1 Introduction

Viruses are small obligate intracellular parasites consisting of either DNA or RNA genome surrounded by coat of proteins known as capsid (Gelderblom, 1996). The DNA or RNA genome may consist of single stranded (ss) or double stranded (ds) with complex shape mostly spherical or tubular (Louten, 2016). Different replication strategies are required for the various types of genome. For replication of RNA viruses, at least three types of RNA must be synthesized i.e. the genome, a copy of genome and mRNAs using virally encoded RNA-dependent-RNA-polymerase (RdRp) (Payne, 2017). Viruses need living host cells for replication because they have no ribosomal machinery hence the need to rely on protein synthesis present in their host cells (Walsh *et al*., 2011).

Viruses are known to have emerged from evolution and ecological processes due to interaction with their host over some period of time (Elena *et al*., 2014). For plant virus emergence, they are mostly mediated by changes in agricultural practices and long-distance transportation of plant materials (Rojas and Gilbertson, 2008). They can have either positive or negative impacts on their hosts in several ways. Roossinck, in 2015 reported that, virus may be either beneficial or harmful leading to the death of plants. This means, there can be a mutualistic relationship between virus and host depending on the environments and vice versa. Viruses interact with their host plant and the environment thereby exchanging genetic information between hosts (McLeish *et al*., 2019).

Although viruses are capable of virtually infecting different species of cultivated and wild plants, their host range varies from narrow to wide depending on individual type of viruses (Gergerich *et al*., 2006). The type and severity of the host reactions to virus infections are variable and depends on the virus strains, sources of infection, crop genotypes as well as environmental conditions (Hull, 2014). For example, the *Cucumber mosaic virus* is known to have the widest host range worldwide among plant viruses while *Citrus tristeza* infects only a few species in the genus *Citrus* (Gergerich *et al*., 2006).

Some plant viruses are vector transmissible e.g. fungi, nematodes, aphids and other insects whiles others are transmitted via pollen, seeds and seedlings, grafting, roots and mechanical transmission (Büttner *et al*., 2022). For cultivated woody plants, they are transmitted via vegetative propagation depending on specific properties of the virus (Büttner *et al*.,2022). Depending on the mode of transmission, different strategies for preventing the spread of plant viruses need to be taken into consideration and has been described in chapter 6.

With an estimated annual economic impact of over $30 billion, about 50% of the known plant viruses have been reported to be a contributing factor in agricultural crop losses globally (Sastry and Zitter, 2014). Much research has been done on viruses infecting fruit trees such as grapevine, mango, pome fruit, banana and citrus (Umer *et al*., 2019). Nevertheless, there is little or no knowledge on viruses infecting forest and urban trees (Büttner *et al.*, 2013). Hence there is a need to focus on urban green space since they have immense impact on the health of our populations (Konijnendijk *et al*., 2013). Although viral diseases in trees are widespread, there is a lack of extensive data on viruses infecting forest and urban trees (Büttner *et al.*, 2013).

Forest pathology previously focussed traditionally on insect damage and fungal diseases (Linnakoski and Forbes, 2019) without considering viruses affecting forest and urban trees. Trees for instance, in urban green areas are subject to great stress and often more short-lived than their conspecifics in a natural environment (Roloff, 2013). Not only abiotic stress factors such as nutrient deficiency, pollutants in both air and water, contaminants in soil, fine dust, road salt and ozone influence the health of tree (Czaja *et al*., 2020), but also many biotic factors (e.g. soil microorganisms, insects, bacteria, nematodes, viruses etc.). Viral diseases, which are able to increase the predisposition to other pathogenic factors, have been found on degenerating deciduous trees in cities for over a decade now (Büttner *et al*., 2013). For instance, Cooper and Massalski (1984) observed that, complex symptoms of changes in colour and shape of leaves from some deciduous trees such as *Betula* sp., the loss of branches and crowns are associated with virus infections.

