A new cyanogenic metabolite in Arabidopsis required for inducible pathogen defence

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Thousands of putative biosynthetic genes in Arabidopsis thaliana have no known function, which suggests that there are numerous molecules contributing to plant fitness that have not yet been discovered1–2. Prime among these uncharacterized genes are cytochromes P450 upregulated in response to pathogens3–4. Here we start with a single pathogen-induced P450 (ref. 5), CYP82C2, and use a combination of untargeted metabolomics and coexpression analysis to uncover the complete biosynthetic pathway to 4-hydroxyindole-3-carbonyl nitrile (4-OH-ICN), a previously unknown Arabidopsis metabolite. This metabolite harbours cyanogenic functionality that is unprecedented in plants and exceedingly rare in nature5–8; furthermore, the aryl cyanohydrin intermediate in the 4-OH-ICN pathway reveals a latent capacity for cyanogenic glucoside biosynthesis9–11 in Arabidopsis. By expressing 4-OH-ICN biosynthetic enzymes in Saccharomyces cerevisiae and Nicotiana benthamiana, we reconstitute the complete pathway in vitro and in vivo and validate the function of its enzymes. Arabidopsis 4-OH-ICN pathway mutants show increased susceptibility to the bacterial pathogen Pseudomonas syringae, consistent with a role in inducible pathogen defence. Arabidopsis has been the pre-eminent model system12–13 for studying the role of small molecules in plant innate immunity14; our results uncover a new branch of indole metabolism distinct from the canonical camalexin pathway, and support a role for this pathway in the Arabidopsis defence response15. These results establish a more complete framework for understanding how the model plant Arabidopsis uses small molecules in pathogen defence.

To identify cytochromes P450 potentially involved in the biosynthesis of novel defence-associated small molecules, we obtained raw data sets for all transcriptomics experiments dealing with biotic stress in A. thaliana from the NASCArrays database. We examined CYP genes present in the probe set and selected a candidate, CYP82C2, that is highly expressed under a variety of pathogen treatment conditions, but whose native function in Arabidopsis is unknown (Fig. 1a).

To identify small molecules whose levels change in a CYP82C2-dependent manner, we performed comparative metabolomics with a homozygous transfer-DNA insertion line of CYP82C2. We used the bacterial pathogen P. syringae pv. tomato DC3000 harbouring the avrRpm1 avirulence gene (Psta) as an elicitor since CYP82C2 expression is strongly upregulated 24 h after inoculation with this strain (Fig. 1a). We analysed tissue methanolic extracts of 11-day-old seedlings grown hydroponically in the presence of Psta by liquid chromatography–mass spectrometry (LC–MS), and computationally compared mutant and wild-type (WT) Col-0 metabolomes. From this analysis, we identified 11 compound mass signals that reproducibly and significantly differ between WT and cyp82C2 (Fig. 1b); these mass ions are induced after pathogen elicitation and are not bacterially derived (Extended Data Fig. 1a).

We next sought to obtain clues about the structure of these compounds from their tandem mass spectra (MS/MS). MS/MS analysis revealed that the 11 compounds could be divided into two classes (A and B in Fig. 1b), assigned as indole-3-carboxaldehyde (IAL) derivatives with (B) and without (A) hydroxylated indole systems. Moreover, the fact that the cyp82C2 mutant lacked all the hydroxylated derivatives but accumulated excess amounts of their non-hydroxylated counterparts suggested that CYP82C2 acts as an indolic hydroxylase. However, except for compound A1 (Fig. 2b), which was confirmed to be indole-3-carboxylic acid methyl ester, the structures of these compounds remained elusive.

To facilitate structural analysis, we investigated whether any of these compounds were exuded into the medium in the cyp82C2 mutant seedling experiments (Fig. 1d). Filtered spent medium was loaded onto a C18 silica gel cartridge, and non-polar metabolites were eluted with acetonitrile and analysed by LC–MS. Surprisingly, the profile of spent medium extracted in this manner was notably different from that of tissue methanolic extracts: while small amounts of A2–A7 were present, no A1 could be detected; instead, a new ultraviolet-active compound with m/z = 171.0553 [M + H]+ dominated the LC–MS trace (Fig. 1d). NMR analysis of this compound followed by comparison with a synthetic standard established its identity as the novel metabolite indole-3-carbonyl nitrile (ICN) (Fig. 1c and Extended Data Fig. 2).

