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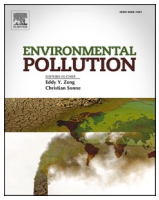

Betula pendula trees infected by birch idaeovirus and cherry leaf roll virus: Impacts of urbanisation and NO_2 levels☆

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ABSTRACT

Viruses are frequently a microbial biocontaminant of healthy plants. The occurrence of the infection can be also due to environmental stress, like urbanisation, air pollution and increased air temperature, especially under the ongoing climate change. The aim of the present study was to investigate the hypothesis that worsened air quality and fewer green areas may favour the higher frequency of common viral infections, particularly in a common tree in temperate and continental climates, *Betula pendula* ROTH.

We examined 18 trees, during the years 2015–2017, the same always for each year, in the region of Augsburg, Germany. By specific PCR, the frequency of two viruses, Cherry leaf roll virus (CLRV, genus Nepovirus, family Secoviridae), which is frequent in birch trees, and a novel virus tentatively named birch idaeovirus (BIV), which has been only recently described, were determined in pollen samples. The occurrence of the viruses was examined against the variables of urban index, air pollution (O_3 and NO_2), air temperature, and tree morphometrics (trunk perimeter, tree height, crown height and diameter). Generalized Non-linear models (binomial logit with backward stepwise removal of independent variables) were employed.

During the study period, both CLRV and BIV were distributed widely throughout the investigated birch individuals. CLRV seemed to be rather cosmopolitan and was present independent of any abiotic factor. BIV's occurrence was mostly determined by higher values of the urban index and of NO_2. Urban birch trees, located next to high-traffic roads with higher NO_2 levels, are more likely to be infected by BIV.

Increased environmental stress may lead to more plant viral infections. Here we suggest that this is particularly true for urban spaces, near high-traffic roads, where plants may be more stressed, and we recommend taking mitigation measures for controlling negative human interventions.

1. Introduction

The Intergovernmental Panel on Climate Change (Cisséet al., 2022) has highlighted microbial diseases (i.e. viruses and bacteria) in human and plants as the top dire biological factor for future generations. At the same time, wind-pollinated plants produce copious amounts of pollen, which – as part of the plant reproductive function – are liberated and become airborne, to achieve a successful reproduction. It is well known that ongoing climate change has been contributing to increases in the number of pollen produced per plants and flower unit (Damialis et al.,

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2011), as well as the concentrations of airborne pollen detected in the atmosphere, particularly for trees (e.g. Ziska et al., 2019). What it is known so far is that viruses can occur within pollen in forest trees and shrubs (Büttner et al., 2011, 2013); yet, pollen-mediated transmission of most of these viruses has not been thoroughly studied. Therefore, it is also unknown which biotic or environmental factors may influence pollen-mediated virus dispersal in infected plants.

Birch trees (*Betula* spp.) are very common in the Northern Hemisphere and are of high ecological importance as pioneer species due to their adaptability to low nutrient soils (Oksanen, 2021). Birch trees are frequently found in urban environments, and their pollen is also a major aeroallergen, especially in temperate and continental climates, causing allergic rhinonjunctivits and asthma in susceptible humans (Biedermann et al., 2019). Studies have documented the infection of birches by a variety of virus species, belonging to badnaviruses, carlaviruses, ilarviruses, idaeoviruses and nepoviruses; co-infection with two or more viruses, e. g., with cherry leaf roll virus (CLRV) and birch idaeovirus (BIV), is very common in birch leaves (Büttner et al., 2023; Massart et al., 2017; Rumbou et al., 2020, 2021).

One of the very common viruses in birch trees is CLRV. The virus is horizontally as well as vertically pollen transmitted (Massalski and Cooper, 1984; Massalski et al., 1988). It belongs to the family Secoviridae and to the genus Nepovirus and was first described in 1933 in sweet cherry (Prunus avium L.) and English walnut (Juglans regia L.) trees (Büttner et al., 2011; Posnette and Cropley, 1955; Wellink et al., 2000). Today CLRV has been detected in plants all over the world. It has a wide host range of more than 36 plant families of which at least 24 are broad-leaved trees, with *Betula* spp. Being one of the most common hosts (Rebenstorf et al., 2006; Walkey et al., 1973). The presence of CLRV in birches is generally of high interest since it has been repeatedly detected in many trees exhibiting strong foliar symptoms, like leaf roll, vein banding and chlorotic patterns with subsequent necrosis of leaves (Rumbou et al., 2016, 2018).

