OZONE TREATMENT: A SOLUTION TO IMPROVE SANITARY AND PHYSIOLOGICAL QUALITY OF VINE PLANT.

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Abstract:

Context and purpose of the study - The vineyard world is faced to a lot of fungal diseases. Grapevine Trunk Diseases (GTD) are some of the major. After exhibiting chronical foliar symptoms, grapevines can die by apoplexy within only few days. A range species of fungi was described to be associated with the apparition of early symptoms of GTD. It is well known that ozone dissolved into water is a powerful disinfectant with no remanence. The main goal of this study was to test the efficiency of this process on different fungal species associated with GTD *in vitro* and *in planta* conditions.

Material and methods – *In vitro sanitary tests:* Eighteen strains of two different species associated with GTD were selected (*Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*). Ozone dissolved into water (4.5 $g.m^{-3}$ according the Henry's law) or autoclaved demineralized water (control) were applied on spore suspensions from strains. Suspensions were then plated on agar medium. Germinating spores were observed after five days. *In vivo sanitary tests:*Cuttings of *Vitis vinifera* Cabernet-Sauvignon clone 15 were drilled until the vascular channels. In each injury, plants received 20 µL of spore suspension (10^5 $spores.mL^{-1}$) of *P. minimum*. Immediately after inoculation, infected wounded damages were treated with 20 µL of ozone dissolved into water (4.5 $g.m^{-3}$ according the Henry's law). The fungal development was evaluated 4, 6 and 9 weeks after inoculation by q-PCR.

Results – *In vitro sanitary tests*: Solution of ozone dissolved into water presented a complete sporicide effect. Indeed, no spore germinated in ozonated treatments whereas water treated controls normally developed. *In vivo sanitary tests:*The anti-fungal abilities of ozone treatment were secondly assessed by quantification of *P. minimum* DNA in woody tissues (via qPCR). Four and six weeks after inoculation, ozone treatment strongly reduced the source of inoculum present in the injury, resulting in more of 50% decrease of the number of *P. minimum* copies per ng of total. After nine weeks, the quantity of DNA is more important in ozone modality than in control modality. These results suggest that ozone treatment slowed down the fungal colonization *via* its primary sporicide effect in cutting-wounding conditions. Finally, consequences on the physiological aspect of the plant after ozone treatment should be discussed during the congress thanks to some news results.

Keywords: Grapevine, Fungi, Ozone, Disinfection, Growth.

1. Introduction.

Ozone treatment: A solution to improve sanitary and physiological quality of vine plant

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Context and purpose of the study

The vineyard world faces to a lot of fungal diseases. Grapevine Trunk Diseases (GTD) are some of the major. A range species of fungi was described to be associated with GTD. It is well known that ozone dissolved into water is a powerful disinfectant with no remanence. The **main goal** of this study was **to test the efficiency of ozonated water on different fungal species associated with GTD *in vitro* and *in planta* conditions.**

Materials and methods

In vitro sanitary tests: Eighteen strains of two different species associated with GTD were selected (*Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*). Ozone dissolved into water (4.5 $g.m^{-3}$ according the Henry's law) or autoclaved demineralized water (control) were applied on spore suspensions from strains. Suspensions were then plated on agar medium. Germinating spores were observed after five days.
In vivo sanitary tests: Cuttings of *Vitis vinifera* Cabernet-Sauvignon clone 15 were drilled until the vascular channels. In each injury, plants received 20 µL of spore suspension (10^5 $spores.mL^{-1}$) of *P. minimum or P. chlamydospora*. Immediately after inoculation, infected wounded damages were treated with 20 µL of ozone dissolved into water (4.5 $g.m^{-3}$). The fungal development was evaluated 4, 6 and 9 weeks after inoculation by q-PCR.
Irrigation tests: Non-infected cuttings were irrigated with ozonated water (4.5 $g.m^{-3}$) or demineralized water (control) in order to follow their growth and development.

Results

In vitro spore germination test (P. min)

Ozonated water has a powerful sporicidal effect.

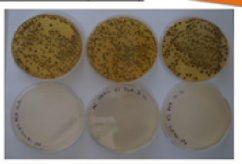

Fig. 1

Solution of ozone dissolved into water presented a **complete sporicide effect**. Indeed, no spore of *P.min* (**Fig. 1**) and *P.ch* germinated in ozonated treatments, either for, whereas water treated controls normally developed.

In vivo quantification of fungal development (P. min)

Ozonated water delayed fungal development.

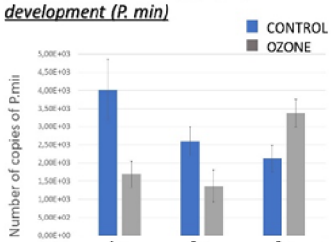

Fig. 2

Ozone treatment **strongly reduced the source of inoculum** present in the injury, resulting in more of 50% decrease of the number of *P. minimum* (**Fig. 2**) and *P. chlamydospora* copies per ng of total. However, after nine weeks, we observed an increase in the number of copies in the ozone modality.

Bud release kinetics

Ozone in low concentrations might favor plant development.

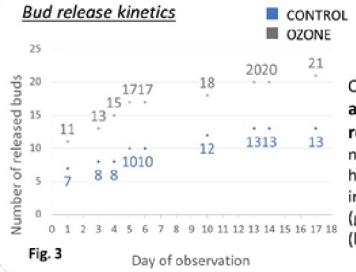

Fig. 3

Ozone treatment results in the **acceleration of the bud release process**, as the number of released buds is higher when plants are irrigated with ozonated water (grey) instead of normal water (blue) (**Fig. 3**).

Root development

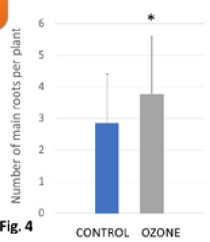

Fig. 4

The number of main roots formed is significantly higher in plants irrigated with ozonated water (grey) than control plants (blue) (**Fig. 4**). Therefore, **ozone might stimulate root growth**.

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