Chemically, the most striking feature of ICN is the presence of a highly reactive α-ketonic moiety that, to our knowledge, has not been found in any plant natural product; however, benzoyl cyanide has been previously identified in the secretions of millipedes16–18. The α-ketonic is susceptible to nucleophilic attack, resulting in the displacement of cyanide ion: in alkaline aqueous solution, ICN degrades to indole-3-carboxylic acid (ICA) (an alternative route to ICA in Arabidopsis has been reported19); in methanol, ICA methyl ester (A1) is formed instead, explaining the presence of A1 and the absence of ICN in methanolic extracts (Fig. 1c). Modifying the tissue extraction procedure by using an acidified 1:1 acetonitrile/water mixture enabled direct detection of ICN by LC–MS; additionally, when deuterated methanol was used, only the deuterated form of A1 was observed (Extended Data Fig. 1b–e). On the basis of its molecular formula and the synthesis of an authentic standard, A6 was shown to be a serine–ICN addition product (see Fig. 2b). However, in the presence of cysteine and structurally related compounds, ICN can undergo spontaneous cycloaddition, resulting in the formation of a thiazoline ring and the net loss of ammonia. This last observation allowed us to determine the structures of and synthesize standards for compounds A2–A5, which are the cycloaddition products of ICN and cysteine (A4) or Cys-Gly dipeptide (A2) and their thiazole analogues (A5 and A3, respectively; see Fig. 2b, Extended Data Fig. 3, and Supplementary Table 1).

The absence of the hydroxylated analogues B1–B6 in the cyp82C2 insertion line pointed to ICN as the likely substrate for this enzyme. Incubation of ICN with yeast-expressed CYP82C2 yielded only a trace amount of hydroxylated ICN, but a significant amount of 4-hydroxy-ICA (4-OH-ICA) (structure shown in Fig. 3), as confirmed by NMR spectroscopy and comparison with a synthetic standard (Extended Data Fig. 4a–d). Since CYP82C2 shows no activity on ICA, we deduced

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Table 3). We performed a metabolomic analysis of the compounds derived from ICN, whether as artefacts of the extraction (A1 and B1), as in vivo addition products (A2–A7, B2–B6).

We next investigated the biosynthesis of ICN, using the CYP82C2 gene as bait for coexpression analysis. For our pathogen data set, the CYP79B2 gene, whose encoded enzyme converts tryptophan (Trp) into indole-3-acetaldoxime (IAOx), has the second highest correlation (Pearson’s r) with CYP82C2 among all genes profiled (Supplementary Table 3). We performed a metabolomic analysis of the cyp79B2 cyp79B3 double knockout line, which is deficient in IAOx production. No ICN-derived metabolites are produced in this mutant (Fig. 2a), indicating that ICN is derived from IAOx.

In searching for the enzyme(s) responsible for further conversion of IAOx to ICN, we postulated a biosynthetic route paralleling that of the cyanogenic glycoside dhurrin: a CYP79-catalysed formation of an aldoxime, followed by a CYP71-catalysed formation of a cyanohydrin intermediate. In the dhurrin pathway, the cyanohydrin is glycosylated to yield the final product, whereas in ICN biosynthesis, a final dehydrogenation is required to produce an α-ketonitrile (Fig. 2c).