Besides the long known CLRV, also new viruses in *Betula* have been identified recently (Rumbou et al., 2018). Two contigs of an unknown virus from birch leaf samples were connected to the genus Idaeovirus, which has been recently assigned to the family Mayoviridae by the International Committee on Taxonomy of Viruses (ICTV; https://ictv.global, accessed January 31, 2023). Rumbou et al. (2020) consequently described this new virus in 2020 as the birch idaeovirus (BIV). The genome of BIV is bipartite with positive-sense single-stranded RNA of approx. 5.5 kb for RNA-1, and >1.5 kb for RNA-2. One of the known idaeoviruses affecting woody hosts, Idaeovirus rubi (raspberry bushy dwarf virus, RBDV), is vertically and unassisted horizontally pollen-transmitted, which is why the same can be assumed for other idaeoviruses. Horizontal transmission of viruses by infected pollen involves the infection of the mother plant trough the fertilized flower. Infection of the pollen mother cell and resultant pollen leads indirectly to embryo infection (vertical transmission). Vertical transmission of viruses is achieved, if the seed becomes infected by the virus, either for instance by attachment of virus particles to the seed surface, or, more commonly, through transfer of viruses during fertilisation of the embryo. However, it cannot be concluded that if a virus is transmitted horizontally by pollen there will also be vertical transmission, or vice versa (Card et al., 2007).

The aim of this work was to assess how two viruses, CLRV and BIV, are distributed in pollen samples throughout a birch population in a temperate climate of central Europe, where the species is widely distributed, namely in the greater region of Augsburg, Germany. While CLRV has been a common disease of birch trees in temperatre and continental Europe, BIV has been a novel virus, with unknown effects and factors that contribute to its spreading. We hypothesised that viral infection may be associated with tree growth parameters and urbanisation-relevant stressors, such as elevated air temperatures and traffic-related air pollution. Elevated temperatures in cities, known as the “urban heat island” effect, can cause heat- and drought stress in susceptible plants (Memon et al., 2009; Kuttler, 2008). It has been documented already that higher temperatures, drought stress, increased levels of CO_2 and other air pollutants contribute to increases in viral transmission directly and indirectly via influences on insect vectors (Chung et al., 2016; Del Toro et al., 2019; Robel et al., 2013; Singh et al., 1988; Szczepaniec et al., 2019; van Munster et al., 2017). It is well known that high levels of NO_2 decreased the fertility of birch pollen and simultaneously increased its allergenicity by nitration of pollen proteins (Cuinica et al., 2014; Shiraiwa et al., 2012). Other known effects of NO_2 on plants include modification of enzymes, stomatal conductance, respiration and even reduction of the photosynthesis rate (Wellburn, 1994). Having said the above, we also hypothesised that air pollution, relevant to urban, high-traffic spaces, might render trees more susceptible to viral infections, and that the combination of both, elevated air temperatures and air pollutants, might also weaken the birch tree’s capability to contain an existing infection and therefore contribute to pollen transmission of CLRV and BIV.

2. Methods

2.1. Trees and pollen samples

18 birch (*Betula pendula*) trees in the region of Augsburg were observed and probed during three successive years (2015–2017). Each year, the onset of flowering was recorded by daily phenological observations according to Kolek et al. (2021). Tree growth-related parameters measured were trunk perimeter (m), crown radius and height (m), and tree height (m). Pollen was extracted by sieving from 100 catkins per tree at the onset of its flowering as described previously by Beck et al. (2013). Pollen samples were stored at −80 °C until processing.

2.2. Environmental parameters

Ambient concentrations of the air pollutants O_3 and NO_2 were measured directly at each tree by passive samplers (PASSAM AG, Männedorf, Switzerland) during 3 weeks before and up to the start of the flowering season per individual tree. The passive samplers contain a reagent that reacts with the substance to be measured as soon as it is exposed to the air. The exact time of exposure is noted and after the exposure is finished and the sampler is sealed, the sampler is analysed in a laboratory. By measuring the concentration of the reagent, the concentration of the substance of interest over the time of exposure can be estimated.

The urban index (UI) was calculated based on CORINE Land Cover 2006 data (European Environment Agency, 2013) using ArcGIS 10.6 and its implemented focal statistics tool essentially as described in Jochner et al. (2012). The UI represents the proportion of predefined built-up areas (e.g., continuous and discontinuous urban fabric, industrial or commercial units) within a radius of 2 km. The index can vary from 0 to 1 (high degree of urbanisation).

Daily data for temperature and precipitation were assessed via the German Weather Service (Deutscher Wetterdienst (DWD), Offenbach, Germany, 2019). The weather station (DWD ID: 232) is situated at Augsburg Airport (48.4254 N; 10.9420 E) to the north of the city of Augsburg. Because there is no knowledge on the timing of birch pollen infection by viruses, we checked both, cumulative temperature of the previous year’s summer (June–August) and cumulative temperature of the 30 days prior to flowering start, in order to cover the entire pollen formation process.

