

Figure 1S. Uncomparted bioassay setup: no contact between the plant roots and mycelium. The seeds of the test plants were placed 4.0 cm above the center of the mycelium plug and root morphology was monitored up to 15 days (A). Regardless of the growth rate of the different mycelial strains, the roots of Arabidopsis (A,B) or Cistus (C) did not enter in contact with the mycelium after a period of 15 days (a stereomicroscope was used to confirm that no hyphae reached the roots). δ = sum of the root length + fungal colony radius.

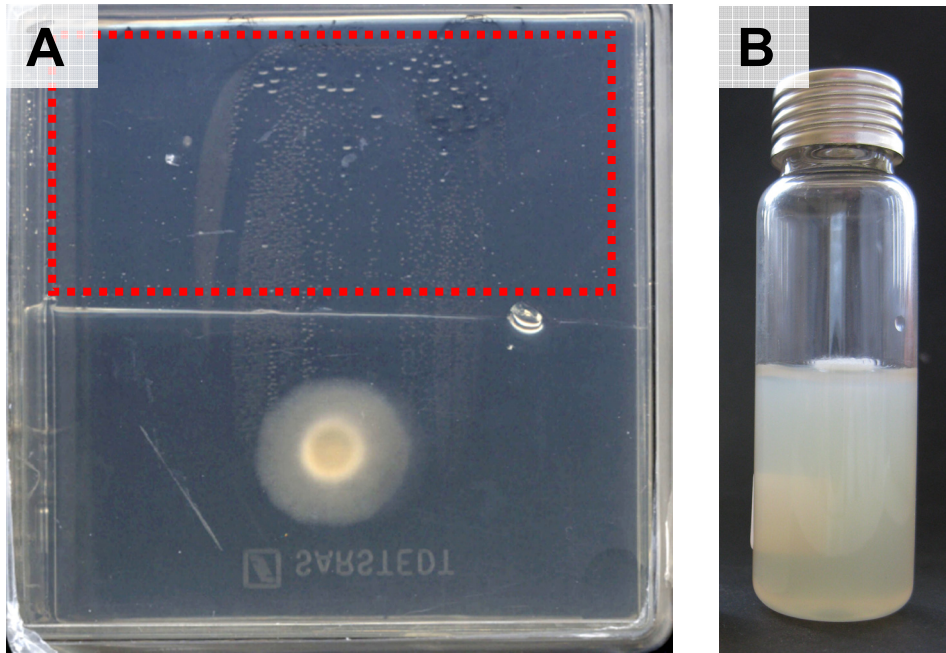


Figure 2S. Experimental setup for IAA and ethylene determination, Since both hormones can be produced by plants, the setup used to determine whether truffles produced IAA and ethylene excluded the test plants. IAA was measured from the MS agar portion of the Petri dish (dashed line ----) **(A)** of plates containing mycelium or mock inoculated with an agar plug. Ethylene was measured in the headspace of a 20 ml vial containing 10 ml agar inoculated either with the mycelium or a an agar plug **(B)**