Correlation analysis implicated CYP71A12, a P450 linked to camalexin biosynthesis, as the most likely candidate gene for the cyanohydrin formation step (Supplementary Table 3). Profiling of the cyp71A12 transfer-DNA insertion line, as well as transfer-DNA insertion lines of its two closest Arabidopsis homologues, CYP71A13 and CYP71A18, demonstrated that the CYP71A12 gene is in fact probably responsible: all ICN derivatives with the exception of A6 are at ~10% of WT levels in the cyp71A12 mutant, but unaffected in the cyp71A13

Figure 1 | Transcriptomic and metabolomic analyses implicate CYP82C2 in the biosynthesis of novel pathogen defence-related secondary metabolites. a, Heat map of relative gene expression levels for cytochrome P450 genes in Arabidopsis under various pathogen stress conditions. The enlarged map shows the top 10 P450 genes after sorting by mean expression level over all conditions. Cytochromes P450 in grey have previously been biochemically characterized. b, Levels of the most significantly differing metabolites identified in seedling comparative metabolomics experiments with cyp82C2. Data represent the mean ± s.d. of six biological replicates. c, ICA methyl ester (A1) and 4-OH-ICA methyl ester (B1) are methanolic degradation products of ICN and 4-OH-ICN. d, High-performance liquid chromatography traces of growth medium for WT and cyp82C2 seedlings, showing Psta-dependent accumulation of ICN and 4-OH-ICN.

Figure 2 | Targeted metabolic profiling of candidate transfer-DNA insertion lines helps uncover the entire ICN biosynthetic pathway. a, Heat map of mean ICN-derived metabolite levels relative to WT in Psta-elicited transfer-DNA insertion lines. Mutants in bold have significantly decreased levels of ICN derivatives. Note that A6 levels are not affected to the same extent as levels of other metabolites in any line except for cyp79B2/B3, hinting at an alternative biosynthetic route from IAOx for this metabolite. b, Structures of all ICN derivatives, confirmed by comparison with synthetic standards (see Extended Data Fig. 3 and Supplementary Table 1). c, Proposed biosynthetic pathway from Trp to 4-OH-ICN and downstream metabolites.
and cyp71A18 mutants (Fig. 2a). Levels of camalexin and other indolic metabolites were only slightly changed in whole-seedling tissue extracts of the cyp71A12 mutant (Extended Data Fig. 5c).

Further correlation analysis using CYP71A12 as bait revealed a cluster of five tandemly arrayed homologous genes, At1g26380–At1g26420, that are highly coexpressed with CYP71A12 (Supplementary Table 3). At1g26380 encodes a flavin-dependent oxidoreductase known as FOX1 (ref. 19). We profiled the corresponding homozygous transfer-DNA insertion lines for these genes and found a three- to fivefold reduction in levels of ICN metabolites in the fox1 mutant, with no significant changes observed for the other mutants (Fig. 2a). Additionally, we observed a build-up of IAL, the expected hydrolysis product of the indole-3-cyanohydrin intermediate (Extended Data Fig. 5d). More strikingly, the fox1 mutant accumulates new mass signals corresponding to indole cyanogenic glycosides (ICGs), not previously observed in plants (Extended Data Fig. 6a–e, structures shown in Fig. 4d). Cyanogenic glycoside compounds are widely distributed in the plant kingdom, but have not yet been detected in Arabidopsis. Disruption of the ICN pathway at the FOX1-catalysed step therefore leads to capture of some portion of the cyanohydrin intermediate by non-specific glycosyltransferases, exactly paralleling dhurrin synthesis.

We sought to confirm the proposed biochemical transformations (Fig. 2c) by reconstituting the complete pathway in vitro. A combination of yeast microsomal CYP71A12 and CYP82C2 and N. benthamiana-expressed FOX1 was sufficient to catalyse the conversion of IAOx to ICN, as illustrated in Fig. 3; the production of 4-OH-ICN is inferred from the accumulation of 4-OH-ICA. We also reconstituted the biosynthesis of 4-OH-ICN in the heterologous host N. benthamiana, using transient expression of the four pathway genes necessary for production of 4-OH-ICN from Trp via Agrobacterium-mediated transient transformation. We observed significant accumulation of B1 (from methanol extraction of 4-OH-ICN) only when all pathway genes were present; however, we also noted background levels of ICA and IAL when only early pathway genes were expressed (Extended Data Fig. 7). Notably, when we expressed CYP79B2 and CYP71A12 but not FOX1, we again observed the accumulation of ICG mass signals (Extended Data Fig. 6f).