2.3. RNA isolation from pollen

To extract total RNA from pollen samples, the method of Boom et al. (1990) was followed. Briefly, 0.01–0.2 g of pollen was mechanically ground in a sterilized mortar in grinding buffer (6 M guanidine-hydrochloride, 0.2 M sodium acetate, pH 5.2, 25 mM EDTA,

1 M potassium acetate, 2.5% (w/v) polyvinylpyrrolidone) and extracted by vortexing after addition of 10% (w/v) SDS, followed by 10 min shaking (800 rpm) at 70 °C. After centrifugation (10 min, 4 °C, 15, 000×*g*) the RNA-containing supernatant was transferred into a fresh tube with 300 µl of 6 M NaI, 0.15 M Na_2SO_3 solution, 150 µl 96% ethanol and 25 µl silica solution (1 g SiO_2 per ml DEPC-treated H_2O), mixed and incubated for 10 min at 20 °C on an overhead shaker to allow the binding of the RNA to the silica particles. The samples were centrifuged (1 min, 4 °C, 6000×*g*), the supernatants discarded, and the pellets washed twice with washing buffer (10 mM tris-HCl, pH 7.5, 0.5 mM EDTA, 50 mM NaCl, 50% ethanol). The tubes were dried for 10 min upside down and the dried pellets resuspended in DEPC-treated H_2O by a brief incubation at 70 °C. A final centrifugation step (4 min, 4 °C, 15, 000×*g*) was carried out to separate the RNA from the silica particles. The RNA was then precipitated from the supernatant with 8 µl 8 M LiCl and 500 µl 96% ethanol overnight at −20 °C. On the next day, the samples were centrifuged (20 min, 4 °C, 15,000×*g*), the supernatants discarded, and the pellets washed once with 70% ethanol. After drying of the pellets in a SpeedVac, they were dissolved in 30 µl of sterile, DEPC-treated H_2O.

The nucleic acid concentration of the samples was determined by NanoDrop™ One/One© Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific). To determine the quality of RNA preparations, the samples were analysed by agarose gel electrophoresis.

2.4. RT-PCR amplification of viral RNA sequences

1,3 µg of RNA was reverse transcribed to cDNA using 100 units of reverse transcriptase and 100 pmol random hexamer primers (Maxima H Minus Reverse Transcriptase™, Thermo Scientific) in the presence of 20 units of RNase inhibitor (Thermo Scientific) and 2 mM dNTPs at a final concentration according to manufacturer's instruction. The cDNA was stored at −20 °C until further processing. To amplify a 627 bp sequence of the CLRV coat protein gene, the following PCR was conducted for CLRV detection (CLRV PCR-1): protocol: 1x (2 min, 94 °C); 35x (30 s, 94 °C; 30 s, 53 °C; 30 s, 72 °C); 1x (7 min, 72 °C), with primers CP350F: GAGAGAAATT TTAGCTTYTCYATG and CP977R: ACTCMACCCTATCAAARTATAYCA as previously described by Langer et al. (2016).

In addition, we amplified a 353 bp sequence of the 3′ untranslated region (3′ UTR) present in both genomic RNAs of the CLRV (CLRV PCR-2); protocol: 1x (2 min, 94 °C); 35x (30 s, 94 °C; 30 s, 53 °C; 30 s, 72 °C); 1x (7 min, 72 °C) applying the following primer set: CLRV-RW2: GTCGGAAAGATTACGTAAAAGG (Werner et al., 1997) and CLRV-RW1 mod: CATGCGACCGGTCCTAGTAGTA, as modified by Bütow et al. (2013).

To amplify a 556 bp sequence of the replicase (RNA 1) of BIV, BIV PCR-1 was performed as previously described for CLRV detection with the following program: 1x (2 min, 95 °C) 35x (20 s, 95 °C; 20 s, 56 °C; 20 s, 72 °C) 1x (5 min, 72 °C) and the primer set: Idaeo00001 F: GGGTCATCTTCGGGGAAGTG and Idaeo00001 R: AAAGTCTCCAGCATG ACGC.

Additionally, we amplified a 199 bp fragment of the movement protein sequence encoded by RNA 2 of BIV by the following detection PCR (BIV PCR-2): protocol: 1x (2 min, 95 °C) 35x (20 s, 95 °C; 20 s, 53 °C; 20 s, 72 °C) 1x (5 min, 72 °C). As BIV-RNA2-specific primers Idaeo 00051 F: GCTGAAGAAGGCTGGAGGT), Idaeo 00051 R: TCGCCCGTCAT GAAATGACA could be established.

For quality control of successful cDNA synthesis from RNA extracts of birch pollen, a 181 bp sequence of the plant NADH dehydrogenase subunit 5 gene was amplified using the primers nad5-sense: GATGCTTCTTGGGGCTTCTTGTT and nad5-antisense: CTCCAGTCACCAACATTGGCATAA as described by Menzel et al. (2002).

2.5. Sanger sequencing of PCR amplicons

To verify the results of the PCR, PCR products amplified with all primer pairs were prepared for sequencing using the Invisorb® Fragment CleanUp kit (Invitek Molecular GmbH, Berlin, Germany) and sent for Sanger sequencing to Macrogen (Amsterdam, Netherlands). The sequence data were analysed using the software BioEdit (version 7.2.6.1; Hall, 1999) and compared to data from the National Center for Biotechnology Information (NCBI) using Blastn at the nr database for identification of reference sequences.