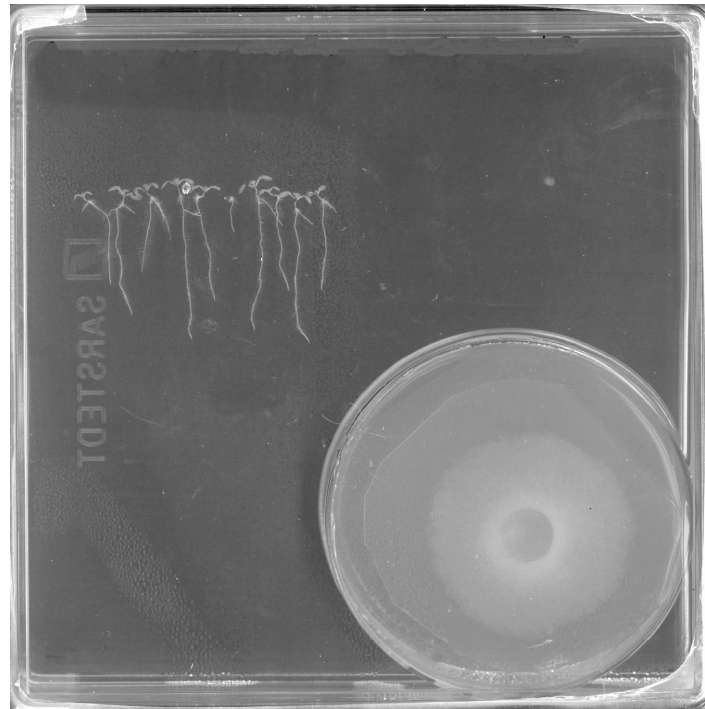


Figure 3S. Compartmented bioassay

Compartmented dual culture bioassay used to test the effect of truffle mycelial volatiles on plants. The small round Petri dishes were filled with malt extract agar containing different concentrations of the ethylene precursor L-methionine (0 mM, 1 mM and 10 mM) and inoculated either with truffle mycelium or mock inoculated with an agar plug. The bioassay was done in the growth chamber at 20 ± 1 °C with a 16 h photoperiod