The Trp-derived metabolites camalexin and 4-methoxy indol-3-yethylglycosinolate (4-methoxylgycobrassicin) have been shown to play a key role in Arabidopsis immunity (Fig. 4d)10,11,18,21. To evaluate whether 4-OH-ICN pathway products also contribute to Arabidopsis disease resistance, we challenged 4-OH-ICN biosynthetic mutants with a diverse panel of pathogens. Using surface inoculation to mimic the natural infection process, we found that, compared with letters denote statistically significant differences (P < 0.05, two-tailed t test). c. Growth analysis of Pst in WT adult leaves pre-immunized with 1 μM flg22 and 100 μM ICN, 4-OH-ICN, camalexin or solvent control (dimethylsulfoxide (DMSO)) for 24 h before infiltration with Pst. Data represent the median ± s.e.m. of three biological replicates. Asterisk denotes statistical significance relative to WT (P < 0.01, two-tailed t test). Experiment was repeated three times, producing similar results. d. Summary of known major Trp-derived secondary metabolites in Arabidopsis and oxidative biosynthetic enzymes that have been used to reconstitute the pathways in vitro or in planta.
WT, the adult leaves of cyp71A12 and cyp82C2 are more susceptible to the virulent bacterial hemi-biotroph Pst (P. syringae pv. tomato DC3000) and comparable to the immuno-deficient fls2 mutant, which cannot perceive the bacterial microbe-associated molecular pattern (MAMP) flg22 (refs 22, 23) (Fig. 4a). Similarly, seedlings of the 4-OH-ICN pathway mutants are more susceptible to Pst than WT in the presence and absence of flg22 (Fig. 4b), indicating a role for 4-OH-ICN in basal disease resistance against a bacterial pathogen. Notably, the adult leaves and seedlings of the camalexin pathway mutants cyp71A13 and pad3 are also more susceptible to Pst infection than WT (Fig. 4a, b), suggesting a previously unrecognized role for camalexin in the antibacterial defence response. To test for a direct role of the ICN pathway metabolites in the plant innate immune response, either as inducible antibacterial or signalling compounds, we measured their protective effect against subsequent bacterial infection by infecting WT adult leaves with Pst after pre-immunizing them with pure compounds and flg22. Compared with a solvent control, pre-treatment with 4-OH-ICN (but not ICN or camalexin) conferred greater bacterial resistance (Fig. 4c), which supports a direct mechanism of action for 4-OH-ICN in inducible plant defence.

We also observed increased disease symptoms in adult leaves of the cyp82C2 mutant upon inoculation with spores from the avirulent fungal necrotroph Alternaria brassicicola (Extended Data Fig. 8e, f) and—consistent with a previous report24—the virulent necrotroph Botrytis cinerea (Extended Data Fig. 8a, b), but not from the obligate fungal biotroph Golovinomyces orontii (Extended Data Fig. 8c, d). Furthermore, purified ICN and 4-OH-ICN have a growth inhibitory effect on B. cinerea and A. brassicicola comparable to that of camalexin25 (Extended Data Fig. 9). However, we cannot rule out the possibility that the role of the 4-OH-ICN pathway in fungal defence is indirect, as adult leaves of the cyp82C2 mutant appear partly impaired in camalexin production after Alternaria treatment (Extended Data Fig. 10).

The camalexin and 4-OH-ICN pathways rely on a pair of paralogous genes, CYP71A12 and CYP71A13, which are members of the CYP71 family linked to innovations in plant metabolism26 (Fig. 4d). Strikingly, the 4-OH-ICN pathway resembles the widespread cyanogenic glucoside pathway that has been lost in the Brassicaceae, and appears to be a metabolic re-invention leading to a novel cyanogenic metabolite type derived from Trp27,28. It is possible that 4-OH-ICN acts in concert with other Trp-derived metabolites, each contributing to protection against overlapping sets of specific pathogens. Collectively, our data provide additional insight into the Arabidopsis defence response and, more generally, how plants use metabolic innovation to expand innate immunity.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear in the online version of the paper.