2.6. Statistical analysis

Differences among years were analysed and visualized by Box-Whisker plots and Kruskal-Wallis test. Occurrence of viral infections of the birch trees was investigated by use of Generalized Non-Linear Models (GLZ; binomial logit distribution) and with stepwise backward removal of the independent variables. The Wald Statistic, significance level at $p < 0.05$ and visualization of the Area Over the Curve (AOC) were applied for the assessment of the associated relationships. The occurrence of each plant virus, CLRV and BIV, was examined separately against the independent variables of urban index, tree morphometrics, air temperature, and air pollution (NO_2 and O_3). Prior to analysis, all datasets were examined for normality (Shapiro-Wilks test). All statistical tests were performed in R4.2.2 (RStudio), Statistica 14.0 (TIBCO Software Inc.), and Graph Pad Prism 9.

3. Results

A population of 18 trees had been sampled each year uninterruptedly from 2015 to 2017. As seen in Fig. 1, in 2015, pollen samples from 18/18 (100%) and 16/18 (89%) trees were tested positive for CLRV and BIV, respectively. In 2016, 17/18 (94%) of pollen samples were positive for CLRV and 7/18 (38%) positive for BIV. Double infections were found in 16/18 (89%) of samples in 2015, in 7/18 (38%) of samples in 2016 and in 8/18 (44%) of samples in 2017. Mono-infections occurred in 2/18 (11%), 9/18 (50%) and 8/18 (44%) of trees in 2015, 2016 and 2017, respectively. Double-negative samples occurred in 0/18 (0%) of trees in 2015 and in 2/18 (11%) of trees both in 2016 and in 2017.

To verify the detection of BIV with both PCR systems, we sequenced the PCR amplicons of a subset of samples ($n = 10$). The PCR products were sequenced from both directions with fragment-specific primers. Consensus sequences were prepared for sequences generated with each primer pair and compared with known sequences in the database of the NCBI by a blast nucleotide comparison (BLASTN, version 2.12.0). The samples showed high identity with BIV reference sequences (on average, BIV 0001^+: 98.49% and BIV 00051^+: 97.47%). Thus, the detection of BIV in the samples was verified. All sequencing results are shown in the supplementary Table S1.

As shown in Fig. 2, NO_2 concentrations were slightly higher in 2015 than in 2016 and 2017, but the difference, based on a post-hoc multiple comparison test, was marginally non-significant ($p = 0.08$). For O_3 concentrations, there was a significant difference between years, with ozone levels of 2015 higher than in the other two years (2015 vs. 2016: $p < 0.05$; 2015 vs. 2017: $p < 0.01$, one-way ANOVA with post-hoc mixed-effects analysis). The cumulative air temperatures before the onset of tree flowering showed a different pattern, with 2017 significantly higher than the previous two years ($p < 0.001$, one-way ANOVA with post-hoc mixed-effects analysis).

To find out whether viral infection shows any statistical correlation with tree-intrinsic or environmental parameters, we performed multiple logistic regression analysis with the dependent variable 'virus infection' (presence-absence) and the independent variables trunk perimeter, crown radius, tree height, crown height, cumulative temperature of 30 days prior to flowering onset, cumulative temperature of the previous year's summer, urban index (UI), NO_2 concentration, and O_3

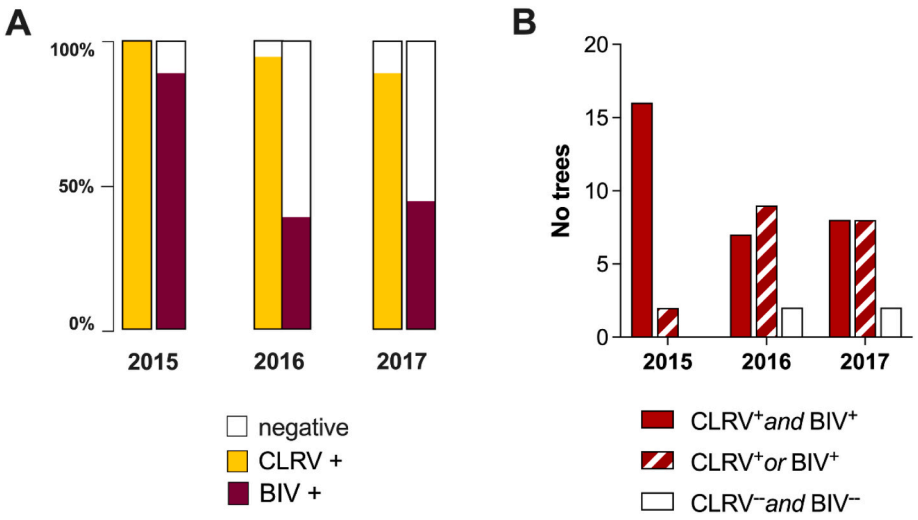

Fig. 1. Virus detection in pollen samples of birch trees. A: Percentages of trees (100% = 18 trees) with virus + pollen are indicated by colored bars (yellow: LRV+; red: BIV+). B: Numbers of trees with pollen samples positive for both viruses (filled bars) or positive for either of the viruses (hatched bars). CLRV: cherry leaf roll virus. BIV: birch idaeovirus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

