Role of stress-related hormones in plant defence during early infection of the cyst nematode *Heterodera schachtii* in Arabidopsis

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Introduction

Sedentary plant-parasitic nematodes are responsible for severe economic and agricultural losses (Abad & Williamson, 2010), whereupon cyst nematodes (CNs) have a pivotal impact on wheat, soybean, potato and sugar beet production. In soil, host root exudates activate the second stage juvenile (J2) from dormancy in the egg shell and attract it to the root. There, it invades the cortex with the aid of its stylet and migrates intracellularly towards the vascular cylinder. It selects and pierces a single cell and releases saliva, triggering a series of cellular changes, such as increased cytoplasm streaming, enlargement of the nucleus, proliferation of plastids and mitochondria as well as dissolution of cell walls towards adjacent cells (Golinowski *et al.*, 1996; Wyss, 1999). Fusion of this initial cell with neighbouring cells leads to the formation of a syncytium that induces as a strong sink within the plant, supporting the nematode with all required nutrients.

*Heterodera schachtii*, a plant-parasitic cyst nematode, invades host roots and induces a specific syncytial feeding structure, from which it withdraws all required nutrients, causing severe yield losses. The system *H. schachtii*-Arabidopsis is an excellent research model for investigating plant defence mechanisms. Such responses are suppressed in well-established syncytia, whereas they are induced during early parasitism. However, the mechanisms by which the defence responses are modulated and the role of phytohormones are largely unknown. The aim of this study was to elucidate the role of hormone-based defence responses at the onset of nematode infection. First, concentrations of main phytohormones were quantified and the expression of several hormone-related genes was analysed using quantitative real-time (qRT)-PCR or GeneChip. Further, the effects of individual hormones were evaluated via nematode attraction and infection assays using plants with altered endogenous hormone concentrations.

Our results suggest a pivotal and positive role for ethylene during nematode attraction, whereas jasmonic acid triggers early defence responses against *H. schachtii*. Salicylic acid seems to be a negative regulator during later syncytium and female development.

We conclude that nematodes are able to impose specific changes in hormone pools, thus modulating hormone-based defence and signal transduction in strict dependence on their parasitism stage.

**Summary**

- *Heterodera schachtii*, a plant-parasitic cyst nematode, invades host roots and induces a specific syncytial feeding structure, from which it withdraws all required nutrients, causing severe yield losses. The system *H. schachtii*-Arabidopsis is an excellent research model for investigating plant defence mechanisms. Such responses are suppressed in well-established syncytia, whereas they are induced during early parasitism. However, the mechanisms by which the defence responses are modulated and the role of phytohormones are largely unknown.
- The aim of this study was to elucidate the role of hormone-based defence responses at the onset of nematode infection. First, concentrations of main phytohormones were quantified and the expression of several hormone-related genes was analysed using quantitative real-time (qRT)-PCR or GeneChip. Further, the effects of individual hormones were evaluated via nematode attraction and infection assays using plants with altered endogenous hormone concentrations.
- Our results suggest a pivotal and positive role for ethylene during nematode attraction, whereas jasmonic acid triggers early defence responses against *H. schachtii*. Salicylic acid seems to be a negative regulator during later syncytium and female development.
- We conclude that nematodes are able to impose specific changes in hormone pools, thus modulating hormone-based defence and signal transduction in strict dependence on their parasitism stage.

**Key words:** defence responses, early infection, ethylene, *Heterodera schachtii*, jasmonic acid, plant-parasitic nematodes, salicylic acid.

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was observed in whole soybean (Alkharouf et al., 2006; Ithal et al., 2007; Mazarei et al., 2011) and Arabidopsis roots (Puthoff et al., 2003) infected with the CNs Heterodera glycines and H. schachtii, respectively. This inducible defence often depends on tissue-specific concentrations of different phytohormones (Denance et al., 2013), which, amongst other functions, trigger and coordinate responses to biotic stresses (Kunkel & Brooks, 2002; Farmer et al., 2003; Pieterse et al., 2009; Goverse & Bird, 2011). For instance, salicylic acid (SA) is a key component in plant defence against biotrophic pathogens (Pieterse et al., 2009), whereas the jasmonic acid/ethylene (JA/ET) pathway acts mainly against necrotrophic pathogens and herbivores (Staswick et al., 1998; Vijayan et al., 1998; Pieterse et al., 2009). Recently, auxins (e.g. IAA) and cytokinins, growth-promoting hormones, were also shown to be involved in the biotic stress responses (Mauch-Mani & Mauch, 2005; Kazan & Manners, 2009; Fu & Wang, 2011). The characteristic feature of phytohormones is their intensive cross-talk, which may be synergistic or antagonistic. Generally synergistic interaction has been observed between JA and ET in the activation of defence against wounding and necrotrophs, whereas competing defence pathways regulated mainly by SA and JA/ET are considered as mutually antagonistic (Glazebrook, 2005). On the one hand, hormone-dependent defence pathways, especially SA, ET and JA, play an important role in defence responses of the host plant to both root-knot nematodes (RKNs) and CNs (Wübben et al., 2001, 2008; Lohar et al., 2004; Ithal et al., 2007; Kyndt et al., 2012; Ali et al., 2013). On the other hand, several studies have shown a positive involvement of plant hormones during nematode feeding site formation (reviewed in Goverse & Bird, 2011; and in Kyndt et al., 2013). In particular, IAA and ET were found to be indispensable for initiation and proper feeding site development (reviewed in Gutjahr & Paszkowski, 2009; and in Goverse & Bird, 2011; Goverse et al., 2000; Karczmarek et al., 2004; Wang et al., 2007; Grunewald et al., 2009; Siewcicka et al., 2009). Further, gibberellins (GAs) have been suggested to maintain nematode feeding sites and act in their maturation (Klink et al., 2007; Kyndt et al., 2012).

Taken together, the different roles of phytohormones vary considerably during feeding site and nematode development, depending on the host, the nematode species and the stage of parasitism. However, their function in mediating attraction and host finding, as well as during early plant defence, is widely unexplored. Therefore, the aim of this work was to elucidate hormone-related defence pathways induced at the onset of nematode parasitism, including attraction, migration and the beginning of syncytium induction. First, we quantified the alterations in hormone concentrations in Arabidopsis roots infected with H. schachtii. Subsequently, we evaluated the expression of specific hormone and defence marker genes during the migratory and early sedentary stage of nematode infection. Further, we observed changes in nematode attraction, infection, and development in plants with altered phytohormone concentrations as well as in hormone-deficient mutant lines. Our results suggest a pivotal role of both JA and ET in the early infection of H. schachtii. JA seems to be a negative regulator for female development, whose signalling is suppressed after successful infection in compatible plant–nematode interaction. By contrast, ET plays a positive role in nematode attraction to the root, whereas SA is primarily involved in subsequent syncytium and female development. These findings reveal an assured involvement of various defence signalling pathways and their specificity during early CN infection in Arabidopsis.

