Calcium phosphate nanoparticles doped with copper ions as efficient tools for downy mildew prevention

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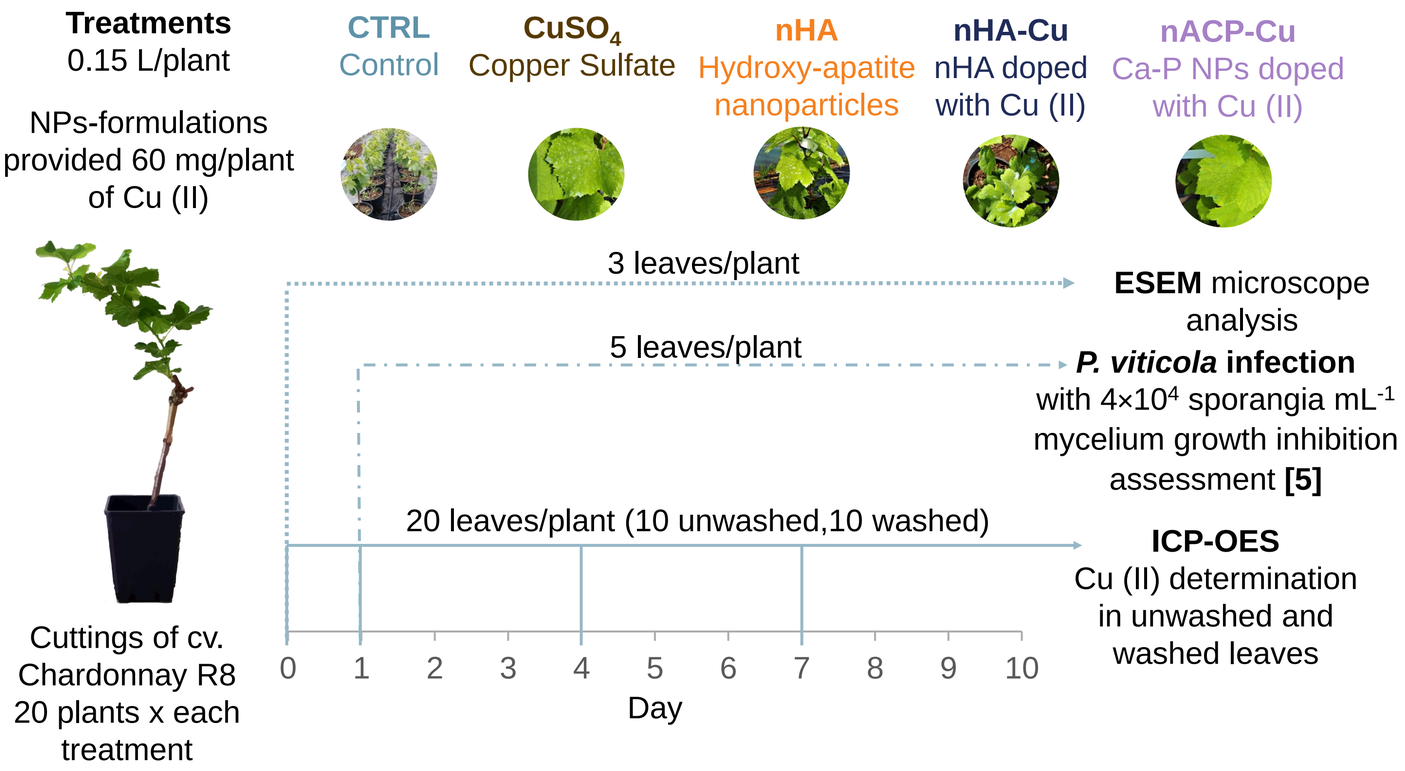
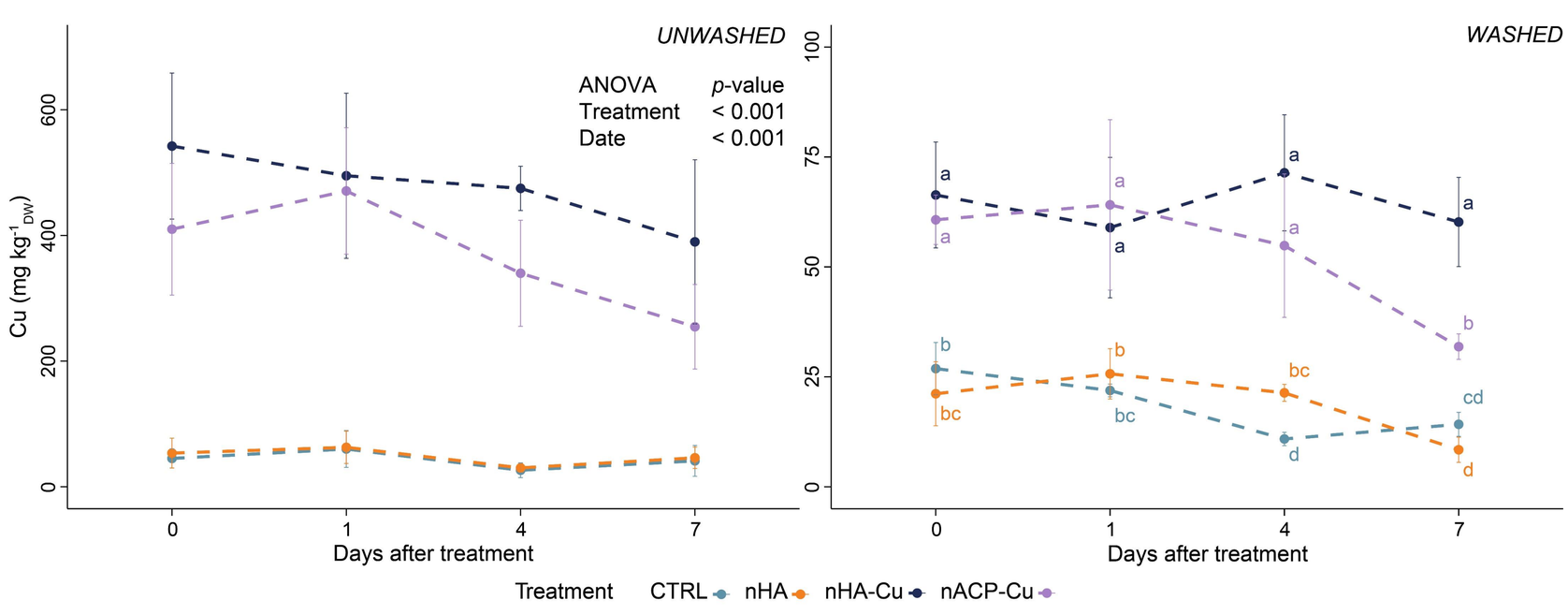
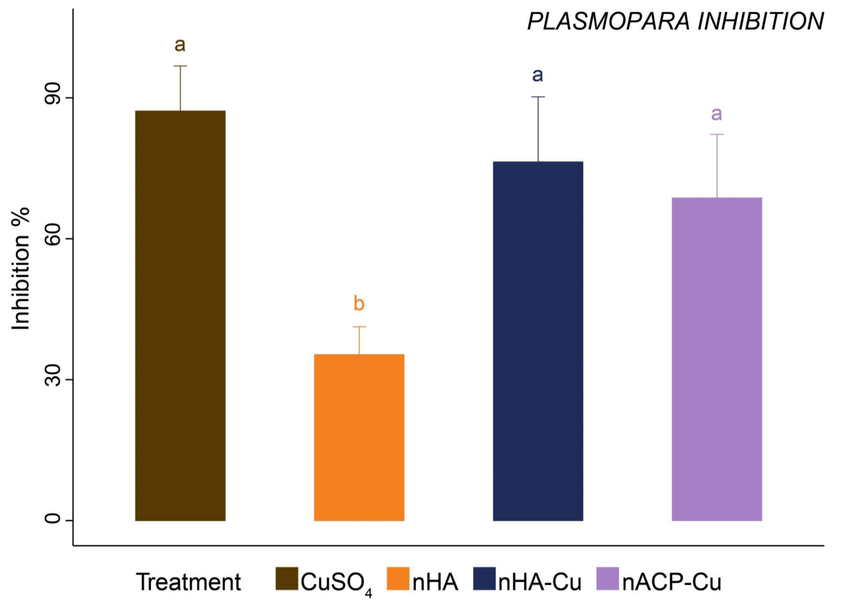
INTRODUCTION Intensive application of agro-chemicals causes an increasing environmental impact in agriculture, especially in high-income crops such as grapevine. Their delivery, however, is highly inefficient, since a considerable amount is leached in the environment **[1]**. For this reason, nanotechnology could represent a sustainable practice able to improve the efficacy of application of agro-chemicals by increasing their permanence on the canopy **[2]**. In vineyard, copper compounds are intensively used as antifungal pesticides, with strict limitations in their application **[3, 4]**. Thus, in the present work, two types of Cu (II)-doped calcium phosphate NPs were applied at low dose on *Vitis vinifera* L. and their efficacy against downy mildew (*Plasmopara viticola*) was compared to that of conventional copper-sulfate treatment.

