Conserved nematode signalling molecules elicit plant defenses and pathogen resistance

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Plant-defense responses are triggered by perception of conserved microbe-associated molecular patterns (MAMPs), for example, flagellin or peptidoglycan. However, it remained unknown whether plants can detect conserved molecular patterns derived from plant-parasitic animals, including nematodes. Here we show that several genera of plant-parasitic nematodes produce small molecules called ascarosides, an evolutionarily conserved family of nematode pheromones. Picomolar to micromolar concentrations of ascr#18, the major ascaroside in plant-parasitic nematodes, induce hallmark defense responses including the expression of genes associated with MAMP-triggered immunity, activation of mitogen-activated protein kinases, as well as salicylic acid- and jasmonic acid-mediated defense signalling pathways. Ascr#18 perception increases resistance in Arabidopsis, tomato, potato and barley to viral, bacterial, oomycete, fungal and nematode infections. These results indicate that plants recognize ascarosides as a conserved molecular signature of nematodes. Using small-molecule signals such as ascarosides to activate plant immune responses has potential utility to improve economic and environmental sustainability of agriculture.

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he recognition of specific molecular patterns has been shown to play a central role in the immune responses of plants and animals<sup>1,2</sup>. Both plants and animals possess pattern recognition receptors that serve to detect several different molecular signatures associated with specific classes of microbes. For example, <i>Arabidopsis</i> recognize bacteria using specific pattern recognition receptors for flagellin, lipopolysaccharide, peptidoglycan and other MAMPs<sup>3,4</sup>. MAMPs are often perceived at low concentrations and induce specific defense responses. Perception of MAMPs, has been shown to involve conserved signal-transduction mechanisms, including activation of mitogen-activated protein kinases (MAPKs), generation of reactive oxygen species and activation of salicylic acid (JA)-signalling pathways<sup>4-6</sup>.

Nematodes are arguably the most numerous animals on earth<sup>7</sup>. They are ubiquitous in soil and parasitize most plants and animals. Plant-parasitic nematodes cause agricultural damage of more than $100 billion annually worldwide<sup>8</sup>. Several studies have shown that plants respond to plant-parasitic nematode inoculation by rapidly activating defense pathways similar to those induced by other pathogens in plants<sup>9-11</sup>. However, the nature of the nematode-derived signals that are perceived by plants has remained unknown. Interestingly, entomopathogenic nematodes, which do not parasitize plants, can also induce plant defenses, including expression of <i>PATHOGENESIS-RELATED PROTEIN-1</i> (PR-1) and increased catalase and peroxidase activity<sup>12</sup>. This suggests that recognition of a conserved nematode signature molecule could be responsible for the plant’s defense response.

Recent work has shown that nematodes rely on an evolutionarily conserved family of signalling molecules, the ascarosides, to regulate development and social behaviours<sup>3-21</sup>. Ascarosides are glycosides of the dideoxysugar ascarylose that carry a fatty acid-derived lipophilic side chain and have been identified exclusively from nematodes. For example, in the model organisms <i>Caenorhabditis elegans</i><sup>22</sup> and <i>Pristionchus pacificus</i><sup>23</sup>, as well as in the insect-parasitic nematode <i>Heterorhabditis bacteriophora</i>, species-specific blends of ascarosides regulate entry into stress-resistant dispersal or infective larval stages. Different structural variants are associated with starkly different activity profiles, and biological activity is frequently observed at very low concentrations.<sup>18</sup>

More than 200 different ascaroside structures from over 20 different nematode species have been identified, demonstrating that ascarosides represent a highly conserved molecular signature of nematodes<sup>18</sup>. These findings suggested that plant and animal hosts of nematodes, as well as nematode-associated microorganisms, may have evolved the means to detect and respond to this ancient nematode signature. Notably, nematophagous fungi, which are natural predators of soil-dwelling nematodes, have recently been shown to initiate the formation of specialized trapping devices in response to ascarosides<sup>24,25</sup>.

In this study, we investigated whether ascarosides are perceived by plants and modulate plant-defense responses. We first characterized the ascaroside profiles of several agriculturally important species of plant-parasitic nematodes, including root-knot and cyst nematodes, which are the two most-damaging groups<sup>26</sup>. We then assessed whether ascarosides produced by plant-parasitic and other nematodes affect plant immunity. Ascarosides were found to trigger conserved defense responses in leaves and in roots, including the SA- and JA-mediated signalling pathways. Moreover, treatment with ascarosides enhances resistance to certain viral, bacterial, fungal and oomyocyte pathogens, as well as to root-knot and cyst nematodes in <i>Arabidopsis</i>.