Materials and Methods

Plant and nematode culture

Seeds of Arabidopsis thaliana L. Heynh. Columbia 0 (Col-0) and mutant lines, dde2 (von Malek et al., 2002) and los6 (Grebenner et al., 2013), were surface-sterilized (0.7% NaClO, 40% ethanol (EtOH)) for 8 min, washed in 70% EtOH and subsequently rinsed three times in dH2O. Ten seeds per dish (94 mm in diameter) were planted on modified Knop medium supplemented with 2% sucrose and subsequently grown at 16:8 h light : dark at 23°C. Each 12-d-old seedling was inoculated with 50 J2s H. schachtii obtained from sterile stock culture (Sijmons et al., 1991). J2s were sterilized in 0.05% HgCl2 for 3 min and immediately washed three times in dH2O. Before inoculation, root length was categorized according to Jürgensen (2001). Inoculated plates were kept in the dark for 24 h, and subsequently transferred into a growing chamber under 16:8 h light : dark conditions. All experiments were repeated independently four times (n = 20). Infection sites were counted at 24, 48 and 72 h after inoculation (hai), and males and females at 14 d after inoculation (dai).

Hormone quantification

Arabidopsis plants were infected with nematodes as described earlier. Shoots and roots were collected at 24 hai, weighed, frozen in liquid nitrogen and stored at −80°C. Hormones were purified and analysed according to Dobrev & Kaminek (2002) and Dobrev & Vankova (2012). Samples (c. 200 mg) were homogenized and extracted with methanol : water : formic acid (15 : 4 : 1, v/v/v). The following labelled internal standards (10 pmol−1 sample) were added: 13C6-IAA (Cambridge Isotope Laboratories, Tewksbury, MA, USA), 2H6-SA (Sigma-Aldrich), 2H2-GA1, 2H2-GA19, 2H6-ABA, 2H5-transZ, 2H5-transZR, 2H5-transZ7G, 2H5-transZ9G, 2H5-transZOG, 2H5-transZROMP, 2H5-DHZ, 2H5-DHZR, 2H5-DHZ9G, 2H6-ip, 2H5-ipR, 2H6-IP7G, 2H6-ip9G, and 2H6-IPRMP (Olchemim, Olomouc, Czech Republic). Extracts were purified using a SPE-C18 column (SepPak-C18; Waters, Milford, MA, USA) and separated on a reverse-phase cation-exchange SPE column (Oasis-MCX; Waters). The first hormone fraction was eluted with methanol (contains ABA and other acidic hormones); the second fraction, eluted with 0.35 M NH4OH in 70% methanol, contained cytokinin metabolites. Both fractions were separated by high-performance liquid chromatography (HPLC; Ultimate 3000, Dionex, Sunnyvale, CA, USA) and the hormones were quantified using a hybrid triple quadrupole/linear ion trap mass
RNA was extracted from whole roots using a Qiagen RNA Plant Mini Kit according to manufacturer’s instructions including DNA digestion with DNase I (Qiagen). RNA was analysed using a Nanodrop 2000c Spectrophotometer (Peqlab, Erlangen, Germany), and cDNA synthesis was performed using SuperScriptIII reverse transcriptase (Invitrogen) according to the manufacturer’s instructions. Primers for hormone and defence marker genes are shown in Supporting Information Table S1. For the reference instructions. Primers for hormone and defence marker genes are reverse transcriptase (Invitrogen) according to the manufacturer’s instructions. The final reaction volume was 25 µl, containing 12.5 µl SYBR Green reaction kit (Invitrogen), 0.5 µl 10 nM primers, 9.5 µl ddH2O, and 2 µl of cDNA template. The PCR reaction was conducted in 40 cycles: 95°C for 10 min, each cycle 95°C for 15 s, 60°C for 60 s. Changes in transcript abundance were calculated using 2^ΔΔCt method (Schmittgen & Livak, 2008). Three independent biological replicates (pools of several individual plants) were tested in technical triplicates (averaged prior calculations).

GeneChip

Twelve-day-old Arabidopsis plants, grown as described earlier, were infected with H. schachtii. Ten hours after inoculation, small root pieces containing nematodes during the migration phase performing stylet movements were cut out. Corresponding root segments from uninfected plants were used as controls. RNA was isolated using a Nucleospin RNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions. The quality and purity of total RNA were confirmed by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA synthesis was performed with NuGEN’s Applause 3’-Amp System (NuGEN, San Carlos, CA, USA) according to the manufacturers’ instructions. NuGEN’s Encore Biotin Module (NuGEN) was used to fragment cDNA. Hybridization, washing and scanning were performed according to Affymetrix 3’ GeneChip Expression analysis technical manual (Affymetrix, Santa Clara, CA, USA). Three chips each were hybridized for control and infected samples, with each microarray representing an independent biological replicate. Primary data analysis was performed with the Affymetrix software Expression Console v1 using the MAS5 algorithm.

Statistical analysis of microarray data

Affymetrix .CDF and .CEL files were loaded into the Windows GUI program RMAExpress (http://rmaexpress.bmbolstad.com/) for background correction, normalization (quantile) and summarization (median polish). After normalization, computed Robust Multichip Average (RMA) expression values were exported as log scale to a text file. Probe set annotations were performed by downloading Affymetrix mapping files matching array element identifiers to AGI loci from ARBC (www.arabidopsis.org). Data were analysed using t-test (P<0.05). The results tables include adj-P-values as indicators of statistical significant difference after correction for multiple testing controlling false discovery rate (Benjamini & Hochberg, 1995). Multiple tests were restricted to a subset of 62 genes involved in JA, ET and SA biosynthesis and signalling, which increases the statistical power of correction. The validation of the GeneChip data with qPCR was performed as described earlier and data analysis was done according to Siddique et al. (2014).

Hormone and hormone biosynthesis inhibitor application

The following hormones or hormone donors were used: methyljasmonate (mJA; Sigma-Aldrich), ethephon (Eth; Sigma-Aldrich) and sodium 2-hydroxybenzoate (NaSa; Sigma-Aldrich). The following inhibitors of hormone biosynthesis were used: salicylhydroxamic acid (SHAM; Sigma-Aldrich), aminooxyacetic acid (AOA; Sigma-Aldrich) and L-2-aminoxyo 3-phenylpropionic acid (PAL-Inh; Wako-chemicals, Osaka, Japan). mJA and SA inhibitor (PAL-Inh) were dissolved in EtOH before preparing the stock solutions. All chemicals were filter-sterilized and prepared as stocks containing 0.02% Tween 20 (mJA, SHAM, Eth, NaSa, PAL-Inh at 10 mM and AOA as 25 mM). Before the experiments, different concentrations of the chemicals were tested for phytotoxicity. The final selected concentration did not result in any phenotypical changes (data not shown). Hormones and hormone inhibitors were applied to the shoots of 11-d-old plants under sterile conditions in two droplets onto two leaves per plant at the following concentrations: 60 µM mJA, 150 µM SHAM, 400 µM Eth, 15 µM AOA, 500 µM NaSa and 75 µM PAL-Inh. Distilled water containing 0.02% (v/v) Tween 20 was used as a control. After application, dishes were put back into the growing chamber.