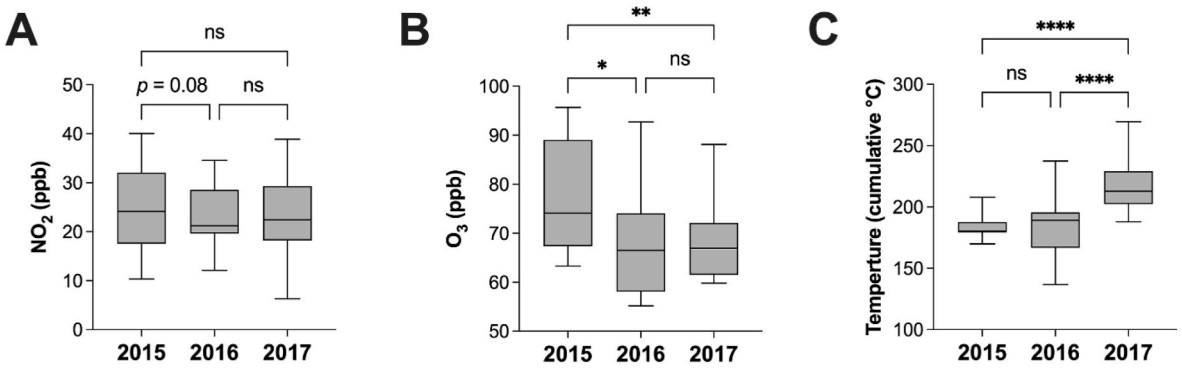

Fig. 2. Comparison of air pollutant levels and air temperatures between the years. NO_2 levels (A), ozone levels (B), and air temperatures (C), measured before the onset of flowering of each tree. *: $p < 0.05$; **$p < 0.01$; ****: $p < 0.0001$, one-way ANOVA with post-hoc multiple pair-wise comparison (mixed effects analysis).

concentration. For CLRV, no factor could significantly explain its variability ($p > 0.05$). For BIV, the resulting model was significant with the area over the curve ROC: 0.89, and with urban index and year-to-year variation being significant (Fig. 3A; model summary in Table 1).

The urban index is positively correlated with the air pollutant NO_2, which showed a similar year-to-year distribution pattern as in the case of

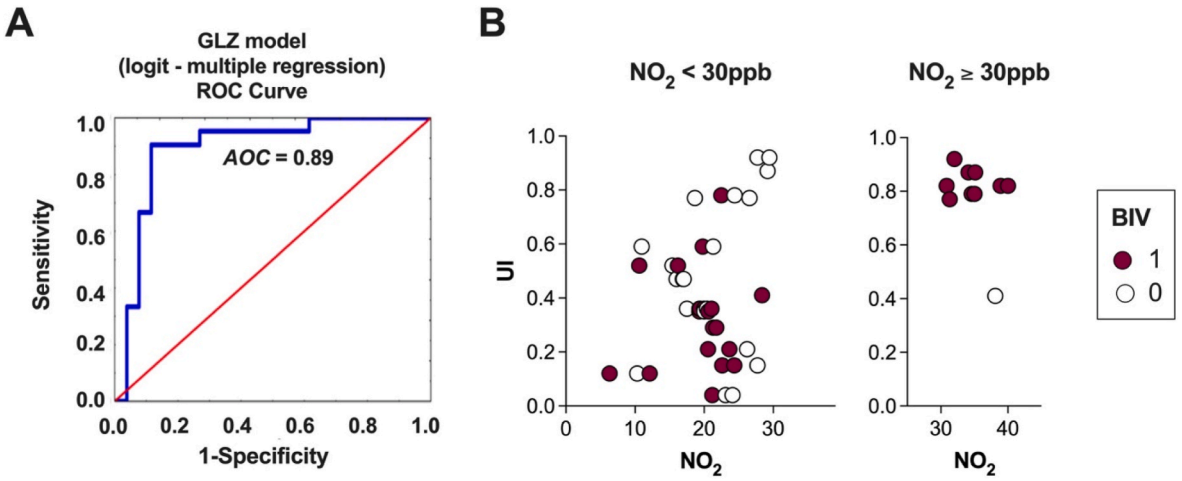

Fig. 3. The urban index (UI) of the tree is associated with BIV infection of pollen samples. Multiple logistic regression analysis resulted in a significant model with urban index as main predictor for BIV infection (A). BIV positive pollen samples were enriched in trees with high urban index (>0.7) and high NO_2 level (≥30 ppb) (B). Legend: BIV: 0: absence, 1: presence.

Table 1
Description of the GLZ model (binomial logit – multiple regression, with backward stepwise removal of independent variables).

Parameter	Wald Statistic	*p*
Intercept	+6.32	0.0119
Urban Index	+11.31	0.0008
Year	+7.29	0.0260

BIV infection. When plotting the NO_2 concentration against the UI, BIV positive samples were clearly enriched in the high UI and high NO_2 samples (Fig. 3B). Indeed, except for one, all samples from trees with an urban index ≥0.6 and an NO_2 level ≥30 ppb were infected with BIV.