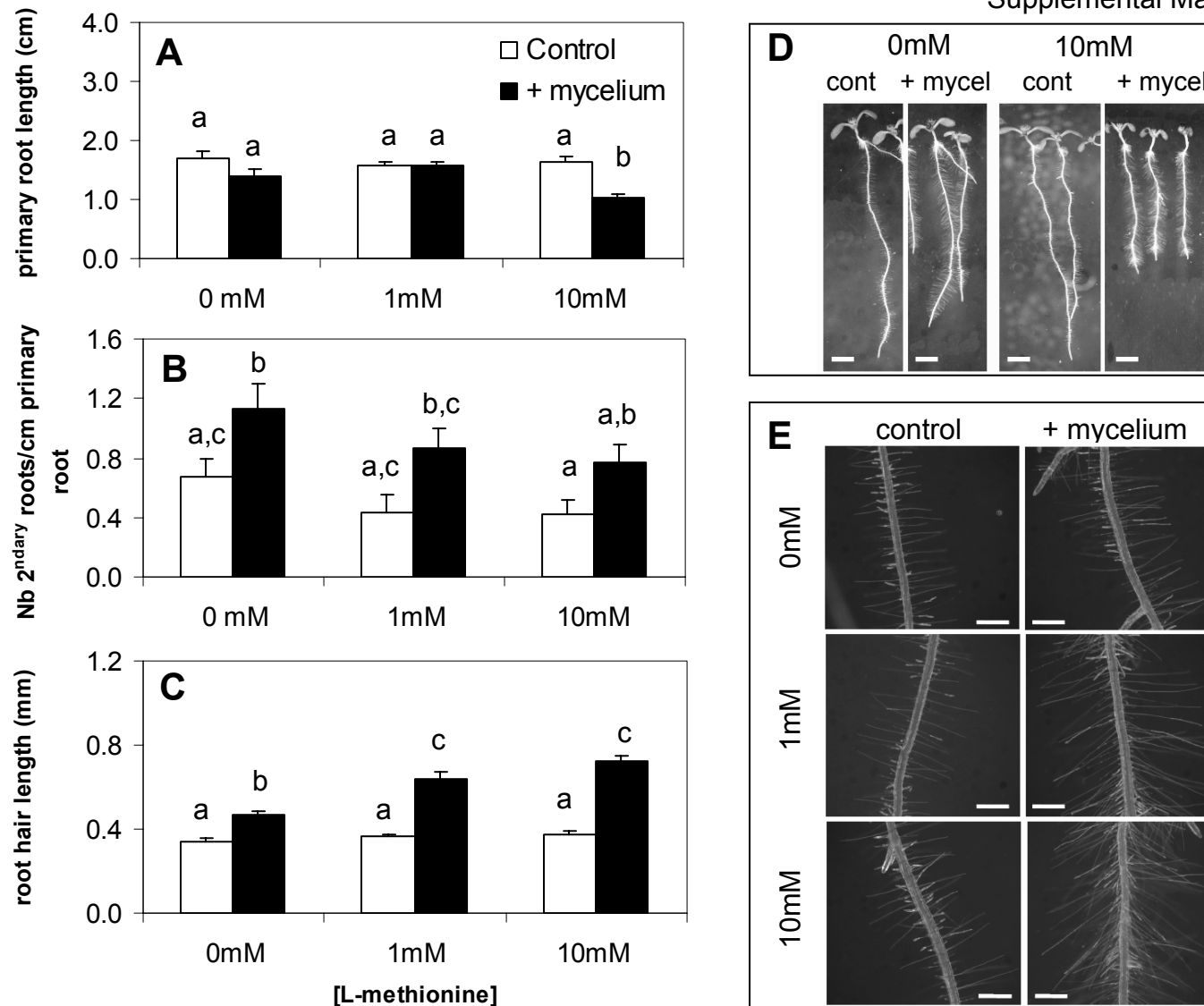


Figure 4S. Effect of mycelial volatiles on *A. thaliana*.

Refer to the bioassay setup in Fig.1S. Volatiles released by the mycelium of *T. borchii* (strain 1) supplied with 0, 1 and 10mM L-methionine modified primary root length (**A, D**), branching (**B**) and root hair length (**C, E**) depending on