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a

![Graph showing ion abundance](image)

- Col-0 Mock
- Col-0 +Flg22

b

- m/z \([M + H]^+ = 176.0706\)

![Chemical structure and spectrum](image)

c

- **CH₃OH extraction**

  - EIC
  - Retention time (min)

- **CD₃OD extraction**

  - EIC
  - Retention time (min)

d

- m/z \([M + H]^+ = 179.0894\)

![Chemical structure and spectrum](image)

e

- **CH₃OH extraction**

  - EIC
  - Retention time (min)

- **CD₃OD extraction**

  - EIC
  - Retention time (min)
Extended Data Figure 1 | Elicitation of compounds identified in metabolomics screen by flg22 peptide and origin of ICA methyl ester as artefact of the methanol extraction method. a, Levels of compounds in Flg22-elicited Arabidopsis Col-0 seedling tissue, quantified as mean [M + H]^+ ion (m/z ± 10 ppm) abundances extracted from raw data; error bars, s.d. based on three biological replicates. Production of these compounds in axenic plant culture demonstrates that they are plant-derived. b, c, Structure and mass peaks of ICA methyl ester (compound A1) seen in LC–MS analysis (b), and EICs for the expected m/z using a standard extraction with 80:20 CH₃OH/H₂O or with 80:20 CD₃OD/D₂O (c). d, e, Structure and mass spectrum peaks seen for the triply deuterated A1 analogue (d), and EICs for the expected m/z using extraction with 80:20 CH₃OH/H₂O, or with 80:20 CD₃OD/D₂O (all EICs are to scale) (e). The presence of the deuterated analogue of ICA methyl ester and the complete absence of the non-deuterated compound in plant extracts when CD₃OD is substituted for CH₃OH show that the methyl ester is not a product of Arabidopsis metabolism, but arises because of the extraction method as a degradation product of ICN.
Extended Data Figure 2 | Comparison of spectra for plant-extracted and synthetic compound establishes identity of ICN as new indolic metabolite produced by A. thaliana. a, Full-range (δ = 10.5 to −0.5) and, b, downfield region partial (δ = 8.5–7.0) 1H NMR spectra in CD3CN. Upfield contaminants in the full-range spectra are presumed to be residual solvent. c, Ultraviolet-visible absorbance spectra obtained via a diode array detector during LC analysis. Note that the prominent peak at 230 nm is due to acetonitrile in the LC mobile phase. d, Targeted MS/MS spectra for the parent ICN [M + H]+ ion (m/z = 171.0550) at a collision energy of 20 V. See Supplementary Table 1 for relative peak intensities at other collision energies. e, Aligned EICs for the ICN [M + H]+ ion for a Col-0 + Pstu tissue sample extracted with DMSO and synthetic compound, showing identical retention times.
Extended Data Figure 3 | Comparison of plant-extracted ICN derivatives, 4-OH-ICN derivatives, and synthetic standards shows identical column elution times for all compounds. Col-0 + Pstα combined EICs were extracted for the relevant compound [M + H]+ m/z values for a DMSO-extracted medium sample (4-OH-ICN trace), or a MeOH-extracted seedling tissue sample (all other traces), while synthetic EICs were extracted for a mixed standard in DMSO. Note that chromatograms are not to scale, and the synthetic standard is not equimolar with respect to all compounds because of partial degradation.
Extended Data Figure 4 | CYP82C2 is an ICN 4-hydroxylase. a, b, $^1$H NMR spectra in CD$_3$OD of synthetic ICA (a) and 4-OH-ICA (b). c, Spectrum for large-scale enzymatic reaction extract of ICN incubated with CYP82C2. In addition to ICA, resulting from hydrolysis of ICN, peaks for a singly hydroxylated analogue of ICA are seen; these are qualitatively consistent with, but shifted slightly upfield (~30–60 Hz) from the 4-OH-ICA spectrum, possibly because of impurities or a pH effect in the enzymatic reaction sample. d, To confirm the identity conclusively, 80 μg of 4-OH-ICA dissolved in CD$_3$OD was added to the enzymatic reaction NMR sample before acquiring another spectrum: no new peaks are seen, while the prior hydroxylated ICA peaks grow in intensity, establishing the product of the enzymatic reaction as 4-OH-ICA. e, EICs for enzymatic reactions of CYP82C2 on ICN or ICA, or empty vector control incubation with ICN. Only trace amounts of the expected 4-OH-ICN product but significant amounts of 4-OH-ICA are seen for the CYP82C2/ICN reaction. No hydroxylated products are seen for the CYP82C2/ICA or empty vector/ICN reactions, indicating that CYP82C2 catalyses only the hydroxylation of ICN to 4-OH-ICN, but 4-OH-ICA is seen as the predominant end product due to rapid hydrolysis of 4-OH-ICN (f). Chromatograms in this figure were obtained using the 20 min LC–MS gradient (see Supplementary Information, Methods section 1.9 LC–MS analysis).