The birch (*Betula* sp.) which is deciduous pioneer tree was selected as a model tree due to the fact that suspected viral symptoms have been observed on them for some years now in urban green (Division of phytomedicine -HUB). *Betula* belonging to the family *Betulaceae* is one of the most relevant broad-leaved trees in Northern and Eastern Europe which contributes to the biodiversity of coniferous forest (Hynynen, *et al*., 2010). The common species in Europe are the *Betula pendula* Roth and *pubescens* Ehrh. (Ashburner and McAllister, 2013). Even though both tree species (*B. pendula* Roth. and *B. pubescens* Ehrh.) are naturally distributed across Europe to central Siberia, *B. pubescens* is widely distributed in the north and eastern regions and grows further north than other deciduous trees whereas the *B. pendula* can reach southern regions such as Iberian Peninsula, South Italy and Greece (Beck *et al*., 2016). The birch is an important tree species in the cities, because they provide habitats for numerous native animal species.

Birch is of considerable value to insectivores, cavity nesters, seed-eaters and field-layer species and has moderately diverse bird community compared to that of mixed deciduous woods (Patterson, 1993). According to (Heydemann; *Der Forst- und Holzwirt, p. 536*, Hanstein 1984), there are a total of 164 types of insect in birch trees, including 11 weevils, 27 longhorn beetles, 10 bark beetles, 9 owl butterflies. *B. pendula* do survive under dry weather conditions with decreasing growth performance whiles the *B. pubescens* are normally not suitable for locations with high temperatures (Asche *et al.,* 2007). Both tree species requires a minimum length of growing season of 110 days with relatively significant characteristics shown in Tab.1.

Tab. 1 Birch wood cultivation with different climatic growth conditions (Asche, *et al*., 2007)

	Minimum length of growing season in days	Trophy level	Very dry	Dry	Moderately dry	Moderately fresh	Fresh	Very fresh	Ground fresh	Basic damp	Damp	Wet	Water logged	Alternative damp	moderate alternating damp	Alternative dry
Betula pubescens	110	3-5														
Betula pendula	110	1-5														

Tree species suitable for the location with good growth performance
Tree species appropriate to the location with decreasing growth performance and/or increasing risk
Tree species not suitable for the site

With a stock volume ranging from 0.5% to 15% of all hardwood standing, depending on the region, *Betula* species play a key role in the forests of Western Europe (Dubois *et al*., 2020). In Germany, they contribute about 5% stock volume of hardwood (Hynynen *et al*., 2010). The *Betula* sp. which has largely been neglected appears to be alternative species for reforestation because they are more adaptable to future climate change and can generate valuable products for future market (Hemery *et al*., 2010; Dubois *et al*., 2020).

Betula sp. can withstand climate changes and tolerate climate fluctuations well if the site requirements of the respective birch species are taken into consideration. Planting birch trees as a pioneer tree also makes economic sense, especially in urban re-cultivation areas. The demands of *Betula* species, which are particularly advantageous in extreme locations such as in the city, are also derived from the distribution area. Wrong site location or selection sites are a major detrimental factor in keeping trees in the city for a long term. Since the *Betula* sp. are sensitive to summer heat (Teskey *et al*., 2015), the location for planting in the city must be carefully considered. This is because both species as well as their hybrids differ, their site location and the choice of species in relation to the birch species play an essential role for growth after transplanting shock and thus for the long-term stability of birch trees in cities.

The general question arises whether *Betula* sp. suffer from viruses in cities more than country side due to city climate or whether trees cultivated for growth in the cities suffer due to poor management practices in the nurseries. Even though *Betula* sp. can adapt to extreme weather conditions, biotic factors such as viruses may influence the vulnerability of trees after being predisposed by abiotic factors. According to reports, agricultural systems, such as monocrops with low genetic diversity and high plant density, which are more susceptible to pathogens; global trade in plant materials; effects of climate change on hosts and vectors; and capacity for rapid evolution and adaptation are factors contributing to the emergence of viruses (Anderson *et al*., 2004; Jones, 2009; Elena *et al*., 2014).