the methionine concentration. Values are shown with std. errors (**A, B, C**) and are the average of $n > 20$ seedlings. Different letters indicate statistically different values ($P < 0.05$, ANOVA on ranks and post-hoc Dunn's test). Scales D = 1.0 mm; E = 0.25 mm.

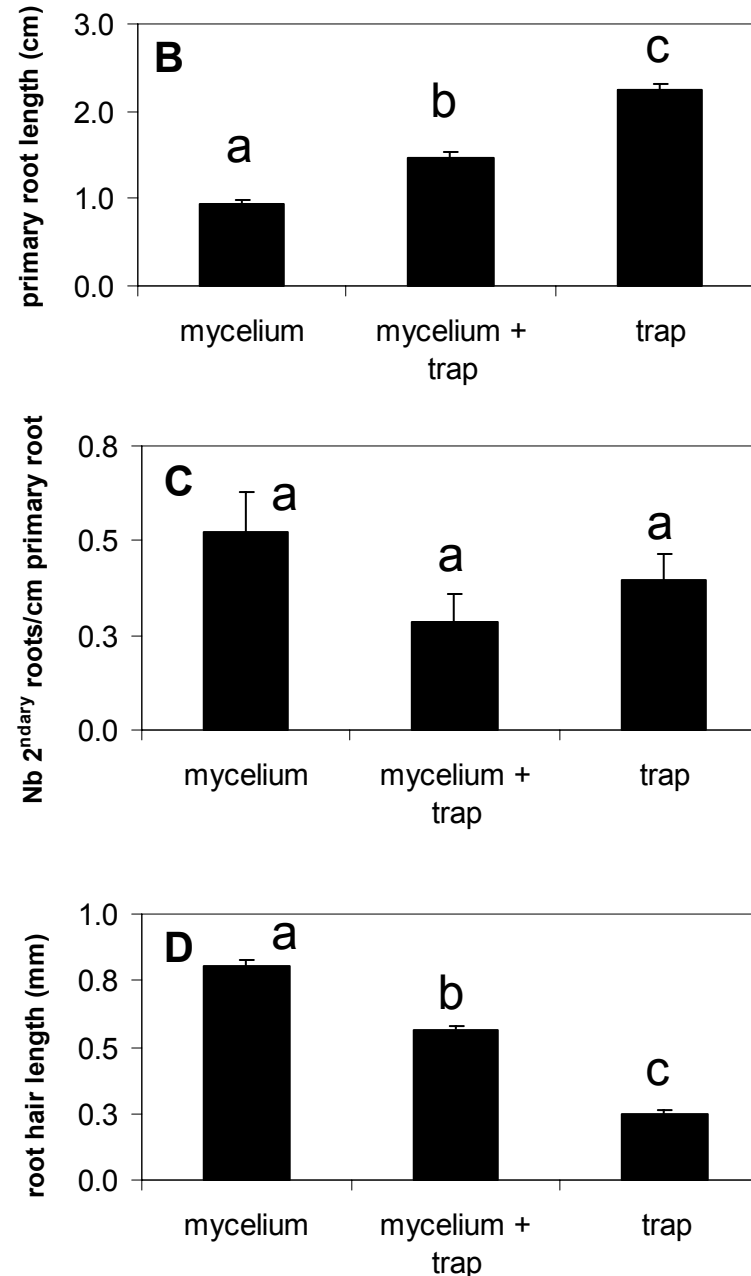
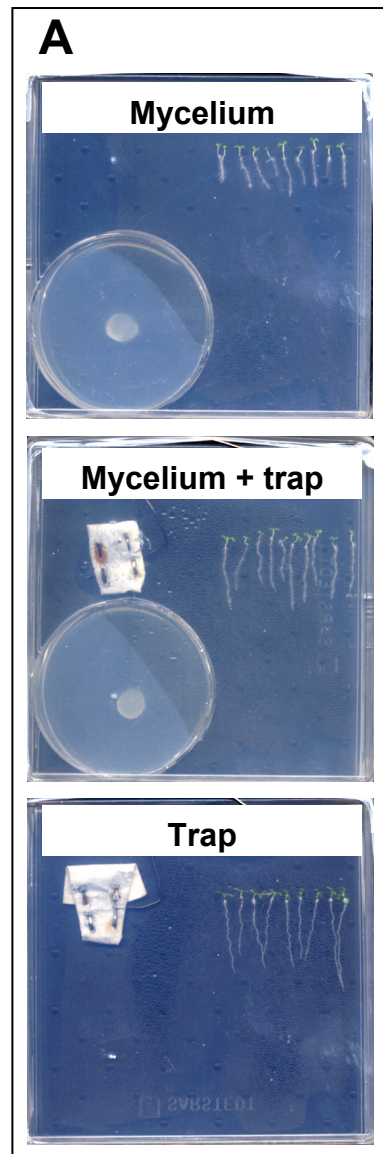