Nematode attraction assays

Nematode attraction assay was performed according to Dalzell et al. (2011). Two percent water agar plates with cylindrical counting wells (8 mm in diameter) connected via cylindrical channels (20 x 2.5 mm) were prepared. Agar discs, containing root exudates from treated and nontreated plants grown on Knop medium as described earlier, were excised from plates in close proximity to the roots. Subsequently they were put into the counting wells. One hundred J2s were placed in the middle of the connecting channel. Six plates for each treatment and each replicate were prepared and placed in the dark at room temperature. After 3.5 h, the number of J2s that reached either one or the other well was counted and classified as attracted by the root exudate of the respective agar disc. Experiments were performed in three independent replicates with six plates each (n = 18). Results were calculated as the attraction rate (%) of the total number of applied nematodes.
Statistical analysis

To test significant differences between the variants, one-way ANOVA and t-test (paired, for attraction assays) were performed using *post hoc* Tukey test. Statistical analysis was conducted using StatGraphics plus 4.0 software (Statpoint Technologies Inc., Warrenton, VA, USA). *P* < 0.05 was used to determine significance.

Results

**Heterodera schachtii** infection triggers changes in endogenous hormone concentrations

Hormone quantification was performed using HPLC-MS to compare nematode-infected and noninfected *Arabidopsis* root. Fig. 1 shows endogenous hormone concentrations at 24 hai covering nematode invasion, migration through the root tissue towards the vascular cylinder and the beginning of syncytium induction. Concentrations of JA and the immediate ET precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), were highly elevated, whereas the concentrations of SA, IAA and active cytokinins (*trans*-zeatin, dihydrozeatin, isopentenyladenine, *cis*-zeatin and their ribosides, act-CKs) were unchanged in the nematode-infected root at this time point. Furthermore, concentrations of ABA and active gibberellin (GA4) were significantly reduced. The concentrations of other hormone metabolites are presented in Table S2.

Early parasitism of *H. schachtii* triggers changes in the transcription of hormone-related genes

To correlate the results of hormone quantification with expression profiles of selected hormone and defence marker genes, time-course qRT-PCR was performed. The following transcripts were determined: *PR-5* (PATHOGENESIS RELATED 5, SA marker), *NPR-1* (SALICYLIC ACID INSENSITIVE 1, key component of SA signalling), *PDF1.2a* (PLANT DEFENSIN 1.2a, JA and ET marker, defence marker), *JAR-1* (JASMONATE RESISTANT 1, jasmonate-isoleucine synthase), *HEL* (HEVEIN LIKE PROTEIN, ET and JA marker, defence marker), and *EIN2* (ETHYLENE INSENSITIVE 2, ET signalling component). This analysis covered nematode root invasion (*c*. 0–6 hai), migration through the root tissue (*c*. 6–12 hai), syncytium induction (*c*. 12–24 hai) and development of young syncytia (*c*. 24–48 hai).

Results shown in Fig. 2 reveal significantly changed expression of *HEL*, which is up-regulated at 12 hai (4.71), subsequently culminates at 24 hai (29.42) and remains elevated until 48 hai (10.59). *EIN2* shows a first slight up-regulation at 24 hai (1.49), followed by its down-regulation at 48 hai (0.71). *PDF1.2a* is first found up-regulated at 24 hai (2.37) and its expression subsequently declines at 48 hai. *JARI* does not show any changes in expression from 6 to 24 hai, whereas at 48 hai it is down-regulated (0.63). *PR-5* shows a slight but not significant up-regulation at 6 hai, whereas at later time points no significant change in its expression is detectable. *NPRI* is slightly up-regulated at both 6 hai (1.67) and 12 hai (1.82), and down-regulated at 48 hai (0.74).

The earlier described experiments showed clear effects of the early parasitism of *H. schachtii* on hormone concentrations, as well as expression of several hormone and defence marker genes in whole nematode-infected roots. To elucidate more specific changes in local gene expression at and around the infection area, we performed a GeneChip analysis. Root segments containing nematodes during the migratory stage at 10 hai were cut out and compared with corresponding uninfected root segments. This particular phase has been chosen, as at this time point, initial significant changes in gene expression were detected. For this study, a subset of 62 genes representing selected JA, ET and SA marker, signalling and biosynthesis genes was extracted (Table 1). To validate these GeneChip results, fold changes obtained for several genes were confirmed by qRT-PCR (Table S3). The entire GeneChip analysis will be published elsewhere (S. Siddique *et al.*, unpublished).

In general, most of the JA-related signalling and biosynthesis genes are up-regulated. In particular, many members of the *LOX* family of enzymes are up regulated at 10 hai (Fig. 2B). *LOX5* and *LOX6*, which are up-regulated by pathogens and jasmonates, are strongly induced at 12 hai (Fig. 2B). This early response is in line with the rapid rise of JA levels observed at 12 hai (Fig. 1).

![Fig. 1](image-url) Hormone quantification (pmol g⁻¹ FW) in *Arabidopsis thaliana* roots infected with *Heterodera schachtii* compared with noninfected control roots. Samples were collected at 24 h after inoculation. JA, jasmonic acid; GA4, gibberellin 4; act-CKs, active cytokinins; SA, salicylic acid; ACC, 1-aminocyclopropane-1-carboxylic acid. Values are means ± SE, n = 4; asterisks indicate significant differences (*, *P* < 0.05).
gene family (e.g. LOX3, LOX4 and LOX6) show increased gene expression, which might indicate the activation of JA synthesis in roots during the migratory stage. We found up-regulation of some ET-related signalling genes, mainly from the ERF gene family (e.g. ERF6 and ERF13). Several genes involved in ET biosynthesis, especially two members of the ACS gene family (ACS2 and ACS6), are up-regulated. SA-related genes show only minor changes in their expression. However, some of the signalling genes and members of the PAL gene family (e.g. PAL1 and PAL2), involved in one of the two possible SA biosynthetic pathways, are up-regulated. We could confirm the up-regulation of the SA-related signalling gene NPR1 during the migratory stage. In the case of two signalling genes, EIN2 and JAR1, GeneChip data confirmed their rather low expression levels. By contrast, ET/JA marker genes HEL and PDF1.2a are up-regulated in the whole infected root as shown by the qRT-PCR; however, according to GeneChip, their expression is not altered locally during the J2s’ migration.