The aim of this thesis is to collect data and generate more knowledge on the frequency and distribution of the viral infections in *Betula* sp. In the case of urban trees there is no information on how long birch population fight this infection already. The question arises whether it's an old or a new problem since they are now being detected in some parts of Europe. The *Betula* sp. population seems to be resisting those viruses already for long time and can combat these viruses under optimal conditions. From my personal observation, natural grown sites and forests had less symptoms compared to *Betula* trees in urban green space. This is probably because in urban areas, there are no optimal conditions and is engulfed with more viruses from all over the world due to artificial ecosystems with man-made diversity.

Again, other plant viruses which are not yet detected in *Betula* can trigger infection. In addition to fungal diseases, viruses are of particular importance, which can manifest themselves predominantly on trees in unsuitable locations under the increasingly extreme climatic conditions in urban green space. Externally, these viral diseases are primarily characterized by the loss of branches, causing possible death of trees which is common in fruit trees but has not yet been shown in birches. The loss of branches influences stability and road safety due to dead branches which causes an increase in management and maintenance cost (Brookes, 2007). The distributions of viruses on road trees are being investigated in this study. Although green urban space is considered as a major positive and cost-effective means of improving the quality of the environment (Navarrete-Hernandez and Laffan, 2019), the complex ecology of urban areas is constantly being disrupted locally by the emergence of new type of species and concentration of chemicals into specific habitats due to human activity (Douglas, 2008).

All these factors mixed with wrong site location, seedlings contaminated with viruses, inadequate attention as well as plant shock affects the resistance of trees to pathogens for years and can often lead to the early death of the trees. In order to fully understand the ecological effects, more research is required. For instance, studies are missing, if virus infected birch trees are more attractive for insects like the birch bark beetle (*Scolytus ratzeburgii*) leading to a fast decline and death of the trees (Tidow, 2020). Plant virus induced attraction by changes in leaf appearance and coloration is known from field crops since a long time (Jones and Barbetti, 2012), hence the need to also focus on urban trees. This effect might be even worse if abiotic stresses are intervening. Strict measures for city planning including protection and examining young tree seedlings diseases such as viruses will be essential in managing these and other difficulties as the world's urban areas increases.

In our basic study the focus was on birch plus trees in seed plantation, as well as younger and old trees in urban green space. In a follow up study, seeds and seedlings from the tree nurseries are to be examined in order to narrow down the ways in which seedlings can be contaminated with viruses. As mentioned before, naturally grown locations of the birch compared with the locations of street trees have noticeable less virus suspected symptoms. This is an indication that the seed-borne viral diseases get into our street trees with the tree nursery material (Cooper, 1979). On the other hand, the means of transmission for most viruses is not yet understood and seems to differ in forest and urban green due to the differences in the ecosystem and the human impact. One of our hypotheses is that, birch populations grown under natural conditions can cope better with viral diseases because only the fittest and /or, virus-free seedlings survives in a given environment. The situation is different with the cultivation of seedlings in the nursery sector, where all seedlings (including infected viral seedlings which would not survive in nature) are processed and cultivated under optimal conditions as much as possible. The transmission routes of pathogens, especially viruses, must be recognized and interrupted, especially if they are spread by human activities during cultivation of trees.

As viruses cannot be treated with pesticides or medication, the major focus in a management concept can only be on early diagnostic of viruses and removal of virus infected trees to interrupt transmission. In this way we can show where the viral diseases come from and open up the possibility of finding and eliminating the sources of infection. Paths for sustainable planting and maintenance of trees free from pathogenic viruses- or infected trees are to be developed. Research has to answer the question if the particular viruses of host plant in a certain virome have a clear pathogenicity and how they contribute to the decline.