Extended Data Figure 5 | Levels of numerous *Arabidopsis* indolic metabolites are altered in ICN pathway gene insertion lines compared with WT plants. a–e, Relative compound levels for mock treatment condition and indicated pathway insertion line mutants, and, f, absolute levels in *Pst*a-treated WT (Col-0) seedlings. For a–e, data bars represent a logarithmically scaled ratio of mean metabolite levels in the indicated line or treatment condition, quantified as [M + H]$^+$ ion abundances by LC–MS analysis with XCMS processing, to levels in *Pst*a-treated WT *Arabidopsis* seedlings. In f, absolute levels for all compounds except RA were quantified by measuring [M + H]$^+$ ion abundances and comparing to standard curves. Error bars, s.d., based on six biological replicates. Cam, camalexin; RA, raphanusamic acid; other abbreviations as detailed previously.
Extended Data Figure 6 | Putative ICGs observed in Arabidopsis and in N. benthamiana expressing ICN pathway enzymes. a, EICs for putative ICGs in WT Arabidopsis and fox mutant elicited with Pstα. The m/z values shown are median values calculated by XCMS. b, Hypothesized structures and theoretical m/z values for the two ICGs identified. c, MS/MS spectrum for ICG1; m/z values and relative abundances are shown above each peak. The ion analysed here (m/z = 691.2210) represents a [2M + Na]⁺ dimer that is significantly more abundant than the [M + Na]⁺ ion. Direct analysis of the [M + Na]⁺ ion (m/z = 357.1057) yielded low abundance spectra that could not be easily analysed. At lower collision energies, the [2M + Na]⁺ ion fragments to [M + Na]⁺, but yields a rich spectrum at 40 V, which is shown. Predicted peak assignments for the ICG1 MS/MS spectrum are shown in the accompanying table. For peaks in bold, exact counterparts could be identified in the dhurrin [M + Na]⁺ 20 V MS/MS spectrum in the METLIN metabolite database. d, MS/MS spectrum obtained for the ICG2 [M + Na]⁺ ion and predicted peak assignments. While the [2M + Na]⁺ peak (m/z = 864.2225) is also seen for this compound (not shown), [M + Na]⁺ is more abundant in this case, and was analysed directly. e, f, Levels of ICG1 and ICG2 in ICN pathway mutants (e) and in WT plants elicited with Pstα and N. benthamiana expressing ICN pathway enzymes (f). For e and f, levels are quantified as mean [M + Na]⁺ ion (m/z ± 10 ppm) abundances extracted from raw data; error bars, s.d., based on six biological replicates.
Extended Data Figure 7 | ICN pathway metabolites are produced in *N. benthamiana* transiently expressing pathway genes. Levels of ICN and 4-OH-ICN derivatives (left axis) and other relevant indolic compounds (right axis), quantified as mean [M + H]$^+$ ion (m/z ± 10 ppm) abundances extracted from raw data; error bars, s.d., based on six biological replicates. The set of transiently expressed genes is indicated for each panel. Background levels of ICA and IAL detected when only the early pathway genes *CYP71A12* and/or *CYP79B2* are expressed indicate potential involvement of endogenous *N. benthamiana* enzymes.
Extended Data Figure 8 | ICN pathway metabolites contribute to disease resistance towards *B. cinerea* but not towards *G. orontii*. a, Top: typical lactophenol trypan blue staining of leaves drop-inoculated with spores from the virulent fungal necrotroph *B. cinerea* to visualize the extent of host cell death (darkly stained areas within and beyond the fungal spore droplet region). Middle: microscopic analysis of stained leaves to visualize the extent of fungal colonization (stained filamentous fungal hyphae within and beyond the fungal spore droplet region). Images were taken at the same magnification ($\times 25$) and are representative of five biological replicates. Bottom: close-up images of the fungal hyphae beyond the fungal spore droplet region for *cyp82C2* and *cyp71A13* mutants. Images were taken at the same magnification ($\times 100$).