Figure 5S. Effect of activated charcoal in the compartmented bioassay. Activated charcoal was used as a volatile trap to decrease the concentration of ethylene produced by *T. borchii* mycelium (strain 1). Activated charcoal was packed in filter paper and suspended inside the Petri dishes with an iron pin (**A**). Seedlings grown in presence of the trap and the mycelium had longer primary roots (**B**) and shorter root hairs (**D**) than those grown without the trap (mycelium). As increasing ethylene concentrations tend to increase root hair length and decrease primary roots, the results (**B**, **D**) suggest that the activated charcoal successfully reduced the ethylene concentration inside the Petri dish. Different letters indicate statistically different results ($P < 0.05$, ANOVA on ranks and post-hoc Dunn's test). For primary root length and branching $n \geq 35$ seedlings, and $n = 300$ for root hairs.

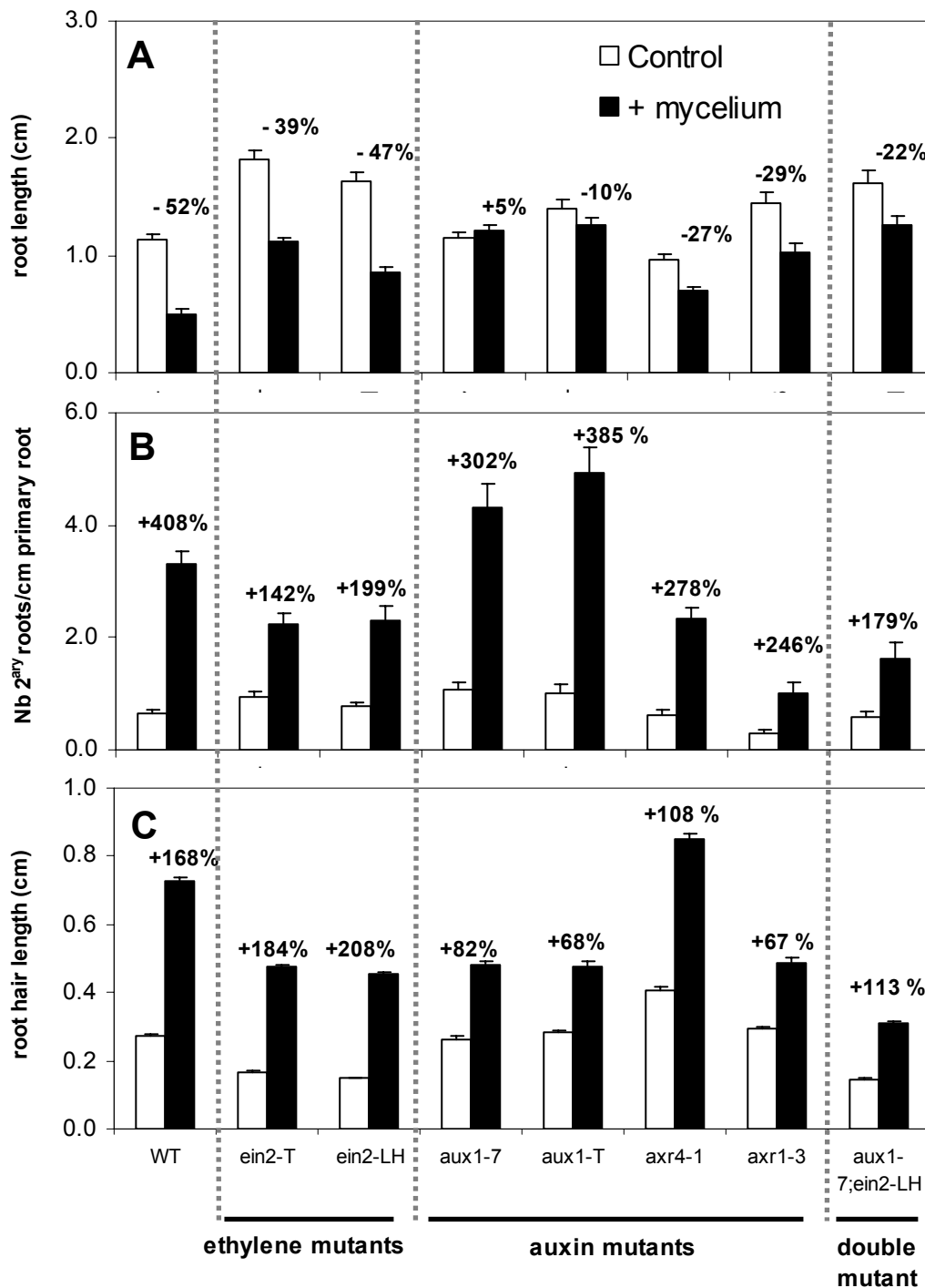


Figure 6S. Screening *A. thaliana* mutants for resistance to truffle metabolites

Using the bioassay setup of Fig.1, various auxin and ethylene mutants were tested for their sensitivity to truffle (*T. borchii* strain 1) metabolites and compared to the WT. (A) primary root length, (B) root branching and (C) root hair length. Bars represent average values (\pm std. errors) for 10 days old seedlings, $n > 20$ seedlings/treatment; for root hair length $n = 300$, 15 days old seedlings. Percentages represent changes versus respective controls.