**Results**

**Plant-parasitic nematodes produce ascarosides.** To investigate the possibility that plants respond to ascarosides, we first characterized the ascaroside profiles produced by several genera of plant-parasitic nematodes. We used media supernatant samples to analyze the excreted metabolome (‘exo-metabolome’) of infective juveniles of three species of root-knot nematodes, <i>Meloidogyne incognita</i>, <i>M. javanica</i> and <i>M. hapla</i>, as well as cyst (Heteroderda glycines) and lesion (<i>Pratylenchus brachyurus</i>) nematodes, using a recently developed sensitive and selective mass spectrometric (MS) screening method<sup>27</sup> (Fig. 1). MS analysis of <i>exo-metabolome</i> samples revealed excretion of similar sets of ascarosides in all analyzed species. In <i>Meloidogyne</i> spp., ascr#18, a compound featuring an 11-carbon side chain, was most abundant, followed by compounds with longer carbon side chains (Fig. 1a–c; Supplementary Fig. 1 and Supplementary Table 1). Concentrations of ascr#18 in the analyzed <i>Meloidogyne</i> spp. culture media samples were variable and ranged from 5 nM to 100 nM. Analysis of <i>H. glycines</i> and <i>P. brachyurus</i> metabolome samples also revealed ascr#18, albeit in smaller amounts than in <i>Meloidogyne</i> spp. (Supplementary Table 1). <i>Exo-metabolome</i> samples of adult <i>M. hapla</i>, <i>H. glycines</i> and <i>P. brachyurus</i> contained trace amounts of ascr#18, whereas the other ascarosides found in infective juveniles could not be detected in adults. These results show that plant-parasitic nematodes, like most other previously analyzed nematode species<sup>19</sup>, produce ascarosides. Notably, the analyzed species from three genera of plant-parasitic nematodes all produce ascr#18 as the most abundant ascaroside. Ascr#18 had previously been identified as a minor component of the ascaroside profile produced by the model organism <i>C. elegans</i><sup>27</sup> and is also produced by entomopathogenic nematodes<sup>19,20</sup>.

Ascr#18 induces defense responses and enhances resistance. On the basis of the finding that ascr#18 is produced by all analyzed species of plant-parasitic nematodes, we asked whether this ascaroside is perceived by plants and affects plant-defense responses to diverse pathogens. Because ascarosides as the nematode signalling molecules may have direct effects on nematode pathogens that could confound detection of plant responses to ascr#18, we began by testing the effect of ascr#18 on defense responses of <i>Arabidopsis</i> to a bacterial (<i>Pseudomonas syringae</i> pv. tomato) and a viral pathogen (Turnip Crinkle Virus—TCV; Fig. 2). Since plants would naturally encounter nematodes via their roots, <i>Arabidopsis</i> roots were partially immersed in water containing different concentrations of ascr#18 for 24 h before leaves were inoculated with the pathogens. Root treatment with 1 µM ascr#18 reduced growth of virulent <i>P. syringae</i>, whereas a higher ascr#18 concentration (5 µM) was less effective (Fig. 2a). Root treatment with ascr#18 at 1 µM also enhanced resistance to virulent TCV (Fig. 2b–d, Supplementary Fig. 2). Viral replication, as measured by the amount of viral coat protein (CP) in leaves (Fig. 2b), was reduced in both inoculated and distal leaves. Moreover, systemic spread of the virus was nearly abolished with only a trace of CP present in distal leaves of ascr#18-pretreated plants. Disease symptoms, including development of chlorosis on the inoculated leaves (Fig. 2c), leaf curling/crinkling and suppression of inflorescence development (Fig. 2d), were also reduced in ascr#18-pretreated plants.

To further characterize <i>Arabidopsis</i> response to ascr#18, we monitored expression of MAMP-triggered immunity (MTI) markers and defense-related genes in leaves at different time points after root treatment with ascr#18. MAPKs and calcium-dependent protein kinase are key components of signalling pathways that regulate recognition of MAMPs by plants<sup>5,28</sup>.
We measured induction of the MAPK-related Flg22-INDUCED RECEPTOR KINASE1 (FRK1) and the calcium-dependent protein kinase-related PHOSPHATE-INDUCED1 (PHI1) MTI marker genes28. Pretreatment of roots with ascr#18 induced increased transcript levels in the leaves of FRK1 at 6 h post treatment (h.p.t.) and PHI1 at 24 h.p.t. (Fig. 2e). In addition, expression of representative biosynthetic or responsive genes for the two major hormones mediating plant immunity, SA and JA, was also affected. Root pretreatment with ascr#18 increased expression of the SA-responsive genes PATHOGENESIS-RELATED-4 (PR-4) and GLUTATHIONE S-TRANSFERASE6 (GSTF6), and the JA-biosynthetic genes LIPOXYGENASE2 (LOX2) and ALLENE OXIDE SYNTHASE (AOS) in leaves (Fig. 2e)6.

