K and Ca concentrations in leaves correlate with ASaV infection in climate change trees (*Fraxinus ornus*) of Hamburg

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Climate change trees (CCT) can represent a valuable and sustainable strategy for addressing the effects of climate change, such as prolonged drought and heat. These are mostly non-native trees whit expected qualities of adaptability used to replace less resilient and susceptible local species. However, the possibility for CCT of getting infections, including viruses, in the new hosting environment should be carefully taken into account. Among the ascertained symptoms, viruses can cause modifications of mineral elements homeostasis in plants, both at the cell and tissue level. Leaves are particularly sensitive to viruses and other biotic stresses, showing increase or decrease of certain elements' concentrations or uneven distributions. Such phenomena can be ascribed to the pathogen's activity and/or to the plant physiological response to the pathogen itself.

Flowering ash (*Fraxinus ornus* L.) has been recently introduced as CCT in the city of Hamburg, Germany. Unfortunately, many of the trees showed the typical symptoms of ash shoestring-associated virus (ASaV), i.e. leaf deformation, shoestrings and spotting.

In this study, leaves from both healthy and ASaV infected (ASaV+, verified by RT-PCR) Fraxinus trees have been sampled for three consecutive years (2019-21) and analyzed with an X-ray fluorescence (XRF) based approach. In particular, μXRF, using both laboratory and synchrotron (SR) X-ray sources, was used to visualize elemental distributions in leaves and identify elements correlations. Compared to healthy samples, ASaV+ leaves showed inhomogeneous distribution and severe depletions of P, S, and Ca in certain regions of the lamina. K instead appeared more concentrated.

Furthermore, SR μXRF allowed to appraise variations in some micronutrients distribution: while Mn increased in the lamina of ASaV+ leaf, Zn decreased. In healthy samples, Ca was more abundant than K; the latter was conversely more concentrated in ASaV+ samples.

Based on these results, 69 healthy and 70 ASaV+ samples were analyzed with a portable XRF (pXRF) instrument, and K and Ca concentrations were quantified: the K/Ca ratio was significantly higher in infected than in healthy samples. It was therefore observed that the K/Ca ratio allowed to correctly classify most of the infected samples, which implies that this parameter could be used, together with visual evaluation of symptoms, for a rapid, non-destructive and cheap indirect pathogen detection.

Such approach using pXRF could be applied also to other types of plants and pathogens thus offering an innovative, fast and in-situ tool for the early detection of plant diseases.