EXPERIMENTAL DESIGN

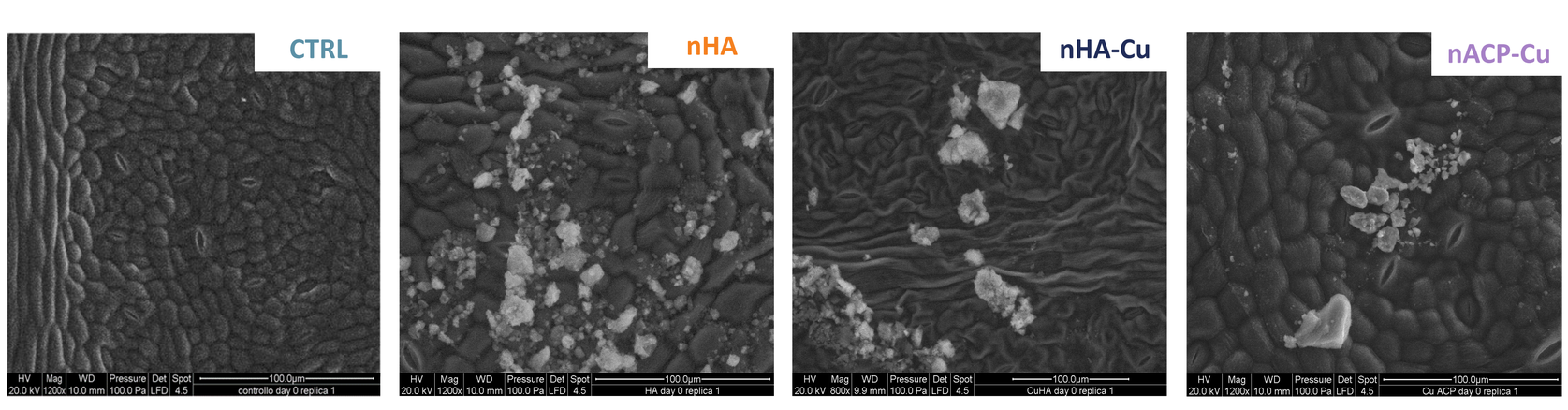






RESULTS AND DISCUSSION The profile of the total concentration of Cu (II) in **unwashed leaves** and that of absorbed Cu (II) (**washed leaves**) was followed for one week. As expected, the application of **nHA-Cu** and **nACP-Cu** caused a significant increase (~11 and 8.6 times, respectively) in the average foliar concentration of Cu (II) compared to **CTRL** or **nHA** alone. Both in **unwashed** and **washed** leaves treated by Cu-functionalized nanoparticles, there was a progressive decrease in Cu (II) concentration over time. It was more evident in **nACP-Cu** treatment, whose pattern was significantly lower if compared to **nHA-Cu** (p<0.001). Regarding the **washed leaves**, a significant decrease occurred during the terminal phases of the experiment in the **nACP-Cu** thesis, if compared to **nHA-Cu**, where Cu (II) concentration remained constant. This fact supports the hypothesis that the **nHA-Cu** was able to maintain a more stable and long-lasting amount of Cu (II) in comparison to the **nACP-Cu** formulation, where Cu (II) is more soluble.

Both Cu-doped formulations were able to prevent *P. viticola* infection by more than 60%, showing no statistically difference with **$CuSO_4$** treatment, even if Cu (II) concentration after its application was 3 times higher (1381.9 ± 512.1 mg kg_{DW}^{-1}). In case of a technical improvement in functionalization, this result opens the possibility of complying with EU regulations by administering low dosage of Cu (II) associated to a nanomaterial. The inhibition was significantly lower in the absence of Cu, as confirmed by treatment with **nHA**.



ESEM analysis showed the presence of Ca-P NPs in aggregates of different sizes (in the range of 0.7-30 µm) in all treatments, except in the control and in **$CuSO_4$** (not shown). The resolution level obtained in low vacuum mode may not have allowed the detection of smaller particles of nanometric size.

CONCLUSION The application of calcium phosphate based nanomaterials doped with Cu (II) is efficient to inhibit the propagation of *P. viticola* at an extent comparable to **$CuSO_4$**, even though the nanomaterial showed a yield distribution in copper of 30% if compared to the conventional treatment. The improvement of the agronomic distribution technique is clearly required. In addition, the results provide evidence that calcium phosphates functionalization allows to deliver ionic elements both on grapevine canopy and at mesophyll level. According to an improvement of the method for the application of Calcium-Phosphate nanoparticles, this technical solution will be promising as a low environmental impact treatment.

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