Because JA and SA signalling interact with the ethylene (ET) and auxin signalling pathways6, we additionally monitored changes in the expression of five genes associated with ET signalling, as well as five genes associated with auxin signalling in response to root treatment with 1 μM ascr#18 at 24 h.p.t. Of the 10 tested genes, only expression of SAUR-LIKE AUXIN RESPONSE PROTEIN34 (SAUR34) was enhanced (Supplementary Fig. 3), suggesting that ascr#18 treatment does not strongly affect auxin- or ET-regulated defense signalling.

Next we assessed whether plants respond to ascr#18 via their leaves. Leaf infiltration of ascr#18 induced activation of the MAPKs, MPK3 and MPK6, which are early markers for the development of MTI (Fig. 2f, Supplementary Fig. 4)5. MAPK activation was monitored via immunoblot analysis using an antibody that detects phosphorylation of the pTE-pY motif29. We observed a transient increase in the phosphorylation of both MAPKs within 10 min after leaf infiltration with 1 μM ascr#18. In addition, transcripts for the prototypic SA-responsive marker PR-1 and prototypic JA-responsive PDF1.2 genes6 were elevated at 24 h.p.t. after infiltration with 0.3 μM or 1 μM ascr#18 (Fig. 2g, Supplementary Fig. 5). Taken together, our results show that in Arabidopsis, local and systemic defenses are activated in response to ascr#18 application to leaves or roots.

Ascr#18 enhances resistance in dicot and monocot crop plants.

To determine whether ascarioside perception is conserved across the plant kingdom and to test for effects on resistance to eukaryotic pathogens, we measured the effect of ascr#18 on defense responses and pathogen resistance of the dicots tomato (Solanum lycopersicum) and potato (Solanum tuberosum), as well as the monocot barley (Hordeum vulgare). In tomato, pretreatment of roots with 1 nM and 10 nM of ascr#18 for 48 h prior to inoculation provided strong protection against the oomycete pathogen Phytophthora infestans as indicated by the reduction in sporangia

Figure 1 | Identification of ascariosides from plant-parasitic nematodes. (a) Examples of ascariosides previously identified from C. elegans and other nematode species. (b) HPLC-MS analysis of M. hapla exo-metabolome samples, showing ion chromatograms scaled to 100% of the ascarioside peak corresponding to m/z = [M-H]− for seven detected ascariosides. (c) Chemical structures of identified ascariosides and relative quantitative distribution as determined by HPLC-MS. For high-resolution MS data, see Supplementary Table 1. Quantitative ascarioside profiles of M. incognita and M. javanica are shown in Supplementary Fig. 1.
number and lesion size (Fig. 3a,b, Supplementary Fig. 6). Similar to the effect of asc#18 on resistance of Arabidopsis to Pst, protection against P. infestans in tomato decreased at higher concentrations of asc#18. However, maximal protection was observed at asc#18 concentrations (1–10 nM, Fig. 3a), much lower than in the case of Arabidopsis (1 μM, Fig. 2a). Root pretreatment with asc#18 at 10 nM also reduced the growth of Arabidopsis roots with 10 nM asc#18 significantly reduced infection of H. schachtii and root-knot (M. incognita) nematodes (Fig. 4c,d), whereas higher concentrations of asc#18 were not effective. Next we assessed whether treatment of Arabidopsis with nanomolar concentrations of asc#18 affected root expression of the defense-related genes PHI1, FRK1 and PDF1.2, respectively, after syringe infiltration of leaves with asc#18, as measured by qRT-PCR. β-Tubulin was used as internal control. *P ≤ 0.05; **P ≤ 0.005; ***P ≤ 0.0005, two-tailed t-test.