It is certainly very difficult to prevent viral infection from trees in case of vector transmitted viruses or if trees are connected to each other by root contacts and thus mixing their microbiome. To protect young trees as long as possible from infection by pathogenic viruses, prophylactic measures should be considered since that might give them a better chance to overcome plant shock and other abiotic stresses. A very hygienic environment for the production of the virus free-trees is by selecting virus-free source material for budding and grafting. The status of viruses in forest trees and the recommendations to combat them can therefore only be determined after basic research in order to get information on mode of transmission of viruses, host range, geographical distribution and other factors.

A concept for dealing with virus-infected trees is also preceded by the identification and detection of the causative pathogens and knowledge of their epidemiology and interactions with the host. On the other hand, trees cannot be ignored as sources of viruses in our ecosystem (Harris and Hill, 2021), as viruses often have a broad range of host plants and can be transmitted to cultivated plants. Based on knowledge on viruses (e.g. *Cacao swollen shoot virus*, *Apple mosaic virus*, *Cherry leaf roll virus*, *Grapevine leafroll-associated virus* etc.) infecting fruit growing trees, it is obvious that viruses can also be found in deciduous trees. These can make the trees sick and must be counted among the pathogens, for example if they reduce the plant quality, the fruit quality, seed germination, the biomass and the longevity of trees. Ultimately, they lead to the degeneration of the entire tree or tree population and thus to long-term damage to the ecosystem.

A severe outbreak of *Citrus tristeza virus* in citrus growing regions during last century is reported to have destroyed almost 100 million trees (Moreno *et al*., 2008). There are also reports on loss of yield efficiency of peach infected with mild isolate of *Plum pox virus* even though tree produced slightly more fruits of smaller size than non-infected trees. A mixed infection of *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), and *Prunus necrotic ringspot virus* (PNRSV) has been reported to reduce growth by exhibiting bark canker, trunk malformation, and tree mortality in some peach cultivars and in reduction of growth rate of seedlings by 2.9 to 69.1% (Nemeth, 1992). Trees on the other hand seem to combat viral infections by developing a kind of equilibrium with its virome. My personal observation in some older individual birch trees (about 80 years) from urban green space from Berlin were found to be infected with several viruses including symptoms but still survive because of growing in a more natural environment.

Symptoms can be named in two ways, whether they are symptoms of virus, diseases, of other infectious diseases or mere physiological or genetic disorders (Bos, L. 1977). Virus induced symptoms are confused with nutrient deficiency, drought or ozone damage or even fungal infection. Often one finds chlorosis (yellowing), subsequent necrosis (drying out, apoptosis), premature leaf-shedding and weak branches as clear symptoms of a viral disease in *Betula* sp. (Büttner *et al*., 2022). The expression and pattern in the leaves depend on the respective viral disease or viral combination. The hypothesis has been generated that the diversity in symptom pattern (i.e. line pattern, chlorotic line pattern, leaf mosaic, small leaves, intercostal chlorosis, leaf necrosis) as shown in (Fig. 1) reflects the diversity of the viruses present in the *Betula* trees. As the correlation of symptoms and viral infection is not examined yet for the mixed infections, it is unknown if the complexity of the virome is the cause of the variability of symptoms.

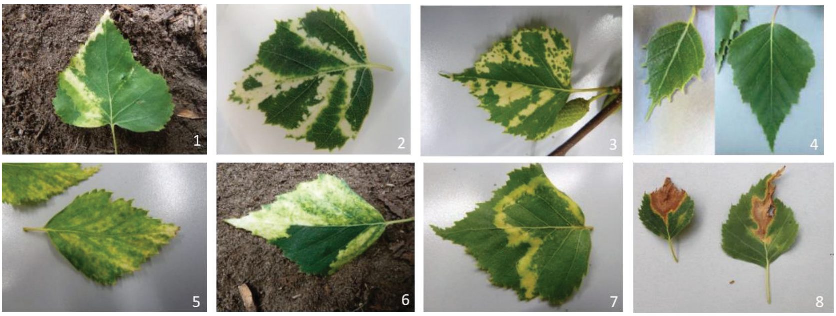

Fig. 1 Diversity of viral *Betula* leaf symptoms; Line pattern (1), chlorotic line pattern (2), leaf mosaic (3), small leaf (4), intercostal chlorosis (5), leaf variegation (6), line pattern (7), leaf necrosis (8). (Division of phytomedicine - Humboldt University of Berlin (HUB).