Modulation of hormone concentrations affects attraction, infection and development of *H. schachtii*

Previous experiments indicated that *H. schachtii* triggers changes in concentrations of several endogenous phytohormones in the root as well as in hormone-dependent gene expression during the early infection. Therefore, the effects of artificially altered hormone concentrations on attraction, infection and development of nematodes were tested. JA, ET and SA concentrations in the roots were modified through foliar application of exogenous hormones or by hormone biosynthesis inhibitors. Increase of hormone concentrations was achieved by application of mJA, NaSa and Eth. As, according to the GeneChip, members of the LOX gene family showed increased expression during the migratory stage, we used SHAM, inhibitor of lipoygenases (LOX), the key enzymes in JA biosynthesis. Further, as members of the ACS family were up-regulated during the nematode migration, we used AOA, an inhibitor of aminocyclopropane-carboxylic acid synthases, rate-limiting enzymes of ET biosynthesis. There are two different biosynthetic pathways of SA. One involves isochorismate synthases (ICSs) and the other phenylalanine ammonia lyases (PALs). According to the GeneChip, some members of the PAL gene family were up-regulated, and hence we used PAL-Inh to specifically block the phenylpropanoid pathway responsible for one branch of SA biosynthesis, besides production of other metabolites such as lignin and flavonoids (Hahlbrock & Grisebach, 1979).

As a proof of concept, endogenous hormone concentrations in the roots of chemically treated and control plants were compared. Hormones were quantified 48 h after chemical application. As with the results of mJA treatment, we observed elevated concentrations of JA in roots. Similarly, NaSa treatment resulted in elevated SA concentrations. As expected, Eth did not have any effect on the concentration of ET precursor ACC, as Eth is directly converted to ET in planta, bypassing ACC. The ET biosynthesis inhibitor AOA affected ACC concentrations, which were significantly reduced. Inhibition of LOX and PAL by SHAM and PAL-Inh, respectively, did not impose significant effects on JA and SA concentrations. It is possible that hormone homeostasis was maintained (in the absence of infection) by conjugate hydrolysis or by activation of the alternative biosynthetic route (in the case of SA) (Fig. 3).

To elucidate whether modified hormone concentrations and altered expression of hormone-related genes might change plant susceptibility towards *H. schachtii*, 24 h before inoculation exogenous hormones or their respective biosynthetic inhibitors were applied onto leaves of 11-d-old plants. Subsequently, a nematode attraction assay with agar discs containing root exudates of treated and control plants was performed. Fig. 4(a) shows that the majority of J2s were more attracted to root exudates of Eth-treated plants than to those of nontreated control plants. By contrast, mJA, NaSa, PAL-Inh, SHAM and AOA treatments did not result in major effects. In the second experiment, attractiveness of plants treated with either a hormone or its respective inhibitor was compared. Similarly to previous tests, Eth-treated plants exhibited a significantly higher attractiveness than
Table 1 Selection of jasmonic acid- (JA), ethylene- (ET) and salicylic acid (SA)-related marker, signalling and biosynthesis genes and their fold changes obtained from a GeneChip representing migratory stage of the Heterodera schachtii J2s in roots of Arabidopsis thaliana

| Function | Gene | Accession no. | Fold change | P-value |
|----------|------|---------------|-------------|---------|
| JA       | PDF1.2 | At5g44420 | 1.1946 | 0.3131 |
|          | PDF2.1 | At2g02120 | −1.5691 | 0.2519 |
|          | PR3   | At3g12500 | 2.2006 | 0.1199 |
|          | PR4/   | At3g04720 | 1.4863 | 0.1815 |
|          | HEL   | At1g72260 | −1.1404 | 0.1071 |
|          | THI2.1 | At1g72260 | −1.1404 | 0.1071 |
|          | COI1  | At2g39940 | −1.2203 | 0.0393 |
|          | JAR1  | At2g46370 | −1.3605 | 0.0061 |
|          | JAZ1  | At1g19180 | 4.5502 | 0.0418 |
|          | JAZ5  | At1g17380 | 6.4781 | 0.0454 |
|          | JAZ6  | At1g72450 | 3.4011 | 0.0376 |
|          | JAZ8  | At1g30135 | 15.8334 | 0.0040 |
|          | JAZ10 | At5g13220 | 12.5437 | 0.0073 |
| Biosynthesis | AOC1 | At3g25760 | 9.7309 | 0.0093 |
|          | AOC2  | At4g25780 | 3.3775 | 0.0092 |
|          | AOC4  | At1g13280 | 1.2134 | 0.0458 |
|          | DDE2  | At5g42650 | 3.1624 | 0.0000 |
|          | LOX2  | At3g45140 | −1.0209 | 0.3362 |
|          | LOX3  | At1g17420 | 3.0767 | 0.0107 |
|          | LOX4  | At1g72520 | 4.3060 | 0.0194 |
|          | LOX5  | At3g22400 | −1.0665 | 0.7320 |
|          | LOX6  | At1g67560 | 2.4060 | 0.0041 |
| ET       | PDF1.2 | At5g44420 | 1.1946 | 0.3131 |
|          | PR3   | At3g12500 | 2.2006 | 0.1199 |
|          | PR4/   | At3g04720 | 1.4863 | 0.1815 |
|          | EIL1  | At2g27050 | −1.0886 | 0.2343 |
|          | EIN1  | At5g03280 | −1.3594 | 0.0201 |
|          | EIN2  | At3g20770 | −1.0489 | 0.2597 |
|          | EER4  | At1g17440 | 1.0217 | 0.8271 |
|          | EER5  | At2g19560 | −1.1555 | 0.0785 |
|          | ERF1  | At3g23240 | 2.7546 | 0.0454 |
|          | ERF3  | At1g50640 | −1.0424 | 0.4908 |
|          | ERF4  | At3g15210 | 1.4687 | 0.0458 |
|          | ERF5  | At5g47230 | 1.6311 | 0.0155 |
|          | ERF6  | At4g17490 | 8.1924 | 0.0052 |
|          | ERF7  | At3g20310 | −1.2335 | 0.1230 |
|          | ERF13 | At2g44840 | 5.9034 | 0.0444 |
| Biosynthesis | ACS2 | At1g01480 | 7.8195 | 0.0046 |
|          | ACS4  | At2g22810 | −1.0032 | 0.9700 |
|          | ACS6  | At4g11280 | 4.1610 | 0.0595 |
|          | ACS7  | At4g26200 | 1.3469 | 0.3159 |
|          | ACS8  | At3g37770 | 1.2855 | 0.1568 |
|          | ACS9/ETO3 | At3g39700 | 1.0784 | 0.2960 |
|          | ACS11 | At4g08040 | −1.0160 | 0.9015 |
|          | ETO1  | At3g51770 | −1.2184 | 0.1041 |
|          | ETO2/AC5 | At5g68500 | −1.0955 | 0.2863 |
| SA       | PR1   | At2g14610 | −1.0640 | 0.3459 |
|          | PR2   | At9g57260 | −1.1129 | 0.2464 |
|          | PR5   | At1g75040 | −1.2143 | 0.0464 |
|          | EDS1  | At3g48090 | −1.0742 | 0.3463 |
|          | EDS5  | At4g39030 | 2.7249 | 0.0068 |
|          | NPR1  | At4g26120 | 4.0646 | 0.0115 |
|          | NPR3  | At5g45110 | 1.8615 | 0.0203 |
|          | NPR4  | At4g19660 | −1.1599 | 0.1578 |
|          | PBS3  | At5g13320 | 2.4500 | 0.1118 |

AOA-treated plants. No differences were found between NaSa and PAL-Inh or between mJA and SHAM (Fig. 4b).