**Asc#18 enhances resistance of Arabidopsis to nematodes.**

Next, we tested whether root exposure to asc#18 affected infection of Arabidopsis by plant-parasitic nematodes. Using a range of asc#18 concentrations, we found that pretreatment of roots with 10 nM asc#18 significantly reduced infection of Arabidopsis with cyst (H. schachtii) and root-knot (M. incognita) nematodes (Fig. 4c,d), whereas higher concentrations of asc#18 were not effective. Next we assessed whether treatment of Arabidopsis with nanomolar concentrations of asc#18 affected root expression of the defense-related genes PHI1, FRK1 and WRKY53, which encodes an immune-modulating transcription factor. We found that all three genes were induced within 6 h of exposure to 10 or 50 nM asc#18, whereas exposure to a higher

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**Figure 2 | Asc#18 enhanced pathogen resistance and activated defense responses in Arabidopsis.** (a) Enhanced resistance to virulent Pseudomonas syringae pv. tomato (Pst) DC3000 after root pretreatment for 24 h with asc#18. Bacterial growth was assayed at 3 d.p.i. Data are averages ± s.d. (n ≥ 3). (b) Quantification of TCV CP in inoculated (local) and uninoculated (systemic) leaves of plants root pretreated for 24 h with asc#18. Leaves were harvested at 2, 3 and 6 d.p.i. for immunoblot analysis with the anti-CP antibody. Coomassie blue staining (CB) served as loading control. (c) TCV-inoculated (local) leaves photographed at 6 d.p.i. (d) TCV-infected plants photographed at 6 d.p.i. (e) Transcript levels as measured by qRT-PCR of defense-gene markers in leaves from plants root pretreated with asc#18 (1 μM). Gene-transcript levels were determined at 6 h.p.t. and 24 h.p.t. Data are average ± s.d. (n = 3). (f) Activation of MAPKs MPK3 and MPK6 in Arabidopsis 10 and 15 min after leaf pretreatment with asc#18. CB served as loading control. (g) Induction of SA and JA marker genes PR-1 and PDF1.2, respectively, after syringe infiltration of leaves with asc#18, as measured by qRT-PCR. β-Tubulin was used as internal control. *P ≤ 0.05; **P ≤ 0.005; ***P ≤ 0.0005, two-tailed t-test.
concentration (300 nM) had no effect or reduced expression (Supplementary Fig. 9).

**Plant responses to other ascarosides.** In order to assess whether plants respond to ascarosides as a compound class, we tested three additional ascarosides whose structures differ from that of ascr#18 in several different ways, for their ability to induce defense responses. For this we selected ascr#3, an ascaroside that includes a conjugated double bond in the side chain, as well as ascr#9 and oscr#9, which both have a much shorter side chain (5 carbon) than ascr#18 (11 carbons, Fig. 1a)17. Similar to ascr#18, leaf infiltration of *Arabidopsis* with either ascr#3 or ascr#9 induced expression of the prototypic SA-responsive PR-1 and JA-responsive PDF1.2 genes, whereas oscr#9 showed no effect (Supplementary Table 2). Moreover, root pretreatment of tomato with ascr#9, like ascr#18, enhanced resistance to *P. infestans*, whereas neither ascr#3 nor oscr#9 affected resistance at the concentration tested. These results show that plants respond to structurally diverse ascarosides, but that responses vary in a structure- and species-dependent manner. Notably, oscr#9, whose structures differ from that of ascr#9 only in the position of the attachment of the side chain to ascaroylase, was inactive in all assays, whereas ascr#9 was active. The possibility that the observed increases in pathogen resistance were due to antibacterial or anti-fungal activity of ascarosides is unlikely since a previous study showed that *C. elegans* metabolome samples containing micromolar to millimolar concentrations of ascarosides have no anti-microbial activity31.

**Discussion**

Our work shows that ascarosides, a class of small molecules specific to nematodes, are perceived by plants as a conserved foreign molecular signature. Analogous to the effects of MAMPs such as flagellin2,28,32, perception of ascarosides by plants as ‘nematode-associated molecular patterns’ leads to activation of conserved immune responses, resulting in enhanced resistance to a broad-spectrum of pathogens and pests. Plants respond to ascr#18, the most abundant ascaroside in plant-parasitic nematodes, at very low concentrations, similar to those required for perception of MAMPs2,23, suggesting that plants have evolved specific receptor(s) for ascaroside perception. Similar to bacterial MAMPs, sensitivity to ascarosides varied between plant species: in tomato, potato and barley 10 nM ascr#18 strongly induced defense-gene expression and enhanced resistance, whereas *Arabidopsis* required much higher concentrations. Notably, in both *Arabidopsis* and tomato, efficacy decreased at the highest tested ascr#18 concentrations. For example, in tomato ascr#18 concentrations of 1–10 nM provided maximal resistance to *P. infestans*, whereas enhancement of resistance was reduced or lost at 100 nM.