Many of the viruses detected in *Betula* (Tab.2) have so far been completely unexplored and their impact on the domestic ecosystem is difficult to assess. It is therefore particularly important to identify these, obtain new knowledge about the pathogenicity of these viruses and to be able to diagnose the pathogens among them in a targeted manner. Obvious virus-suspect symptoms on newly planted trees only show up after a few years or under harmful environmental conditions. For tree nurseries or seed plantations, it is very difficult and expensive to find out about a tree infection after a couple of years cultivating diseased trees.

Especially if those viruses belong to pathogens affecting fruit quality or tree health. The trees must be removed from field and cannot be sold without the consequence of losing the quality standards of the nursery and resulting in immense financial losses. Therefore, the diagnostic of pathogenic viruses as early as possible in the production process is of utmost importance. However, the longevity of trees is the basis for the long-term establishment of many organisms such as pathogens in the tree ecosystem (Lindenmayer *et al*., 2017). If the longevity is reduced from the outset during planting by pathogenic viruses contained in the planting material (young trees or seedlings), a long-term existence of trees in the ecosystem cannot be established. For this reason, targeted virus diagnostics in the plant material is particularly important, which has not yet been carried out due to a lack of knowledge and research into pathogenic viruses of deciduous trees. This is a different situation in production of fruit trees such as grapes or berries, where the production is already based on an efficient virus management.

Tab. 2 Viruses detected in host plant (*Betula* sp.)

Host plant	Detected virus	Reference
Betula sp.	*Apple mosaic virus*	Bandte *et al*., 2009; Cooper and Massalski, 1984, Gotlieb and Berbee 1973.
Betula sp.	*Arabis mosaic virus*	Bandte *et al*., 2009; Polák and Zieglerová, 1997; Polák and Procházková, 1996; Hardcastle and Gotlieb, 1980; Gotlieb and Berbee, 1973.
Betula sp.	*Birch leaf roll virus*	Rumbou *et al*., 2018.
Betula sp.	*Cherry leaf roll virus*	Büttner *et al*., 2011; von Bargen *et al*., 2009; Jalkanen *et al*., 2007; Jones *et al*., 1990; Nienhaus and Castello, 1989; Cooper and Atkinson, 1975.
Betula sp.	*Tobacco necrosis virus*	Cooper and Massalski, 1984.
Betula sp.	*Tomato ringspot virus*	Cooper and Massalski, 1984.

In field crop production, the loss of plants and the economic impact due to viruses is about 50% before virus management (Jones *et al*., 2019). Unfortunately, it is very difficult to show an economic loss due to viruses in the production of deciduous trees. Mainly this has something to do with the long-life span of trees compared to field crops, which require long term experiments and research project financing. Financing of this need deep interest of the society in the topic.

As long as tree diseases are hardly recognized, the research on tree viruses is focussing on obvious symptoms as represented by the Birch leaf roll disease (BLRD). The improvement of the sustainability of tree planting, virus diagnostics, control management of viral diseases and prophylactic hygiene measures in maintenance are to be mentioned as necessary steps towards an effective virus management. Basic knowledge on the biology of the viral pathogen is the prerequisite to gain information on how to interrupt life cycle of viruses or to block viral transmission in a given population. Epidemiology and pathogenicity of the newly discovered viruses in birch as well as in other hosts, host specificity, life cycle, mode of transmission, host plant range and phylogeny, are totally unknown and have to be investigated especially for so far unknown viruses.