As shown in the previous experiments, H. schachtii triggers an elevation of JA and ACC concentrations in roots and induces an elevated expression of ET- and JA-related genes. Moreover, J2s are more attracted to root diffusates collected from Eth-treated plants. In order to investigate the effects of hormone alterations mediated by H. schachtii infection on further nematode attraction, we performed an additional assay using agar discs containing root exudates sampled from infected and noninfected plants. The results show that significantly more J2s moved towards the discs with root diffusates from infected plants (Fig. 4c).

Subsequently, an infection assay was conducted to test whether alterations in endogenous hormone concentrations affect the infection and development of H. schachtii. Infection sites were monitored at three time points: 24, 48 and 72 hai. Males and females were counted at 14 dai and the female : male (F : M) ratio was calculated. This parameter is a meaningful indicator of the developmental conditions provided by the host. It is known that more females than males can develop under optimal nutritional and environmental settings (Triantaphyllou, 1973). Eth treatment is beneficial for nematodes, which infect more quickly (with a c. 20% higher infection rate at 24 hai) when compared with control plants (Fig. 5a). This effect subsequently declines, resulting in no difference in the number of infection sites at 3 dai (data not shown). No significant changes in male and female development (Fig. 5b,c) or in F : M ratio (Fig. 5d) were detected for Eth-treated plants.

Lower concentrations of ACC triggered by AOA treatment resulted in reduced numbers of J2s infecting the roots (Fig. 5a). Accordingly, the counts of female and male nematodes were significantly decreased (Fig. 5b,c); however, there was no effect on F : M ratio (Fig. 5d). After foliar application of mJA, infection rates were lower compared with nontreated plants during the first 24 hai (Fig. 5a). Although the total number of developing nematodes was not altered (males and females together; Fig. 5b), the female counts were significantly reduced (Fig. 5c), leading to decreased F : M ratio (46% lower compared with controls; Fig. 5d). No differences in nematode infection were observed in plants treated with the JA inhibitor SHAM. NaSa treatment, although proven to elevate SA concentrations in roots (Fig. 3b), did not show any significant effects on infection and development of nematodes, except for a significantly reduced F : M ratio (15% lower compared with controls; Fig. 5d). PAL-Inh did not show any impact on H. schachtii development.
Impaired JA biosynthesis has an effect on *H. schachtii* development

Experiments with hormone biosynthesis inhibitors, SHAM, PAL-Inh and AOA, revealed that only AOA significantly affected nematode development. Concerning JA-related mutants and their impact on nematodes, only limited information is available. The foliar SHAM application did not change the JA concentrations in the root and did not trigger any effects on nematodes. Taking this into account we decided to analyse nematode development on JA biosynthesis mutants: *dde2* (allene oxide synthase, a key enzyme in JA biosynthesis) and *lox6* (lipoxygenase involved in the early steps of JA biosynthesis in plastids). As shown in Fig. 6, in roots of both mutant lines, a significantly greater number of females could develop (Fig. 6a) and the F : M ratio in the *lox6* mutant was significantly higher in comparison with the wild-type (Fig. 6b).

**Discussion**

Sedentary plant-parasitic nematodes comprise a large group of important biotrophic endoparasites. In contrast to foliar pathogens, there is considerably less knowledge concerning signalling pathways involved in plant defence effective against these root parasites (Wubben et al., 2008). In mature feeding sites, the plant defence responses are generally suppressed (Szakasits et al., 2009), whereas at the onset of the parasitism they are induced (Puthoff et al., 2003; Alkharouf et al., 2006; Ithal et al., 2007; Mazarei et al., 2011). Hence, these early defence mechanisms are critical and can decide about success or failure of the nematode. However, there is still only limited information on how nematodes challenge plant stress responses at the onset of infection and which mechanisms at later stages lead to the impairment of plant defence. Phytohormones have been proposed to be involved in these processes (reviewed in Goverse & Bird, 2011); however, their exact role during juveniles’ root attraction, penetration, migration and initiation of syncytium formation has been studied only to a minor extent. Therefore, the aim of this study was a broad analysis of possible functions of the main stress-related phytohormones during attraction and early parasitism of *H. schachtii* in Arabidopsis.

**JA triggers early defence responses against *H. schachtii***

We showed that endogenous JA concentrations are strongly elevated during early nematode parasitism, which is additionally
confirmed by the significant up-regulation of several genes associated with JA biosynthesis. In addition, our expression studies revealed an up-regulation of some JA/ET marker genes such as PR-3 and PR-4 (HEL) during the migration stage. However, Hamamouch et al. (2011) did not observe any changes in their expression at later stages of *H. schachtii* infection (5 dai and later). Interestingly, the same authors showed an elevated expression of PR-3 in roots infected with RKN. Ithal et al. (2007) performed a study of the CN *H. glycines* on soybean and, in contrast to our results for the migratory stage (6–12 hai), observed a down-regulation of genes encoding JA biosynthetic enzymes in developing syncytia. The authors concluded that an active suppression of JA biosynthesis and signalling plays an important role during syncytium formation and thus nematode development. Similarly, Ji et al. (2013) also detected suppression of JA biosynthesis in 7 and 14 dai giant cells of RKN. On the other hand, RKN susceptibility of tomato does not depend on JA biosynthesis but requires an intact JA signalling pathway via Coi-1 (Bhattarai et al., 2008).

These differences in the activation of JA-dependent pathways are intriguing. They are a result not only of the different plant species used but also of the very distinct way of infecting the host root by CNs and RKNs. Infective juveniles of RKNs cause much less tissue damage as a result of intercellular migration within the root in comparison to intracellular invasion of CNs. This could explain the different roles of JA in signalling during early parasitism of both genera in different hosts.