Trees whose pollen had tested positive for both viruses in the PCR (CLRV+ and BIV+; "double positive") were significantly more likely to be located at a site with higher urban index than trees whose pollen had tested positive for only one of the two viruses (CLRV + or BIV+; "single-positive") ($p < 0.001$; Mann-Whitney test). As in our cohort were only three samples that had been double-negative (CLRV- and BIV-) by PCR in all years, this group could not be tested (Fig. 4).

Finally, we mapped the 18 common trees with respect to their environment, i.e., the vicinity to high-traffic roads, which may be an indicator of elevated NO_2 levels. Fig. 5 shows the distribution of the birch trees under study, categorised based on the urban index of the locality they grow and their vicinity (or not) to main roads is also depicted. In Fig. 5, six out of 10 trees (white circles) in the 'urban-high traffic category' exhibited UI > 0.75, and these specific trees were also located in high-speed/high-traffic roads and/or in crossroads (distance to roadside <10 m). In contrast, seven out of the 8 trees (black triangles) were located in large rural or forest areas, with a proximity less than 500 m in all cases.

White circles: Trees very close to high-traffic roads (<10 m) and/or in the city center or city areas with high levels of sealing. Black triangles: Trees away from high-traffic roads and/or outside city center, in close proximity to forest areas or within parks.

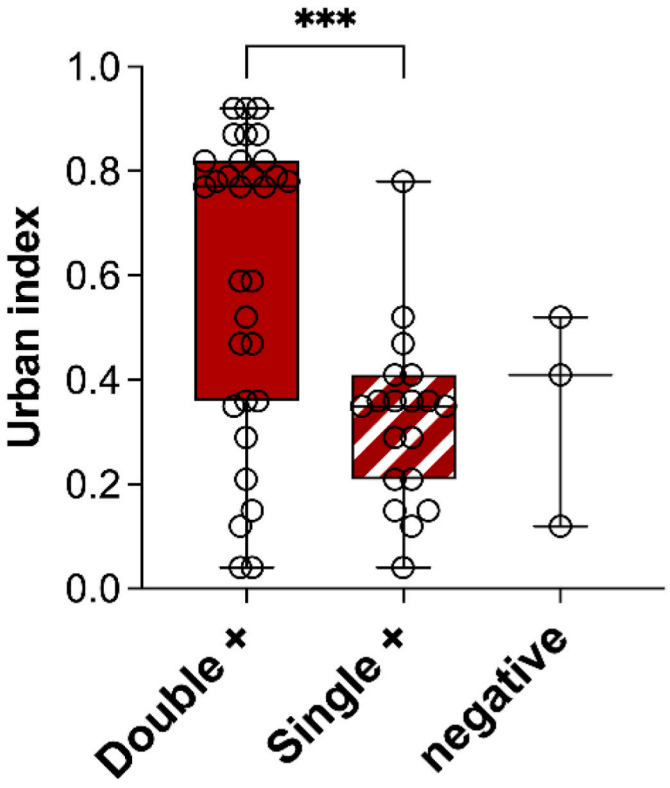

Fig. 4. Urban index of trees with double, single and no infection. Double + pollen samples (CLRV$^+$ and BIV$^+$) were associated with a higher urban index of the respective tree than single + samples (CLRV$^+$ or BIV$^+$) or pollen samples which were negative for both viruses. ***: $p < 0.001$, Mann Whitney test.

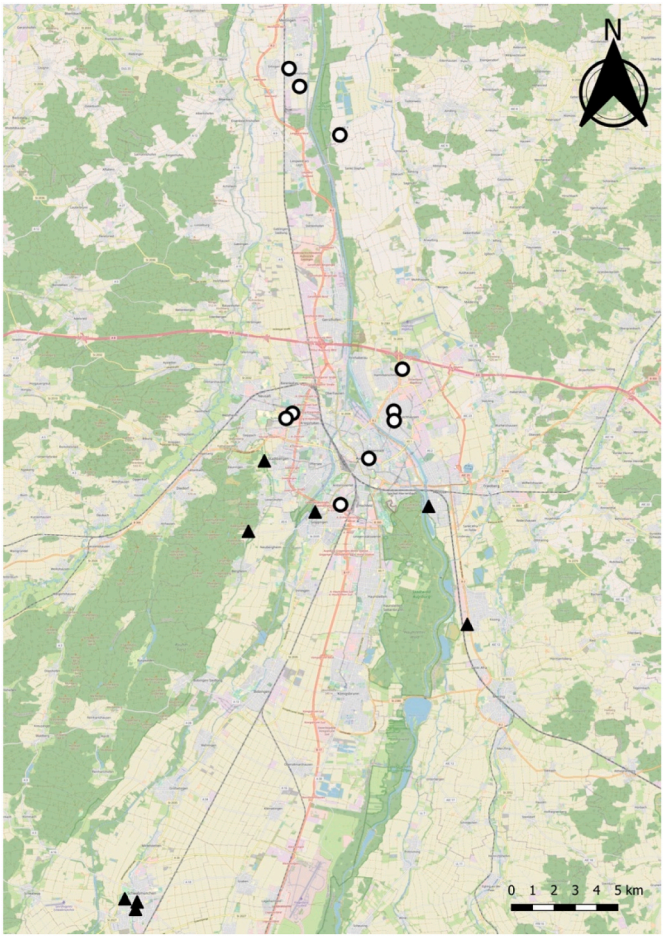

Fig. 5. OpenStreetMap of birch trees in relation to the urban index and their vicinity to main roads in the region of Augsburg.