Knowledge of the viral status of gene banks, nurseries, forest stands, and trees in urban green space, such as roadside trees, can add supporting information on the biology of the viruses. This knowledge can be used for developing control plans and diagnostic tools for tree management. Many so far unknown viruses such as *Birch leaf roll associated virus* (BLRaV) have been identified by application of High-throughput sequencing (HTS) in deciduous trees (Rumbou *et al*., 2018). BLRaV is associated with severe symptoms in birch leaves (i.e. chlorosis, leaf rolling, vein banding and necrosis) and shall be further investigated in this study to underline its contribution to the Birch leaf roll disease (BLRD). Therefore, one chapter is particularly reviewing the knowledge on BLRaV and other badnaviruses.

The hypothesis rises that; viral diseases in woody trees begins from “mother” trees and tree nursery stock that are grown with inadequate selection and under poor hygienic condition. Since the tree seedlings are not certified as virus-free unlike the fruit growing trees, there is a possibility of increased incidence of viral diseases, which are then transferred to the urban green areas and contributes to the decline of city climate stressed trees. One important way viruses are transmitted is from seeds and seedlings onto urban green which weakens trees in the long term. Another way of increasing viral infection in urban green is linked to the observation that, birch populations that have developed under natural conditions can cope better with viral diseases. This is of great importance to clarify whether the investigated viral diseases get into our street trees by inadequate seedling selection. One can assume other ways of transmission depending on the individual properties of the viral species. For instance, a transmission by pollen or decaying leaves or vectors has to be considered as well. Furthermore, there are indications that plant viruses could be a contributing factor to the decline of *Betula* sp. depending on the choice of species, health status of planting material as well as location.

The distribution of viruses affecting *Betula* trees in seed plantation might give information if viral infections starts from seed plantation. Leaf samples showing virus suspected symptoms from streets of bigger cities (Berlin) can give information on the dimension of viral diseases already distributed in the urban green space. In order to fill a huge gap of knowledge about viral diseases, such studies can monitor how widespread are viral symptoms in *Betula* trees in the city.

As the correlation of symptoms and viral infection is not examined yet, it is unknown if the complexity of the virome is the cause of the variability of symptoms. Based on the investigations, better decisions can be made in order to plant and maintain healthy *Betula* trees in the urban green space in the future. To this end, diagnostic procedures must be developed and refined. Only then can the importance of the different viruses currently being discovered in the *Betula* trees and their damage to the ecosystem be recognized. In our ecosystem's, organisms are clustered into parasites and symbionts (Rynkiewicz *et al*., 2015). This separation applies for viruses as well, but beneficial viruses are even more difficult to determine because an infection and replication in a host cell by a virus implies per se a negative impact on the tree even if the beneficial impact is of a secondary mode for instance by protecting the host from a more aggressive viral strain (Roossinck, 2015). Those beneficial viruses might exist in a complex mixture of viruses as we find it in *Betula* and they might drive the tree towards equilibrium with its microbiome. This is supported by the infection by viruses in older trees, which find ways to combat it.

The situation in mixed viral infected trees is highly complex but reflexes the true situation in nature, where we find lots of viruses associated with natural populations of plants (Harris and Hill, 2021). With less information known on plant viruses affecting deciduous woody plants, the HTS has become a reliable tool for further diagnostic and discovery of viral agents in woody hosts which is more reliable (Massart *et al*., 2017; Roossinck *et al*., 2015) like particle isolation, enrichment and bioassay followed by molecular characterization. The use of HTS hasproven as good as or better than bioassays in detecting viruses and viroids in woody plants (Rott *et al*., 2017; Al Rwahnih *et al*., 2015). For example, about 21 new viruses infecting forest trees have been discovered within the last five years (Rumbou *et al*., 2021) via HTS including the genus badnavirus. Several steps such as; nucleic acid extraction and virus sequences enrichment, library preparation, automated sequencing, data analysis are needed to be taken into consideration when applying HTS technologies.