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Effects of nitric oxide on sunflower seedlings: A balance between defense and development

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Nitric oxide (NO) is a major plant signaling molecule that plays key roles during plant-pathogen interactions and plant development. Previous work showed the participation of NO in the development and lignin composition of sunflower roots. Thereby, we have hypothesized that NO applications could control the attack of the fungal pathogen *Verticillium dahliae* in sunflowers. Seedlings growing hydroponically were pretreated with NO donors and further inoculated with the fungus. Evaluation of disease symptoms showed that NO pretreatments could not reduce Verticillium wilt. Strikingly, NO donors appear to promote the fungal infection. These results indicate that NO applications were unable to protect sunflowers from *Verticillium* attack and highlight the role played by the fine tuning regulation of NO levels required to balance plant responses between development and defense.

Keywords: fungal control, *Helianthus annuus*, lignin, nitric oxide

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Nitric oxide (NO) is a diffusible gas involved in many physiological processes related to plant growth and development.[1] We have recently reported that endogenous NO is involved in the definition of root architecture and lignin monomeric composition in sunflower.[2] Besides, NO has been shown to display key defensive roles during plant-pathogen interactions.[3-4] Since the first reports describing NO functions as a signal molecule in plant disease resistance[5] and its participation in the hypersensitive response,[6] several lines of evidence have reinforced the concept that NO can help plants to defend themselves from microbial invasions. The analyses performed in different plant species have focused on the defense contribution of a rapid NO burst induced by the attack of fungal pathogens.[7-10] However, to our knowledge, no evidence has been presented demonstrating that applications of NO could increase tolerance to a fungal attack *in planta*.

Verticillium dahliae is a soil-borne pathogen with a broad host range which infects susceptible plants principally through the root tip and enters the cortex tissues to move from base to apex along the vascular system.[11] Affected plants progressively wilt and eventually die. The multiplicity of NO actions involved in plant adaptive responses to environmental constraints[12] together with our demonstration of NO-dependent reprogramation of gene expression in sunflower roots including the induction of stress-responsive genes,[2] prompted us to hypothesize that NO applications could help to control the attack of *V. dahliae* in sunflower. To test this hypothesis sunflower seedlings growing hydroponically[2] were inoculated through the roots with *V. dahliae* conidia. The typical symptoms of wilting, chlorosis and necrosis were observed, with more intense signs in the lower leaves. In order to evaluate the ability of NO to reduce *Verticillium* attack 2 different NO donors were applied previous to the inoculation: sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO). Briefly, 3-days-old seedlings were treated for 2 d with 20 μM SNP or 50 μM GSNO in the hydroponic medium and then transferred to fresh milieu containing *V. dahliae* conidia. Visualization of the symptoms revealed that SNP- and GSNO-treated seedlings exhibited severe leaf wilting (**Fig. 1A**) and were more affected by *V. dahliae* than inoculated ones but non-pretreated with NO donors. Additionally, dose-response analyses showed that