A probit GLZ model was run separately on the two groups, again with BIV occurrence as output variable. Fig. 6 shows a surface plot of BIV occurrence versus urban index and NO_2 for the "urban" trees. According to the model, there was a significant positive correlation between BIV occurrence and both, UI and NO_2 level ($R^2 = 0.33$; $p < 0.001$). No significance was found for the rest of the trees labelled as "not urban", i.e., trees away from roads, in rural areas, or both. As seen in Fig. 6, for the study area, this threshold seems to be more than approx. 30 ppb NO_2 and more than 70% of the urban index.

4. Discussion

In the present study, we generated data on the distribution of two plant viruses infecting birch in urban and rural populations, the well-characterised CLRV and the recently discovered BIV. Our key finding is that urban index and NO_2 levels, in combination with close (<10 m) proximity to roadside, were associated with higher occurrence of the novel BIV in the pollen samples of the trees.

In general, both viruses were widely distributed within pollen samples obtained from the studied birch population. The high incidence of CLRV in urban birch trees is not surprising. Landgraf et al. (2016) had previously confirmed CLRV in 63 out of 73 samples of symptomatic leaves from birch trees in Berlin. This is the first comprehensive study about prevalence and abundance of the novel idaeovirus BIV in birch pollen samples. One explanation for the wide distribution of both viruses

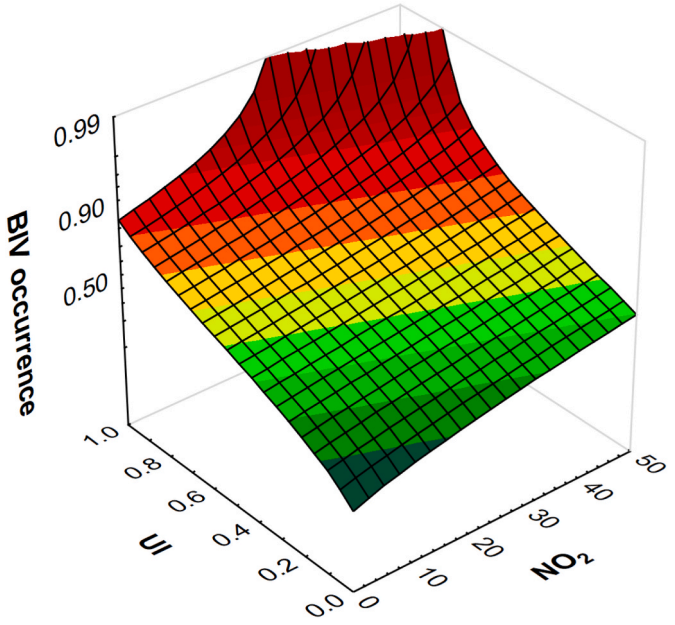

Fig. 6. Surface plot (logistic regression) of BIV occurrence against urban index (UI) and NO_2 levels. Results shown for the birch trees in urban environment or close vicinity to major roads in the study area, or both ($R^2 = 0.33$; $p < 0.001$).

in the samples examined in this study could be horizontal transmission. It is known that CLRV is horizontally and vertically pollen-transmitted (Cooper et al., 1984; Massalski and Cooper, 1984). The same has to be assumed for BIV, since pollen transmission had previously been observed for the related Idaeovirus -RBDV (Converse, 1991; MacLeod et al., 2004). Some viruses are known to increase the replication of unrelated viruses in a mixed infection (Xu et al., 2022). A similar mechanism could explain the high degree of co-detection of CLRV and BIV in our samples.

While a high occurrence of both CLRV and BIV was observed, the distribution of both viruses differed within the investigated population, depending on the tree's location and the year of sampling. A significantly higher urban index was associated with trees whose pollen were infected with both viruses than with trees whose pollen only carried one of the two viruses. The trees whose pollen were uninfected by either of the two viruses were mostly located in rural areas.

A higher urban index means that a specific site has a high degree of sealed surfaces, which is usually accompanied by increased temperatures, decreased water and nutrient availability (Armson et al., 2013), and higher pollution of air and soil (Gregg et al., 2003). Only the pollen from three trees were tested negative for both viruses in all studied years, and all those trees were located in natural or agricultural environments with low urban index, illustrating the high impact of urbanisation on the susceptibility of birch trees to viral infection. The microclimate around a tree is expected have an added impact on the tree's susceptibility and responsive ability to infection, compared to the larger scale of 2 km used for the calculation of the urban index. The calculation of this index on a smaller radius could be useful. However, such low-scale data would also result in higher statistical errors and therefore require larger sample sizes.