A similar decrease of activity at higher concentrations has been observed for ascaroside-mediated phenotypes in nematodes. For example, attraction of male *C. elegans* to hermaphrodite-produced ascarosides, as well as aggregation of *C. elegans* hermaphrodites in response to indole ascarosides is maximal at picomolar concentrations, but decreases markedly at higher concentrations15,34. The cause of the observed decrease in activity at higher ascaroside concentrations is not known. However, it has been suggested that high concentrations of small-molecule ligands can result in unproductive engagement of receptors, for example, by interfering with formation of receptor dimers35, which have been shown to be required for ascaroside perception in *C. elegans*36.

The ascr#18 concentration providing best protection against root infection with the root-knot and cyst nematodes (10 nM) in
Arabidopsis was much lower than the concentration needed for best protection against leaf infection with bacterial and viral pathogens tested (1 μM). This may reflect higher expression of ascaroside receptors in the roots or differences in the distances between the sites of infection from the site of ascr18 application or perception. In addition, the observed reduction of nematode infection levels may be due to direct effects of ascr18 on these nematode species, which remain to be investigated. However, the induction of defense-associated genes in the roots at the effective ascr18 concentrations suggest that the increased resistance is, at least in part, mediated by enhanced immunity. Nanomolar concentrations of ascr18 are representative of those found in culture media samples of the analyzed plant-parasitic nematode species, supporting the biological significance of ascr18 in plant-nematode interactions.

The systemic induction of defense genes and pathogen resistance in leaves following root application suggests that ascr18 itself (or a metabolite of ascr18) moves from the roots to the leaves, and/or that ascr18 induces synthesis of a mobile signal in the roots that then travels to the leaves to activate immune responses. We have not been able to detect ascr18 in the leaf tissue of root-treated plants, but this could be due to limited uptake of the already very low concentrations of ascr18 used in the assays. Although plants primarily encounter ascarosides via their roots, leaf infiltration and foliar spraying with low ascr18 concentrations were also effective at inducing the defense responses. This finding suggests that ascaroside receptors are also present in leaf tissue. Further characterization of plant responses to ascarosides and identification of cognate receptors may reveal how perception of nematode-associated molecular patterns, MAMPs and other molecular patterns converges on triggering plant immunity. The use of small-molecule signals, such as ascarosides, to activate plant immune responses could help improve the economic and environmental sustainability of agriculture.

Methods
Worm sample preparation. Nematode eggs (M. hapla strain VW9, M. incognita VW6, M. javanica VW4 and P. brachyrhizus) were extracted from greenhouse-grown tomato plants, and surface-sterile juveniles were prepared as described in Branch et al.37. H. glycines was raised on greenhouse-grown soybean. Batches of ~ 30,000–100,000 freshly hatched juveniles were incubated for 24 h in an 8 ml sterile water with rotation. After brief centrifugation, the supernatant was filtered through a 22-μm filter then frozen. Filtered supernatants and worm pellets were lyophilized, each extracted with 2 × 5 ml methanol and filtered over cotton wool. Extracts were concentrated in vacuum and resulting residues were resuspended in 150 μl methanol and filtered prior to MS analysis.

MS analysis. Low-resolution HPLC-MS and HPLC-MS/MS was performed using an Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (4 × 250 mm, 5 μm particle diameter) and connected via a diode array detector to a Quattro II mass spectrometer (Micromass/Waters) using a 101 split. A 0.1% acetic acid–acetonitrile solvent gradient was used at a flow rate of 3.6 ml min⁻¹, starting with an acetonitrile content of 5% for 5 min which was increased to 100% over a period of 40 min. Nematode metabolite extracts (prepared as described above) and synthetic samples (see Supplementary Methods and from Reus et al.27) were analyzed by HPLC-ESI-MS in negative and positive ion modes using a capillary voltage of 3.5 kV and a cone voltage of ~ 40 and +20 V respectively. HPLC-MS/MS screening for precursor ions of m/z = 73.0 (negative mode) and neutral loss of 130.0 (positive mode) was performed using argon as collision gas at 2.1 mtorr and 30 eV. To confirm elemental composition of the identified compounds, samples were additionally analyzed by high-resolution mass spectrometry, using a Waters nanoACQUITY UPLC System equipped with a Waters AcQUITY UPLC HSS C18 column (2.1 × 100 mm, 1.8 μm particle diameter) connected to a Xevo G2 QToF Mass Spectrometer. MassLynx software was used for MS data acquisition and processing.