Further, we showed that artificially elevated JA concentrations in roots led to a reduction, whereas JA biosynthesis mutants led to an enhancement, of female development. Similar to these results, it was shown that exogenous JA treatments of spinach, oat and tomato increased resistance against different nematode species (Thurau et al., 2003; Soriano et al., 2004; Cooper et al., 2005). The JA pathway was also found to play a significant role in defence against the RKN *Meloidogyne graminicola* in rice, while mJA application reduced gall number and JA-deficient mutant plants were more susceptible (Nahar et al., 2011).
Here, we demonstrate that the invaded plant produces elevated amounts of JA during nematode migration to defend itself, which is supported by an up-regulation of several JA biosynthetic genes, such as LOX and AOS. At the same time, the nematode attempts to switch off the JA-based plant defence responses by triggering the up-regulation of genes that are known to suppress plant JA signalling, such as JAZ8 (Shyu et al., 2012), as well as the repression of JA signalling via Coi1 and Jar1. In the later stages, as a result of the down-regulation of, for example, LOX genes, the JA biosynthesis is suppressed in soy plants infected with *H. glycines* (Ithal et al., 2007). Based on these results, we suggest that in compatible interactions at the onset of CN infection, the nematode is able to suppress JA-dependent plant defence. This is supported by the fact that the artificial elevation of JA concentrations interferes with nematodes’ ability to suppress JA biosynthesis and signalling, resulting in a significantly decreased number of developing females. On the other hand, to elucidate the effects of decreased JA concentrations on *H. schachtii*, we performed an infection assay with two mutants deficient in JA biosynthesis, dde2 (AOS) and lxo6. As expected, the results revealed an increased female development on both mutants in comparison to the wild-type. In addition, lxo6 showed a significantly enhanced F : M ratio. Our results are in line with findings recently published by Ozalvo et al. (2014) showing another lipoygenase mutant (*LOX4*) to be similarly more susceptible to *H. schachtii*. We conclude that in compatible plant–nematode interactions, JA is a main player during early plant defence; however, the nematode contrived to suppress and overcome JA-related defence responses and successfully infect the host.

**ET plays a pivotal and positive role in nematode attraction**

The existing data concerning the role of ET in nematode defence and development are complex and often contradictory, very likely because of its pleiotropic role in defence and development, where ET seems to play different roles at different stages of nematode infection (reviewed in Kyndt et al., 2013). For instance, Wubben et al. (2001) found root exudates of ET-overproducing mutants to be more attractive to J2s. Similarly, in our attraction assays, Eth-treated plants were more attractive and could be infected much faster, resulting in a higher infection rate. Thus, the high attractiveness of roots with elevated ACC/ET concentrations towards infective J2s suggests a pivotal role of the ET pathway during the host-finding process. Moreover, in preinfected plants, this indirect effect might be a result of altered plant defence as well as significantly elevated ET concentrations, which are perceived by following juveniles as clear evidence that the roots are already successfully infected and therefore that the plant is most probably a suitable host. In contrast to CN, Fudali et al. (2013) found that Arabidopsis roots treated with an ET inhibitor are more attractive, whereas ET-overproducing mutants are less attractive to juveniles of the RKN *Meloidogyne hapla*. These disparities might be the result of a differing experimental setup (‘medium plugs’ vs living roots, respectively), and the fact that CNs have a narrow host range and probably respond to a different set of factors responsible for the attraction to host roots than RKNs do (Fudali et al., 2013).

Interestingly, although ET plays a positive role in CN attraction, at later stages its suppression in syncytia indicates a rather negative function for nematode development, which is supported by studies of Mazarei et al. (2003) and Ali et al. (2013). The ethylene-responsive element binding protein gene GmEREBP1 was found to be down-regulated in susceptible soybean infected with CN, whereas it is up-regulated in resistant roots (Mazarei et al., 2003). Similarly, it was shown that the ET-responsive RAP2.6 gene is down-regulated in syncytia and its overexpression leads to enhanced resistance, which the authors suggest is a result of the activated JA pathway and callose deposition (Ali et al., 2013).

On the other hand, older studies by Glazer et al. (1983, 1985) showed, for RKN, that enhanced ET concentrations support giant cell enlargement, whereas chemical blocking of ET inhibits their development. Similarly, for CNs, Goverse et al. (2000) and Wubben et al. (2001, 2004) found evidence that an intact ET pathway is required for successful root colonization and syncytial development. The authors showed that ET-insensitive Arabidopsis mutants as well as the application of an ET inhibitor led to a significant decrease in the number of *H. schachtii* females, similar to our results after AOA application. Moreover, ET-overproducing mutants and the application of ACC increased female development (Wubben et al., 2001, 2004). The authors suggest that the hypersusceptibility could be partially attributed to the increased juvenile attraction, rather than to better developmental conditions. After Eth treatment, we did not observe any differences in female development, which could be a result of the application method used and the following time-dependent attenuation of the effect.

Based on our results and previous studies, we conclude that the role of ET is strongly dependent on the stage of parasitism, shifting from a positive role in attraction and root invasion to a negative role in further nematode development.

**SA acts as a negative regulator during syncytium and female development**

It is generally accepted that the SA pathway is predominantly effective against biotrophic pathogens (Pieterse et al., 2009). Wubben et al. (2008) showed that SA marker genes PR-2 and PR-5 are induced in infected roots from 3 dai on. PR-1 expression as well as endogenous SA concentration, however, remained unaltered. Hamamouch et al. (2011) found up-regulation of PR-1, PR-2 and PR-5 in roots infected with *H. schachtii* at 5 dai and at later time points. Here, we show that neither the concentrations of endogenous SA in nematode-infected roots at 24 hai nor the transcript abundance of the SA marker gene PR-5 from 6 hai on were significantly altered. As shown by Wubben et al. (2008) on mutants defective in SA accumulation or signalling (*sid2-1, pad4-1* and *NahG*), a significantly greater number of *H. schachtii* females could develop. To test whether elevated SA concentrations affect host susceptibility during early infection, we performed nematode attraction and infection assays on NaSa-treated plants. This treatment, similar to the results of Wubben et al. (2008), triggered a lower F : M ratio but did not cause any other effects on *H. schachtii* attraction and infection rates. In the
case of Pal-Inh, blocking one of the two SA biosynthetic pathways as well as other metabolites produced by PAL, for example lignin, we would expect an increase in plant defence against CN. This treatment, however, did not show any significant effects on nematodes. Therefore, we speculate that either PAL does not play an eminent role during early infection of *H. schachtii* or our experimental setup was not optimal. Foliar application of NaSa and PAL-Inh might trigger only short transient effects in roots. The use of mutant lines or growth medium directly supplemented with the chemicals ensuring their constant delivery to the root could result in significant changes in nematode susceptibility.

Taken together, these results suggest that well-established parasitism of CN requires a local suppression of SA signalling and that SA does not play a major role during early *H. schachtii* infection but rather acts as a negative regulator during later phases of parasitism, when syncytium matures and nematodes differentiate sexually.