b, Measurement of the disease lesion diameters in infected leaves. Data represent the median ± s.e.m. for five biological replicates. Asterisks denote statistical significance relative to WT ($P < 0.05$, two-tailed t test).

c, Typical lactophenol trypan blue staining of fungal conidiophores (spore-bearing structures) formed in leaves infected with the adapted powdery mildew *G. orontii*. The pad4-1 mutant is more susceptible to fungal growth by *G. orontii* and thus produces significantly more conidiophores. Images were taken at the same magnification ($\times 100$) and are representative of three biological replicates.

d, Measurement of the number of conidiophores in infected leaves. Data represent the mean ± s.d. for three biological replicates.

e, Top: typical disease symptoms 3 days after drop inoculation of leaves with spores from the avirulent fungal necrotroph *A. brassicicola*. Bottom: microscopic analysis of infected leaves after lactophenol trypan blue staining confirming that disease symptoms are consistent with extent of fungal colonization (lightly stained fungal hyphae extending from the fungal spore droplet region) and host cell death (darkly stained areas along and beyond the border of the spore droplet region). Images were taken at the same magnification ($\times 25$) and are representative of ten biological replicates.

f, Measurement of the disease lesion diameters in infected leaves. Data represent the median ± s.e.m. of eight (top graph) or ten biological replicates (bottom graph). Different letters denote statistically significant differences ($P < 0.05$, two-tailed t test).
Extended Data Figure 9 | ICN and 4-OH-ICN but not their degradation products inhibit fungal growth in vitro. a, b, Fungal growth inhibition assays on *B. cinerea* SF1 (a) or *A. brassicicola* FSU218 (b) with the tested compound (or compound combination) indicated. For compound combinations, the concentration indicated is for each compound; the given combinations approximate the hydrolysis products of ICN or 4-OH-ICN. Growth of fungi in potato dextrose broth on a microplate was quantified by measuring absorbance at 600 nm (OD$_{600}$ nm) 72 h after spore inoculation and subtracting the absorbance at 0 h; see Methods for further details. Error bars, s.d. based on three biological replicates. Note that the half-maximum inhibitory concentrations (IC$_{50}$) for both camalexin and ICN are approximately 25 μM against *B. cinerea* and 50 μM against *A. brassicicola*. For 4-OH-ICN, the inhibitory effect is not as pronounced, possibly because of rapid degradation of 4-OH-ICN in potato dextrose broth (see Supplementary Table 2).
Extended Data Figure 10 | Levels of indolic compounds in leaves of mature plants after mock treatment or fungal infection. Tissue extracts were analysed by LC–MS 7 days post-infection for *A. brassicicola* FSU218 and 5 days post-infection for *B. cinerea* SF1. a–e, Levels of indicated compound, quantified as EIC integral for the [M + H]⁺ ion (m/z ± 10 ppm) and converted to absolute amounts by comparison with a standard curve. f, Ion count integrals for indole glucosinolates ([M−H]⁻ ion, m/z ± 10 ppm). Error bars in all panels, s.d. based on six biological replicates.