Plant material and growth conditions. Unless otherwise stated, Arabidopsis italiana ecotype Col-0, tomato (Solanum lycopersicum) cultivar M82 and potato (S. tuberosum) cultivars Desiree, Eva and Yukon Gold plants were grown in a growth chamber under 16-h light/8-h dark (22 °C) regime and 70% relative humidity. Tomato and potato plants were grown in growth chambers for 3 weeks and then transferred to greenhouse conditions until they were used.

Ascaroside treatments. Ascarosides were dissolved in ethanol to prepare millimolar stock solutions. Final aqueous ascaroside dilutions were prepared fresh on the day of the experiment. Control solutions contained equal amounts of ethanol (<0.1% for most experiments). For root treatment, plant pots were placed in a tray containing control solution or water supplemented with ascr18. For leaf treatment, three leaves of 3.5-weeks-old Arabidopsis plants were syringe infiltrated with buffer (20 mM Tris pH 6.5) supplemented with an ethanolic solution of ascr18 or buffer containing an equivalent amount of ethanol. For spray treatment, leaves were sprayed with a aqueous 0.02% Tween-20 solution to which either an ethanol sample was added. Sample preparations were separated by electrophoresis in 8% SDS-PAGE. MAPK activation was detected by immunoblot analyses using 1:1,000 dilutions of polyclonal primary antibodies against phospho-p44/42 MAPK (Cat et al.49, 50).
transcripts were amplified using IQ SYBR Green Supermix (Bio-Rad) from 5 elongation factor 1 for constitutively-expressed Control reactions to normalize RT-PCR amplifications were run with the primers Technologies) and amplified using gene-specific primers (Supplementary Table 3). DNAse treatment was done with DNase I (Life Technologies). First-strand cDNA was synthesized from 50 ng of mRNA using ProtoScript II Reverse Transcriptase (NEB). The qRT-PCR assay was carried out in an iCycler iQ Real-Time PCR Detection System (Bio-Rad) and template amplifications were performed using Taq Universal SYBR Green Supermix (Bio-Rad) from 1/10 of 20 × diluted cDNA in a total 20 μl reaction using 1 μl of each 10 μM gene-specific primer (Supplementary Table 3). All assays consisted of three technical replicates for each RNA sample. Data was analyzed using the iCycler iQ Real-Time PCR Detection System Software version 3.0a (Bio-Rad). The Arabidopsis ACP gene (AT4G01000) was used as an endogenous reference gene. PCR was started with an activation and DNA denaturation step (95°C for 3 min), followed by 40 cycles of 95°C for 20 s and 60°C for 40 s. The relative fold change was calculated according to the 2−ΔΔCt method.

For the RNA analyses in roots, Arabidopsis roots were collected from 20–30 seedlings for each treatment and cDNA was isolated using the Dynabeads mRNA DIRECT Kit (Life Technologies). DNA contamination was removed by treatment with DNase I (Life Technologies). First-strand cDNA was synthesized from 50 ng of mRNA using ProtoScript II Reverse Transcriptase (NEB). The qRT-PCR assay was carried out in an iCycler iQ Real-Time PCR Detection System (Bio-Rad) and template amplifications were performed using Taq Universal SYBR Green Supermix (Bio-Rad) from 1/10 of 20 × diluted cDNA in a total 20 μl reaction using 1 μl of each 10 μM gene-specific primer (Supplementary Table 3). All assays consisted of three technical replicates for each RNA sample. Data was analyzed using the iCycler iQ Real-Time PCR Detection System Software version 3.0a (Bio-Rad). The Arabidopsis ACP gene (AT4G01000) was used as an endogenous reference gene. PCR was started with an activation and DNA denaturation step (95°C for 3 min), followed by 40 cycles of 95°C for 20 s and 60°C for 40 s. The relative fold change was calculated according to the 2−ΔΔCt method.

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P.M., M.M., D.F.K. and F.C.S. designed the research; P.M., M.M., A.K., S.H.v.R., F.K. and S.C. performed the research; F.K., A.C., and V.M.W. contributed reagents, S.H.v.R., F.K. and F.C.S. analyzed nematode metabolomes, R.J.M and S.H.v.R. synthesized ascarosides; P.M., M.M., S.H.v.R., D.F.K., A.K., S.C., X.W., K.-H.K. and F.C.S. analyzed the data; and D.F.K., M.M., F.K., V.M.W., and F.C.S. wrote the paper.

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