The role of other stress-related hormones during early nematode infection

In addition to the three main stress hormones, ABA, GA, auxins and cytokinins play pivotal roles in fine-tuning of hormone-based signalling in plants, for instance as modulators of the SA/JA signalling backbone (Pieterse et al., 2012). Here, we show that concentrations of ABA, together with its catabolites, as well as the IAA precursor IAN and oxidated IAA, active GAs and cytokinins were decreased in nematode-infected roots compared with control roots. Generally, ABA is associated with responses to abiotic stresses (Cutler et al., 2010), but it has also recently been suggested to act as an important fine-tuning regulator of defence responses in various plant–pathogen interactions (Mauch-Mani & Mauch, 2005; Studham & McIntosh, 2012). ABA is known to counteract the SA and ET/JA basal defence and to suppress ET action after pathogen infection. Nahar et al. (2012) described the negative role of ABA in rice defence against the migratory nematode *Hirschmanniella oryzae*, while Karimi et al. (1995) found a lower reproduction rate of *Meloidogyne incognita* on ABA-treated potato plants. Our results suggest that reduced ABA concentrations in *H. schachtii*-infected Arabidopsis roots might result from the ABA/ET counteraction.

Gibberellins are known to regulate many developmental processes throughout the plant’s life (De Bruyne et al., 2014), amongst others, plant immunity, where they allow appropriate modulation of defence responses (Denance et al., 2013; De Bruyne et al., 2014). Accordingly, Kyndt et al. (2012) found strongly induced GA concentrations in rice infected with the RKN *M. graminicola* at 3 dai, and Klink et al. (2007) detected an up-regulation of GA biosynthesis genes in soybean infected with *H. glycines*. By contrast, in our system at an earlier time point, GA concentrations are significantly reduced, which is in line with findings by Bar-Or et al. (2005) and Puthoff et al. (2003) showing genes involved in GA deactivation to be up-regulated in RKN- and CN-infected plants. These rather fragmentary findings show that the putative role of GA in defence against nematodes in different plant systems varies and needs to be studied in more detail.

Similarly, auxins and cytokinins are known to function in regulation of plant growth and in response to abiotic stresses, and have recently emerged as key players in plant–pathogen interactions (Mauch-Mani & Mauch, 2005; Kazan & Manners, 2009; Fu & Wang, 2011). In the case of CNs (Goverse et al., 2000; Grunewald et al., 2009) and RKNs (Karczmarek et al., 2004; Kyndt et al., 2012), it was shown that feeding site initiation and development are auxin-dependent. Here, we found slightly reduced concentrations of IAA and a significantly reduced concentration of the IAA precursor IAN at the very early phase of *H. schachtii* parasitism. In accordance with many reports, we propose that auxin does not play a central role during early defence, but rather later in syncytium induction and development. Cytokinins, which act in combination with auxin, are suppressed by the RKN *M. graminicola* infection at 3 dai, whereas at later developmental stages, their levels are induced (Lohar et al., 2004; Kyndt et al., 2012). Kyndt et al. (2012) postulated that changes in cytokinin concentrations caused by RKNs could be important for swelling of the root meristem and might additionally be necessary for the conversion of galls into nutrient sinks. As, at 24 hai, we also found reduced concentrations of cytokinins, we propose that they play a rather minor role in defence, but that their function in *H. schachtii* infection might be similar to their role in galls induced by RKNs.

The presented results clearly show that JA does play an important role during early plant defence against *H. schachtii*. However, in compatible interactions, the parasite is able to suppress and overcome JA-related defence responses and successfully infect the host. For ET, we conclude that its role is strongly dependent on the stage of parasitism. Elevated ET concentrations in roots are highly attractive to the nematode; however, existing data suggest its negative role in further nematode development. Further, CN establishment requires local suppression of SA signalling, whereas SA does not play a major role during early *H. schachtii* attraction and infection, but rather acts as a negative regulator during later phases of parasitism. Taken together, our results indicate that nematodes are able to trigger sophisticated changes in hormone biosynthetic and signalling pathways in strict dependence on their parasitism stage.

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References

Abad P, Williamson VM. 2010. Plant nematode interaction: a sophisticated dialogue. *Advances in Botanical Research* 53: 147–192.

Ali MA, Abbas A, Kreil DP, Behlmann H. 2013. Overexpression of the transcription factor RAP2.6 leads to enhanced callose deposition in syncytia and enhanced resistance against the beet cyst nematode *Heterodera schachtii* in Arabidopsis roots. *BMC Plant Biology* 13: 1471–2229.
Both induction and morphogenesis of cyst nematode feeding cells are mediated by
Heterodera schachtii in Arabidopsis thaliana. By 2013, Grebner W, Stingl NE, Oenel A, Mueller MJ, Berger S. 2013. Lipoygenase 6-
dependent oxylipin synthesis in roots is required for abiotic and biotic stress resistance of Arabidopsis. Plant Physiology 161: 2159–2170.

Grunewald W, van Noorden G, Van Isterdael G, Beeckman T, Gheysen G. Mathiessus U. 2009. Manipulation of auxin transport in plant roots during Rhizobium symbiosis and nematode parasitism. Plant Cell 21: 2553–2562.

Gutjahre P, Paszkowski U. 2009. Weights in the balance: jasmonic acid and salicylic acid signaling in root-biotrophic interactions. Molecular Plant – Microbe Interactions 22: 763–772.

Halberek K, Grisebach H. 1979. Enzymic controls in the biosynthesis of lignin and flavonoids. Annual Review of Plant Physiology 30: 105–130.

Hamamouch N, Li C, See PJ, Park CM, Davis EL. 2011. Expression of Arabidopsis pathogenesis-related genes during nematode infection. Molecular Plant Pathology 12: 355–364.

Hofmann J, Grundler FWM. 2007. Identification of reference genes for qRT-
PCR studies of gene expression in giant cells and syncytia induced in Arabidopsis thaliana by Meloidogyne incognita and Heterodera schachtii. Nematologica 53: 317–323.

Ithal N, Recknor J, Nettleton D, Maier T, Baum T, Mitchum MG. 2007. Developmental transcript profiling of cyst nematode feeding cells in soybean roots. Molecular Plant–Microbe Interactions 20: 510–525.

Ji H, Gheysen G, Denil S, Lindsey K, Topping JF, Nakar K, Haegeman A, De Vos WH, Trooskens G, Van Creveld W et al. 2013. Transcriptional analysis through RNA sequencing of giant cells induced by Meloidogyne graminicola in rice roots. Journal of Experimental Botany 64: 3885–3898.

Jung C, Wys U. 1999. New approaches to control plant parasitic nematodes. Applied Microbiology and Biotechnology 51: 439–446.

Jürgens K. 2001. Untersuchungen zum Assimilat- und Wassertransfer in der Interaktion zwischen Arabidopsis thaliana und Heterodera schachtii. PhD thesis, Christian-Albrechts Universität, Kiel, Germany.

