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Increased metabolic fluxes in tomato stems infected by *Botrytis cinerea*

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Metabolic reprogramming is part of the plant's defence response following infection by necrotrophic fungi. However, the nature and intensity of metabolic changes contributing to resistance are not well understood. Flux balance analysis (FBA) predicts metabolic flux distributions at steady state, using a constraint-based model relying on a stoichiometric network and optimization with a defined objective function, which hypothesizes optimal biological functioning in this network (Orth et al. 2010, *Nature Biotechnology*, 28, 245–248). FBA was successfully used to study plants physiological behaviour (Colombié et al., 2015, *Plant Journal*, 81, 24-39), but has not yet been applied to plant-necrotrophic fungi interactions. We used nitrogen (N) nutrition to modulate the susceptibility of tomato stems to *Botrytis cinerea*, and examined at each N level the predicted metabolic fluxes in healthy stem tissues adjacent to lesions, in comparison with those of mock-inoculated plants.

The model encompasses 298 metabolic reactions, including the central metabolism, co-factors and redox homeostasis, and pathways towards main components of the biomass as well as main defence-associated secondary compounds. Quantitative metabolomics and other biochemical approaches were used to measure, before and 2, 4 and 7 days after inoculation, the concentrations of 46 “external” compounds, towards which fluxes were adjusted by derivation of their time-courses. These fluxes served as constraints in the model, which predicted the remaining 242 “internal” fluxes as well as C and N imports and gaseous exchanges, under the additional assumptions of internal steady state and the minimization of fluxes as an objective function. Radial growth was assessed by the measurement of stem diameters at different time points.

The model predicted increased fluxes in several pathways of *Botrytis*-inoculated plants in comparison to mock-inoculated ones, whatever the N level. This was driven by an enhanced growth after *B. cinerea* inoculation, which surprisingly increased with N deficit. When the fluxes were normalized according to C input, the model better highlighted distinct metabolic profiles according to the N regime, which could, at least partly, explain the observed range of susceptibilities. This approach provides new insights for the comprehension of resistance to *B. cinerea* in tomato stems, and its link with N availability.