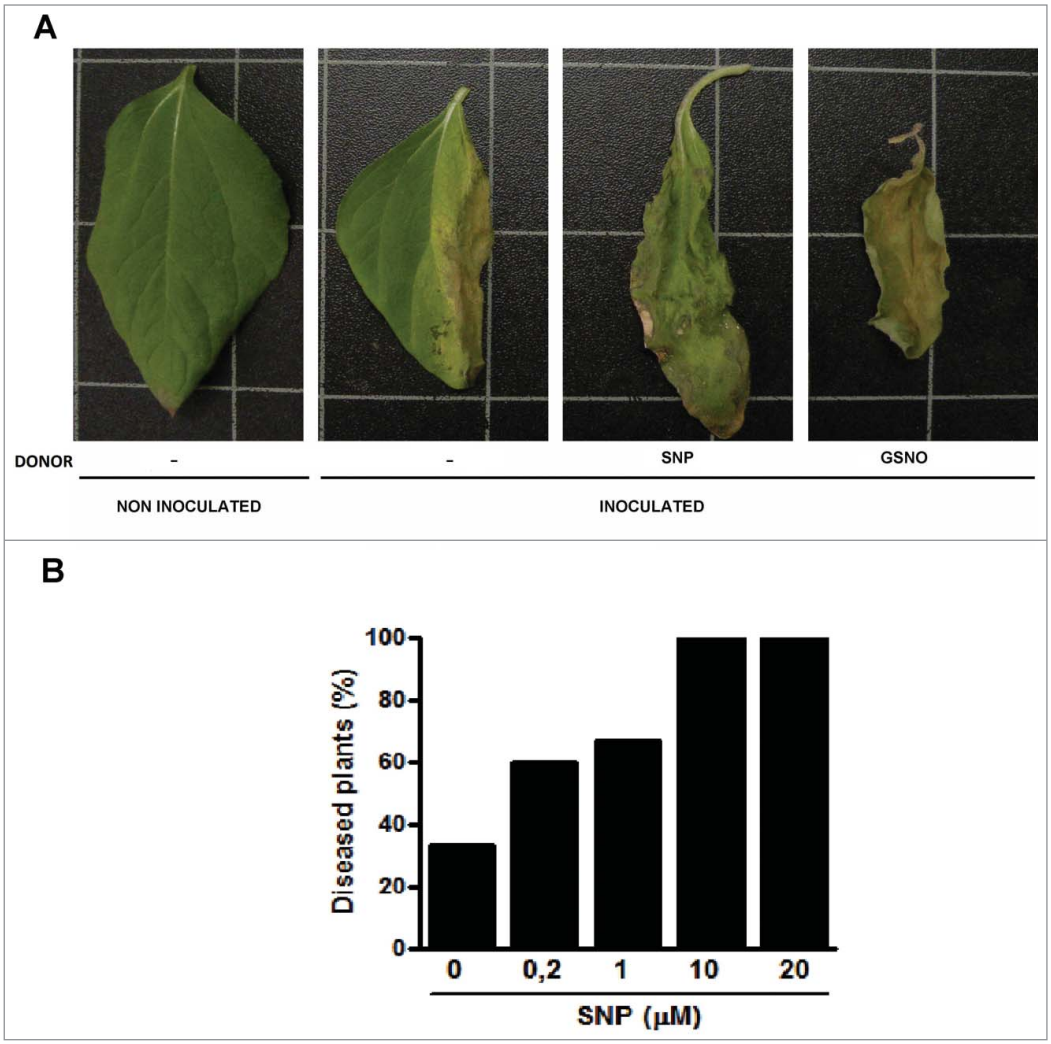

Figure 1. Effect of NO donors on the evolution of Verticillium wilt in sunflower (**A**) 3 days-old *Helianthus annuus* seedlings growing hydroponically were pretreated with 20 μM SNP, 50 μM GSNO or standard nutrient solution (control)[2] and further inoculated with *V. dahliae* by dipping the roots for 18 h in a suspension of 10^7 conidia/ml. Lower leaves were excised and photographed. Leaves from control non-inoculated plants are also shown. Leaves from plants treated with NO donors but non-inoculated present the same aspect as control ones. (**B**) Seedlings were pretreated for 2 d with different concentrations of SNP followed by inoculation with *V. dahliae*. Symptoms were evaluated 20 d after inoculation. Results of a typical experiment are shown ($n = 3$). *V. dahliae* strain America (Plant Pathology Laboratory, FCA-EEA INTA, Balcarce, Argentina) was kindly provided by G. Clemente.

increasing concentrations of SNP from 0.2 to 20 μM resulted in the elevation of the percentage of plants with disease symptoms (**Fig. 1B**). Overall, the results show that NO donor applications increased Verticillium wilt in sunflower.

Recently we have shown that depletion of NO in sunflower seedlings deeply affected root developmental responses and changed gene expression, particularly involving genes related to the lignin biosynthetic pathway.[2] A biochemical approach also showed a parallel change in lignin monomeric composition, increasing the G/S ratio.[2] Lignification is essential for the integrity of plant cell walls and changes in the composition of lignin have been shown to determine differential properties. Moreover, changing the lignin content and monomer composition and cross-linking to reinforce the cell wall is a well known response that plants adopt as an effective mechanism to restrict the pathogen entry and spread.[13] Xu et al.[14] showed that the lignin G/S ratio in a resistant cotton increased upon inoculation with *V. dahliae* while this ratio decreased in a susceptible variety. Those results confirm that increased lignification and cross-linking correlate with resistance to *Verticillium* disease and it could be reasonably assumed that the increased susceptibility of sunflower reported here may be a consequence of changes in lignin properties upon NO application. In conclusion, despite the large bulk of cellular-biochemical evidence suggesting that NO may be a booster of plant defense and adaptation, our results highlight that this molecule cannot be considered as a general protectant for fungal control *in planta* since many other aspects of the plant physiology are regulated by NO. The multiplicity of NO actions may determine different responses for specific pathosystems and an adequate balance between developmental and stress responses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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