

Title:

roGFP - Revealing robust anti-oxidant defences of a mycoparasitic *Trichoderma* species

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Abstract (300 words maximum): :

The fungal genus *Trichoderma* contains a vast array of species well known for their high opportunistic potential and adaptability to various ecological niches. The ability of many *Trichoderma* species to both colonize the rhizosphere and parasitize plant pathogenic fungi has led to their use in biological pathogen control for several decades. Reactive oxygen species (ROS) are linked to both the antagonism imposed by the mycoparasite *Trichoderma* and the elicited defence reaction by its fungal hosts during the mycoparasitic interaction. *Trichoderma* spp. likely tolerate higher levels of ROS compared with some of their host species, thereby giving them an advantage during the mycoparasitic interaction.

In the present study, we investigated glutathione redox dynamics using the fluorescent reporter Grx1-roGFP2 stably expressed in *Trichoderma asperellum* following electrotransformation. Grx1-roGFP2 undergoes reversible changes in its excitation spectrum in response to variations in the cellular glutathione redox potential, providing a real-time indication of intracellular oxidative load. Considering the putative importance of ROS in mycoparasitic interactions, we performed live-cell imaging of the *T. asperellum* reporter strain interacting with the cereal pathogen *Fusarium graminearum*. Surprisingly, the glutathione redox potential did not change during this mycoparasitic interaction. We found no evidence that host-induced tip growth arrest within *T. asperellum* hyphae is induced by intracellular ROS accumulation. Furthermore, we show that the *F. graminearum* mycotoxins deoxynivalenol and zearalenone do not induce detectable changes in glutathione redox potential, even at very high concentrations. We infer that *T. asperellum* has a robust anti-oxidant defence system, supported by the observation that high concentrations of H₂O₂ are required to fully oxidize the reporter during in vivo calibration. We cannot rule out a role for ROS as a signal during mycoparasitic interactions, but, if present, this does not appear to be mediated by glutathione redox potential.