It is well known that many air pollutants, especially NOx, are more abundant in urban than in rural areas. In contrast, ozone (O_3) concentrations are typically higher in rural areas, a phenomenon called 'ozone paradox' (Bocci et al., 2009): in cities, sunlight and high air temperatures induce photolysis of NO_2 to form O_3 (NO_2 + UV-A + heat → NO + O; O + O_2 → O_3). On the other hand, O_3 then readily reacts again with NO (O_3 + NO → NO_2 + O_2), thus, tropospheric ozone is constantly turned over. In rural areas, O_3 is formed under low NOx concentrations, which is why it can accumulate to higher levels than in the cities (Sillman, 1999). Air pollutants such as O_3 have been discussed to play a crucial role in the "disease triangle" of environment, pathogen and host susceptibility (Chappelka and Grulke, 2015). Although in the present study, O_3 levels showed a year-to-year variation pattern similar to that of BIV, O_3 did not turn out as significant predictor for BIV infection in the multiple logistic regression model. Instead, we observed that the levels of NO_2 measured at the trees was associated with BIV infection of the pollen. For CLRV, the model did not run due to the low numbers of CLRV-negative trees.

Although the urban index resulted as the only statistically significant predictor in the multiple logistic regression, the urban index by definition incorporates a variety of parameters, including, but not limited to, air temperature and NO_2. From Fig. 6 (and in combination with Figs. 3 and 5), it is evident that UI and NO_2 appear to have a synergistic effect and the combination of both, and only this, contributes to the higher occurrence of BIV, namely in more urban localities, with higher NO_2 and most frequently close to major roads. Proximity to roadside (<10 m distance) was a key factor in our analysis. This might be explained by the fact that a semi-urban tree located very close to a busy road might have a low UI but still be exposed to comparatively high air pollution levels. In contrast, a tree in an urban park or garden growing more distant to the nearest roadside might be less exposed, although with a similar UI. Undoubtedly, further research is need on the subject, if one wishes to differentiate the isolated impacts and their magnitudes of effect.

The frequent detection of both viruses in urban pollen samples could also reflect higher virus titers in urban samples. In this respect, the documentation of the tree's symptomatic appearance, like chlorotic or necrotic alterations on leaves or bald branches, could be helpful in the future. However, symptom manifestations caused by the presence of other pathogens or insects can easily be confused with symptoms of viral infections, particularly during later stages in the vegetation period (Büttner et al., 2013, 2023). Mixed infections with several viruses could also occur frequently.

Finally, the global commercial trade with plants and seeds contributes to the spread of viral diseases, e.g., via insufficient disinfection of tools used for tree management in urban areas (Bandte et al., 2022; Büttner et al., 2023). It is possible that many of the trees in which CLRV and/or BIV was detected originate from the same nursery. In this case, those trees could have been already infected with the viruses before being planted in their urban locations.

While the results of the study here provide quite clear evidence on the environmental factors affecting the occurrence of plant viruses, there are certain limitations. Even though we originally sampled an almost triple number of trees, the final sample size is comparatively small. Also, viral infection was focused only on pollen samples, and it cannot be ruled out that other tree organs may also be infected. On the other hand, whenever a virus was not detected in a sample, this might either indicate that the tree was not infected, or that there was a low virus titer in the sample. The PCR systems used in this work were not yet optimized for sensitivity and no lower detection limit is known. Thus, the data on infection as assessed in this work is qualitative-only. Another limitation of the study is lack of data on other environmental factors that might explain the high BIV occurrence in urban trees, such as soil compaction, water availability, etc.

The high incidence of the two different viruses in urban trees, investigated in this study, in addition to the urbanisation-associated abiotic stress, could carry the risk of eventual loss of those trees. Urban green spaces fulfill a wide range of services. They contribute to the cooling and shading of urban areas and increase the quality of life as well as the biodiversity (Millenium Ecosystem Assessment, 2005). Urban plants can filter CO_2 and particulate matter from the air (Bolund and Hunhammar, 1999; Strohbach and Haase, 2012), reduce noise (Nowak and Dwyer, 2007) and increase water drainage and humidification (Moser et al., 2017). All these beneficial effects will gain importance with respect to climate change, which underlines the importance of

protective measures for urban trees against diseases, including viral diseases like CLRV and BIV infection. Preventive measures in tree nurseries are essential to avoid the distribution of infected cuttings or seedlings.

5. Conclusion

Under the ongoing climate change, emerging plant pathogens and their interaction with human-health-related (bio)aerosols may pose a new threat for both the environment and human. While the approach of One Health has gained momentum recently, there are still huge gaps in multi-exposome and quantified impacts of human to environment and vice versa. As direct conclusion from our study, we suggest that more attention should be paid to the occurrence and spread of microbes in urban tree populations. Virus infection should be controlled by enforcing protective interventions, e.g. the reduction of traffic-related air pollution in urban areas.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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