Karczmarek A, Overmars H, Helder J, Goverse A. 2004. Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. Molecular Plant Pathology 5: 343–346.

Karimi M, Van Montagu M, Gheysen G. 1995. Exogenous application of abscisic acid to potato plants suppresses reproduction of Meloidogyne incognita. Mededelingen van de Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent 60: 1033–1035.

Karan K, Manners JM. 2009. Linking development to defense: auxin in plant–pathogen interactions. Trends in Plant Science 14: 373–382.

Klink VP, Overall CC, Alkharrouf NW, MacDonald MH, Matthews BF. 2007. Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean (Glycine max) roots infected by the soybean cyst nematode (Heterodera glycines). Plant Pathology 56: 1389–1409.

Kunkel BN, Brooks DM. 2002. Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology 5: 325–331.

Kvndt T, Denil S, Haegeman A, Trooskens G, Batters L, Van Creveld W, De Meyer T, Gheysen G. 2013. Transcriptional reprogramming by root knot and migratory nematode infection in rice. New Phytologist 196: 887–900.

Kvndt T, Vieira P, Gheysen G, de Almeida-Engler J. 2013. Nematode feeding sites: unique organs in plant roots. Plant Science 238: 807–818.

Lohar DP, Schaff JE, Laskey JG, Kiefer JJ, Bilyeu KD, Bird DM. 2004. Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. Plant Journal 38: 203–214.

Mazarei M, Lennon KA, Puthoff DP, Rodermel SR, Baum TJ. 2003. Expression of an Arabidopsis phosphoglycerate mutase homologue is localized to apical meristems, regulated by hormones, and induced by sedentary plant-parasitic nematodes. Plant Molecular Biology 53: 513–530.
Mazarei M, Liu W, Al-Ahmad H, Arelli PR, Pantalone VR, Stewart CN Jr. 2011. Gene expression profiling of resistant and susceptible soybean lines infected with soybean cyst nematode. *Theoretical and Applied Genetics* 123: 1193–1206.

Nahar K, Kyndt T, De Vleeschauwer D, Hoft M, Gheysen G. 2011. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant Physiology* 157: 305–316.

Nahar K, Kyndt T, Nzogela YB, Gheysen G. 2012. Abscisic acid interacts antagonistically with classical defense pathways in rice–migratory nematode interaction. *New Phytologist* 196: 901–913.

Ozalvo R, Cabrera J, Escobar C, Christensen SA, Borrego EJ, Kolomiets MV, Castresana C, Iberkleid I, Brown Horowitz S. 2014. Two closely related members of Arabidopsis 13-lipoxygenases (13-LOXs), LOX3 and LOX4, reveal distinct functions in response to plant-parasitic nematode infection. *Molecular Plant Pathology* 15: 319–332.

Pieterse CM, Leon-Reyes A, Van der Ent S, Van Wees SC. 2009. Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology* 5: 308–316.

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC. 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* 28: 489–521.

Pathoff DP, Nettleton D, Rodermel SR, Baum TJ. 2003. *Arabidopsis* gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. *Plant Journal* 33: 911–921.

Schmittgen TD, Livak KJ. 2008. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* 3: 1101–1108.

Shyu C, Figueroa P, Dpew CL, Cooke TF, Sheard LB, Moreno JE, Katsis L, Zheng N, Browse J, Howe GA. 2012. JAZ8 lacks a canonical degron and has an EAR motif that mediates transcriptional repression of jasmonate responses in *Arabidopsis*. *Plant Cell* 24: 536–550.

Siddique S, Matera C, Radakovic ZS, Hasan MS, Gutbrod P, Rozanska E, Sobczak M, Torres MA, Grundler FMW. 2014. Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen species that limit plant cell death and promote infection. *Science Signaling* 7: 320.

Sijmons PC, Grundler FMW, von Mende N, Burrows PR, Wyss U. 1991. *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. *Plant Journal* 1: 245–254.

Soriano IR, Asenstorfer RE, Schmidt O, Riley IT. 2004. Inducible flavone in oats (*Avena sativa*) is a novel defense against plant-parasitic nematodes. *Phytopathology* 94: 1207–1214.

Staswick PE, Yuen GY, Lehman CC. 1998. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. *Plant Journal* 15: 747–754.

Studham ME, MacIntosh GC. 2012. Phytohormone signaling pathway analysis method for comparing hormone responses in plant–pest interactions. *BMC Research Notes* 5: 392.

Swiecicka M, Filipecki M, Lont D, Van Vliet J, Qin L, Goverse A, Bakker J, Helder J. 2009. Dynamics in the tomato root transcriptome on infection with the potato cyst nematode *Globodera rostochiensis*. *Molecular Plant Pathology* 10: 487–500.

Szakasits D, Heinen P, Wieczorek K, Hofmann J, Wagner F, Kreil DP, Sykacek P, Grundler FM, Bohlmann H. 2009. The transcriptome of syncytia induced by the cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. *Plant Journal* 57: 771–784.

Thurai T, Kifle S, Jung C, Cai D. 2003. The promoter of the nematode resistance gene *Hil1pro*–1 activates a nematode-responsive and feeding site-specific gene expression in sugar beet (*Beta vulgaris* L.) and *Arabidopsis thaliana*. *Plant Molecular Biology* 52: 643–660.

Triantaphyllou AC. 1973. Gametogenesis and reproduction of *Meloidogyne graminisi* and *M. asteronomi* (Nematoda: *Heteroderidae*). *Journal of Nematology* 5: 84–87.

Vijayan P, Shockey J, Levesque CA, Cook RJ, Browse J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* 95: 7209–7214.

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* 17: 1784–1790.

Wubben MJ, Jin J, Baum TJ. 2008. Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. *Molecular Plant – Microbe Interactions* 21: 424–432.

Wubben MJ II, Rodermel SR, Baum TJ. 2004. Mutation of a UDP-glucose-4-epimerase alters nematode susceptibility and ethylene responses in *Arabidopsis roots*. *Plant Journal* 40: 712–724.

Wubben MJ II, Su H, Rodermel SR, Baum TJ. 2001. Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in *Arabidopsis thaliana*. *Molecular Plant – Microbe Interactions* 14: 1206–1212.

Wyss U. 1997. Root parasitic nematodes: an overview. In: Fenoll C, Grundler FMW, Ohl SA, eds. *Cellular and molecular aspects of plant nematode interactions* vol. 10. Dodrecht, the Netherlands: Kluwer Academic Publishers, 5–24.

**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Table S1** Primers for hormone and defence marker genes used in quantitative real-time (qRT) PCR

**Table S2** Hormone quantification in *Arabidopsis thaliana* roots infected with *Heterodera schachtii* in comparison with non-infected roots

**Table S3** Validation of GeneChip data by quantitative real-time (qRT) PCR

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