JA-Ile-lactones uncouple growth and defense in wild tobacco

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Electronic Supplementary Information

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General information.

Reactions were carried out in oven-dried glassware. All solvents, the catalyst and substrates were commercially available and used as received. NMR spectra were taken on a Bruker Avance III HD 400 NMR Spectrometer and analyzed employing the ACD/Spectrus processor software v.14. Chemical shifts are relative to the residual solvent peak as an internal standard. GC-MS spectra were recorded on a ThermoQuest CE Instruments GC 2000 Series coupled to a ThermoQuest Finnigan Trace MS mass spectrometer. GC column: ZB-5 capillary column (15 m × 0.25 mm ID × df 0.25 µm, Phenomenex). Injection port: 250 °C; flow, 15 mL min^{-1} with a split ratio of 10 mL min^{-1}; temperature programme: 50 °C (2 min), 15 °C min^{-1} to 220 °C (2 min), 10 °C min^{-1} to 280 °C (2 min). Helium at 1.5 mL min^{-1} served as a carrier gas. The ionization method was electron impact (70 eV) in positive mode (EI^+). HRMS (ESI^+) was performed on a Bruker Daltonics maXis Ultra High Resolution TOF equipment.

Synthesis of JA-Ile-lactones 9 and 9a.

Synthesis of (±)-**10** was carried out as previously described.[1] Briefly, a 10 mL schlenk-flask was charged with commercially available (±)-**2** (1.02 g, 4.5 mmol), but-3-en-1-yl acetate (3.05 g, 27 mmol, 6.0 *equiv.*) and catalyst **11** (0.14 g, 0.22 mmol, 5 mol %, Scheme S1). The flask was connected to a vacuum line (*ca.* 400 mbar, dynamic vacuum) and gentle warming (45 °C) was applied under stirring. After 5 h the vacuum was stopped and the reaction was quenched by adding 5 mL solution of ethyl vinyl ether (3 M in CH_2Cl_2). Stirring continued for 30 min when the mixture was concentrated at reduced pressure and purified by flash chromatography (AcOEt : *n*-pentane, 3:7). Product (±)-**10** (1.05 g; 84 %, *Z*:*E* = 94:6) was obtained as a pale yellow oil.
(±)-**10** (0.38 g, 1.3 mmol) was dissolved in MeOH (3 mL) and NaOH (*ca.* 0.3 M, 9.3 mL, 2.7 mmol, 2.1 *equiv.*) was added. The mixture was heated to 50 °C for 1 h and allowed cool to room temperature for an additional hour when water (20 mL) was added. The pH was adjusted to *ca.* 3 with HCl (1 M) and the aqueous phase extracted three times with AcOEt (20 mL). The organic extract were combined, washed with brine (40 mL) and dried over $MgSO_4$. Evaporation of the solvent yielded the product (±)-**3** (0.26 mmol; 88 %), which was used in the next step without further purification. Coupling of (±)-**3** to L-isoleucine and macrolactonization of (±)-**8** were carried out following the procedures previously described.[2] Spectroscopic data of compounds (±)-**3**, (±)-**8**, **9**, **9a**, and (±)-**10** were consistent with previous reports.[1,2]

Plant material and planting conditions.

Seeds of *N. attenuata* Torr. Ex. Watson were germinated on Gamborg's B5 medium as previously described.[3] Ten days later, seedlings were transferred to Teku pots (Teku JP3050/104T, Pöppelmann GmbH & Co. KG, Lohne, Germany) for ten days. Plants were then transplanted into 1 L pots filled with soil. All plants were grown at 45-55% relative humidity, 23-25 °C during days and 19-23 °C during nights under 16 h of light (6am-10pm). Plants were watered daily by a flood irrigation system.

Plant treatments, herbivory and plant growth.

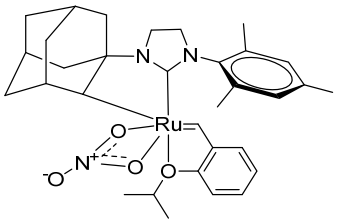
Plant treatments were carried out by applying compounds dissolved in lanolin paste as previously described.[2] Briefly, twenty days after planting plants on 1L pots filled with soil, compounds dissolved in lanoline paste were applied to the petioles of rosette-stage plants. The treatments were repeated every other day for five days to obtain nine treated leaves (0.8 µmol of each compounds per plant) in total. Lanolin-treated plants were used as controls.[4] For secondary metabolite measurements, the leaves of all plants were harvested 24 h after the last treatment, flash frozen in liquid nitrogen and analyzed as described (N = 5).[5,6] For the insect performance experiment, plants (N = 13) were infested with three *Manduca sexta* neonates 24 h after the last chemical treatment and larvae were allowed to feed freely for 10 days. After this period, caterpillars were recaptured and weighed. For the plant growth experiments, plants were treated only once. Stem length was measured four and five days after induction (N = 21). Seed capsules were counted regularly during the entire reproductive period.

Statistics.

Data were analyzed by analysis of variance (ANOVA) using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA). Normality and equality of variance were verified using Shapiro-Wilk and

Levene's tests, respectively. Holm-Sidak *post hoc* tests were used for multiple comparisons. Datasets from the experiments that did not fulfill assumptions for ANOVA were natural log-, root square- or rank-transformed prior to analysis.

Supplementary figures and schemes.



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Scheme S1 Grubbs *Z*-selective catalyst employed in the synthesis of **(±)-10**.

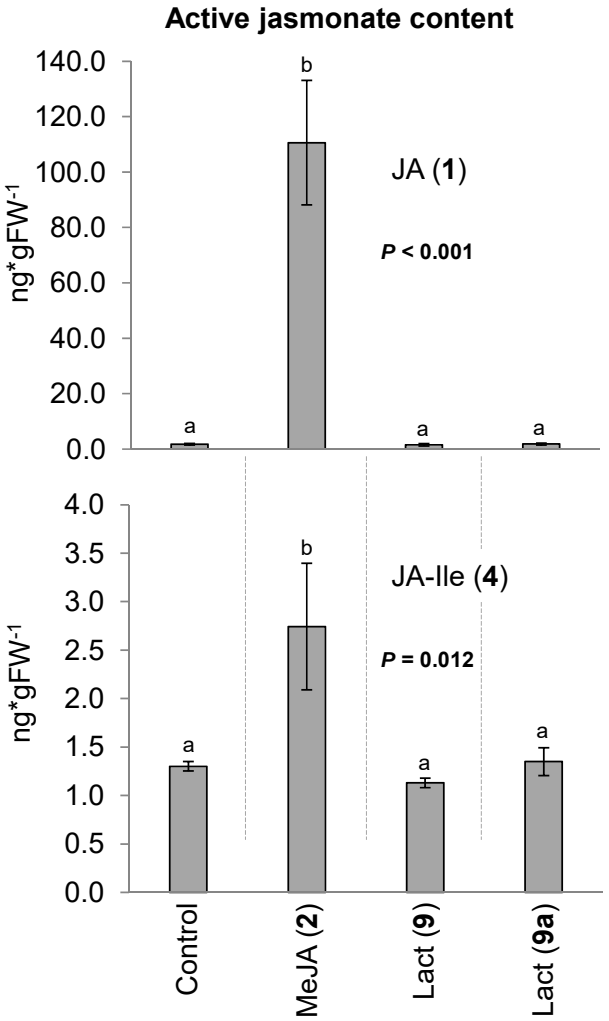

Fig. S1 Jasmonic acid JA (**1**) and JA-Ile (**4**) content in WT plants 24 h after the last treatment with: lanolin (control), **2**, **9**, or **9a**.

References.

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