	0.042	0.040	0.041	0.046	0.042	0.046	0.052
D	0.108	0.101	0.106	0.101	0.043	0.036	0.042	0.034	0.043	0.043	0.051	0.041
E	0.098	0.113	0.091	0.079	0.046	0.043	0.054	0.043	0.044	0.037	0.042	0.044
F	0.094	0.088	0.080	0.071	0.042	0.038	0.054	0.042	0.042	0.042	0.042	0.045
G	0.071	0.073	0.077	0.070	0.047	0.053	0.044	0.043	0.047	0.051	0.047	0.050
H	0.075	0.083	0.079	0.088	0.042	0.054	0.047	0.040	0.043	0.054	0.044	0.041

Reduction Settings
Optical Density
Wavelength Combination : !Lm1

Settings Information
Endpoint
Lm1 405
More Settings
Shake Off
Calibrate On
Carriage Speed Normal
Column Priority

Read Information
SpectraMax 190
ROM v2.0.16 2012-01-31
Start Read : 13:19 02.05.202
Mean Temperature : 23,7 °C

Fig. a: Printout of ELISA- microplate reader for DSMZ-TBRV-reagents



IC-50

TBRV 28/4-2022

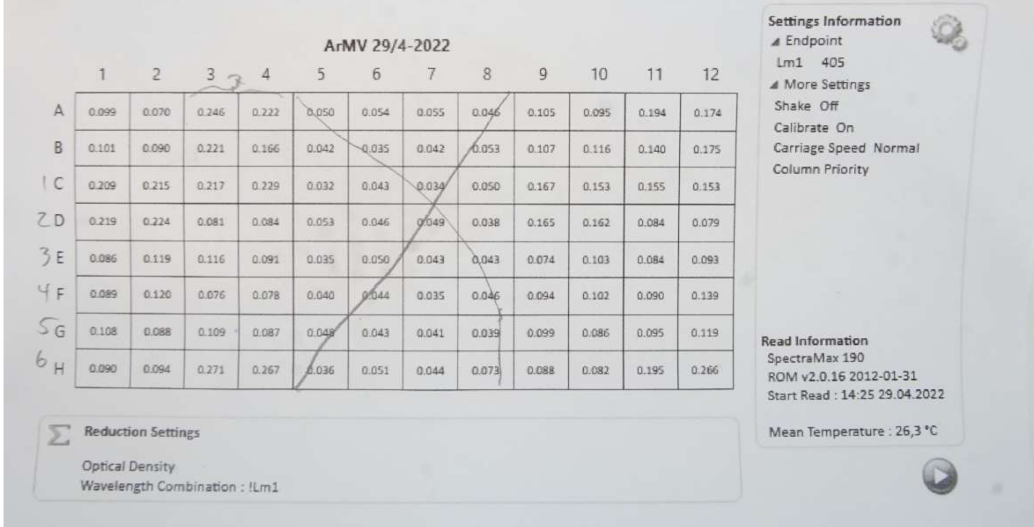
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.064	0.094	0.088	0.065	0.057	0.071	0.060	0.056	0.049	0.059	0.056	0.042
B	0.368	0.330	0.186	0.208	0.054	0.075	0.053	0.058	0.053	0.057	0.056	0.058
C	0.197	0.190	0.543	0.561	0.060	0.074	0.059	0.055	0.057	0.045	0.057	0.059
D	0.560	0.498	0.470	0.578	0.054	0.053	0.060	0.060	0.070	0.056	0.043	0.080
E	0.566	0.507	0.481	0.550	0.056	0.059	0.061	0.053	0.050	0.053	0.052	0.070
F	0.595	0.552	0.083	0.082	0.055	0.056	0.051	0.056	0.041	0.045	0.041	0.039
G	0.112	0.089	0.087	0.082	0.054	0.061	0.058	0.056	0.054	0.043	0.040	0.043
H	0.108	0.087	0.097	0.098	0.049	0.089	0.056	0.058	0.060	0.041	0.044	0.053

Reduction Settings
Optical Density
Wavelength Combination : !Lm1

Settings Information
Endpoint
Lm1 405
More Settings
Shake Off
Calibrate On
Carriage Speed Normal
Column Priority

Read Information
SpectraMax 190
ROM v2.0.16 2012-01-31
Start Read : 14:06 28.04.2022
Mean Temperature : 28,1 °C

Fig. b: Printout of ELISA- microplate reader for Bioreba AG-TBRV-reagents



IC-50

ArMV 29/4-2022

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.099	0.070	0.246	0.222	0.050	0.054	0.055	0.046	0.105	0.095	0.194	0.174
B	0.101	0.090	0.221	0.166	0.042	0.035	0.042	0.053	0.107	0.116	0.140	0.175
1 C	0.209	0.215	0.217	0.229	0.032	0.043	0.034	0.050	0.167	0.153	0.155	0.153
2 D	0.219	0.224	0.081	0.084	0.053	0.046	0.049	0.038	0.165	0.162	0.084	0.079
3 E	0.086	0.119	0.116	0.091	0.035	0.050	0.043	0.043	0.074	0.103	0.084	0.093
4 F	0.089	0.120	0.076	0.078	0.040	0.044	0.035	0.046	0.094	0.102	0.090	0.139
5 G	0.108	0.088	0.109	0.087	0.048	0.043	0.041	0.039	0.099	0.086	0.095	0.119
6 H	0.090	0.094	0.271	0.267	0.036	0.051	0.044	0.073	0.088	0.082	0.195	0.266

Settings Information
Endpoint
Lm1 405
More Settings
Shake Off
Calibrate On
Carriage Speed Normal
Column Priority

Read Information
SpectraMax 190
ROM v2.0.16 2012-01-31
Start Read : 14:25 29.04.2022

Mean Temperature : 26,3 °C

Reduction Settings
Optical Density
Wavelength Combination : !Lm1

Fig. c: Printout of ELISA- microplate reader for Bioreba AG-ArMV-reagents

Fig. d: Photo-series of Campanula isolate 2008 at 25 DPI . From left to right: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1, *N. clevelandii*

Fig. e: Photo-series of Raspberry isolate 2008 at 25 DPI . From left to right: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1, *N. clevelandii*

Fig. f: Photo-series of Peperomia isolate 2000. From left to right: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1 and *N. clevelandii*

Fig. g: Photo-series of Begonia isolate 1996. From left to right: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1 and *N. clevelandii*

Fig. h: Photo-series of Aubretia isolate 1996. From left to right: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1 and *N. clevelandii*

Fig. i: Photo-series of Soil isolate 2009. From top to bottom: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1 and *N. clevelandii*

Fig. j: Photo-series of Soil isolate 2007. From top to bottom: *C. quinoa*, *N. tabacum* cv. ‘Xanthi’, *N. benthamiana*, *N. occidentalis* P1, *N. clevelandii*

Fig. k: Photo-series of Raspberry isolate 2022. From top to bottom: *C. quinoa*, *N. tabacum* cv. 'Xanthi', *N. benthamiana*, *N. occidentalis* P1 and *N. clevelandii*

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

Postboks 5003
NO-1432 Ås
Norway