	Experiment title: Elucidating Cu-based nanoparticles' fate on and in grapevines' leaves for the rational design of more efficient Cu-fungicides.	**Experiment number**: ES1237
Beamline: ID21	**Date of experiment**: from: 12/04/2023 to: 18/04/2023	**Date of report**: 24/06/2023
Shifts: 12	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report:

The goal of this experiment was to understand the behavior of nanoparticulated forms of copper oxide (CuONPs) when deposited on grapevine leaves, and assess the Cu speciation in different plant compartments, namely leaves and petiole. In particular, we aimed at understanding the role of the nano-formulation (CuONPs *vs* ionic control, $CuSO_4$) and the chitosan-BSA capsule (CuONPs *vs* Chi-CuONPs) in the dissolution and translocation of these materials on and in the leaf, at two different collection times (7 *vs* 26 days).

For this, grapevines germinated and grown under laboratory conditions were exposed to the copper treatments, namely CuO-NPs with and without a chitosan-BSA capsule, and $CuSO_4$ for 7- and 26-days prior to the beamtime analysis. The treatments were dropped deposited in the abaxial side of the leaves, at a concentration of 0.11 g/L, in a total volume of 120 µL and 13.2 µg of copper, per leaf.

The exposed leaves were harvested and cut to obtain a 0.28 cm^2 sample containing the whole Cu-exposed area. The leaf disks were then embedded in OCT resin, flash frozen in liquid nitrogen, and shipped to the ESRF facilities in a travel dewar. At the ESRF facilities, the cryo-conserved samples were vertically cross-sectioned (20 µm thick) by Hiram Castillo-Michel using the cryomicrotome at ID-21 and loaded in the cryo-holder of the beamline. The samples were then analyzed in the beam ID-21 at Cu K-edge (8.98 keV).

Ionic copper was found in the abaxial side of plants that were exposed to $CuSO_4$, the ionic control, at both collection points. In the 7-days Cu exposed plants, ionic Cu was also found in the vasculature. These results suggest that a portion of the added $CuSO_4$ may be taken up and translocated into mesophyll while another portion remains on the leaf surface, within the 26-days exposure period (Fig. 1A). Chi-CuONPs were found only at the exposed leaf side, abaxial side, at both collection times, suggesting that this Cu treatment allows

higher retention in the leaf, thus decreasing Cu uptake and translocation to non-exposed tissues (Fig. 1B). In contrast, CuO-NPs exposed leaves showed that the Cu remained on the applied leaf surface (abaxial side) after 7-days exposure. While after 26-days, the Cu was found on the opposite side, namely de adaxial surface (Fig. 1C). No copper was found in the non-Cu control (Fig. 1D). Preliminary data treatment of the µ-XANES spectra performed in the experiment seems to indicate that this is Cu bound to some organic ligands. These results suggest that the CuO-NPs enter the leaf and were exudated to the opposite site within 26 days-exposure period.

These results are relevant for the application of Cu-based nano-fungicides in the viticulture industry as there are still some concerns in the implementation of NPs based treatments as fungicides. With these results we were able to observe that the chitosan encapsulation Cu retention on the applied area for up to 26-days, demonstrating that the capsule played a role in the retention of the Cu in the intended exposed area. Moreover, this knowledge will help to design Cu-based nano-fungicides that can more efficiently protect grapevines from specific pathogens. In the case of downy mildew, Chi-CuONPs look like a promising solution due to the lower surface leaf retention.

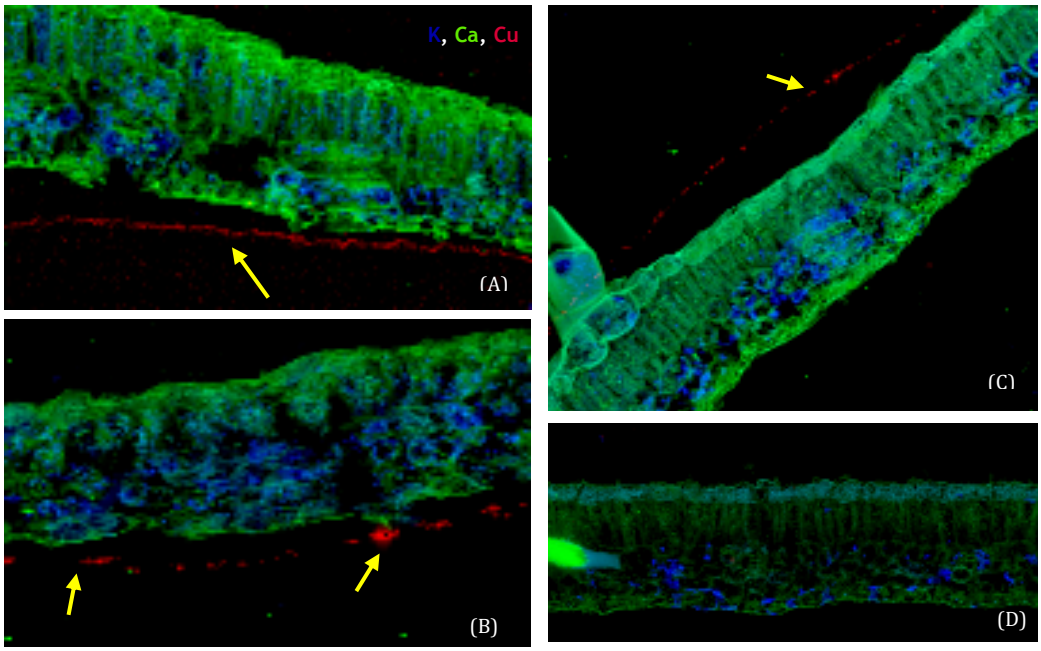

Fig. 1 – Leaf cross-section of germinated grapevine exposed to $CuSO_4$ (A), CuO/BSA/Chi-NPs (B), CuO-NPs (C), and MQ control (D) from data set obtained for the 1-month exposure period. Yellow arrows point at the copper.