Inhibition of jasmonate-mediated plant defences by the fungal metabolite higginsianin B

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Supplementary Figure S1 – Screening assay for modulation of salicylic acid signalling pathway. *Arabidopsis* seedlings expressing GUS reporter under the control of *PR1* promoter, a marker of SA-mediated plant defences, were pre-treated with fractions or pure compound higginsianin B for 1h followed by SA treatment (200 µM) for 24 h. Bars represent means *PRIp:GUS* activity of 5 independent seedlings, ± SD from one representative experiment performed twice. None of the tested fractions or compound were significantly different from the mock control (adjusted *P*-value = 0.25, Kruskal-Wallis with Conover-Iman test).
Supplementary Figure S2 – HPLC-ELSD comparison of four fractions of an active crude extract of *Colletotrichum higginsianum*. LSU, light scattering unit; ELSD, evaporative light scattering detector. Blue arrows = higginsianin B.
Supplementary Figure S3 – Pre-treatments with compounds structurally related to higginsianin B (higginsianins A, C and 13-epi-higginsianin C) do not influence the MeJA-induced degradation of the JA sensor J9V. Seedlings were pre-treated with either mock or 30 µM of the indicated compound for 30 min, following with a further 30 min treatment with either mock or 30 µM MeJA. Immunoblots depict 40 µg of total protein extracts from 60 seedlings. J9V was assayed with anti-GFP antibodies; ACTIN (assayed with anti-actin antibodies) and Ponceau S represent loading controls. Protein molecular mass is shown on the right. Higginsianins A, C and 13-epi-higginsianin C show very weak activity if any. ctrl refers to untreated Jas9-Venus seedlings. E-64 and epoxomicin, respectively a highly selective cysteine protease inhibitor and a specific proteasome inhibitor, were used as controls. Only epoxomicin could prevent MeJA-induced Jas9-VENUS degradation.