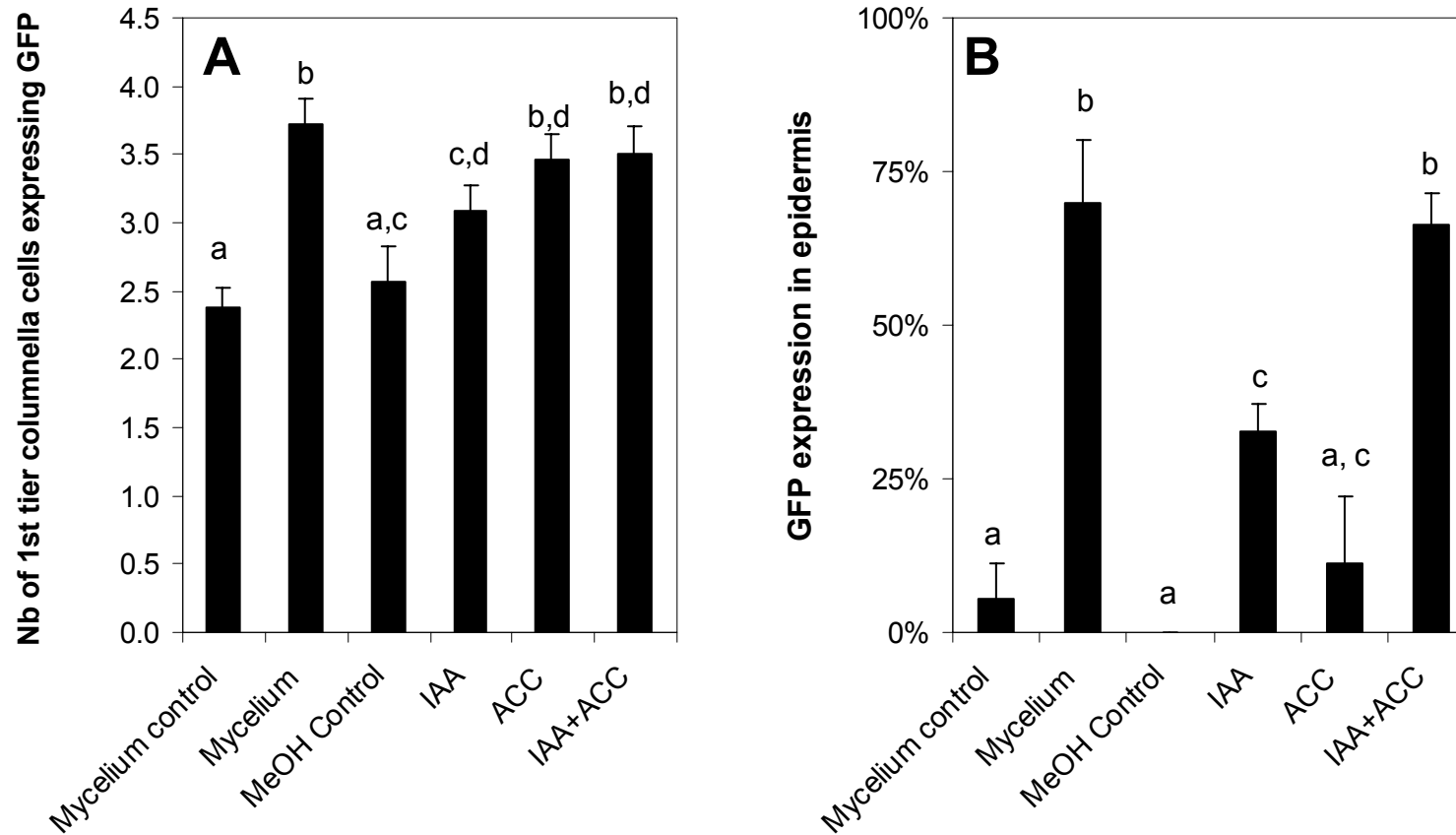


Figure 7S. GFP quantification in root tips of *DR5::GFP Arabidopsis*. For the experimental setup and hormone concentrations refer to Fig. 6. Mycelial metabolites significantly increased the GFP signal in the first tier columnella cells and this increase was fully mimicked by ACC alone or in combination with IAA (**A**). Mycelial metabolites also induced a GFP signal in the root epidermis which was only fully mimicked by the additive effect of both hormones (IAA + ACC) (**B**). Different letters indicate statistically different values ($P < 0.05$, Mann-Whitney for (A), t-test for B; $n \geq 12$ root tips examined from 3 Petri dishes).