Roots of Resistance: *Solanum sisymbriifolium* Produces Nematicidal Metabolites

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Introduction

Plant-parasitic nematodes are one of the most important agricultural pests. While susceptible to nematicides, it is crucial to pursue other avenues of control that can contribute to integrated pest management. To this end, a wild solanaceous plant *Solanum sisymbriifolium*, which can induce hatching yet is resistant to the pale cyst nematode *Globodera pallida* as well as the root-knot nematodes *Meloidogyne incognita, M. hapla* and *M. chitwoodi,* has been under investigation by researchers for over two decades. While most groups focus on plant receptors participating in the two-tiered defense response utilizing PAMP-triggered immunity and effector triggered immunity, we decided to investigate the downstream defenses - the secondary metabolites that directly harm nematodes.

Hypothesis:
Secondary metabolites contribute to the broad anti-nematode resistance of *S. sisymbriifolium*.

To address the hypothesis, we performed an untargeted metabolomic analysis on *M. incognita*-infected and uninfected *S. sisymbriifolium* and two potato cultivars: 'Red polenta' and 'Désirée'.

Fig. 1 *S. sisymbriifolium* plant grown hydroponically in a growth chamber. This species features pronounced prickles and innate resistance to root-knot and cyst nematodes, giving it a distinctly defensive character.

Materials and Methods

Plant inoculation and sample collection:

- 5-week-old *S. sisymbriifolium,* 'Red polenta' and 'Désirée' were inoculated with 400 freshly hatched *M. incognita* stage 2 juveniles (J2). N = 4 biological replicates per treatment, using two independently infected root masses per replicate.
- Roots were harvested 7 days-post-inoculation, flash frozen with liquid nitrogen and shipped to Creative Proteomics (Shirley, NY).

Metabolomic analysis:

- Root metabolomes were profiled by UPLC-Q Mass Spectrometry, after methanol extraction.
- Metabolites differing ≥3-fold between species and ≥2-fold between infection states were prioritized for further analysis.

Assaying J2 sensitivity to metabolites

- A 500 µL solution of approximately 50 freshly hatched J2s was combined with equal amount of stock of selected metabolites and added to 4 wells in a 24 well tissue culture plate
- Nematodes were counted each 24 hours over 3 days under a dissecting microscope by touching them with a prodding tool and observing motility. Data was analyzed in R using GLMM.

Results

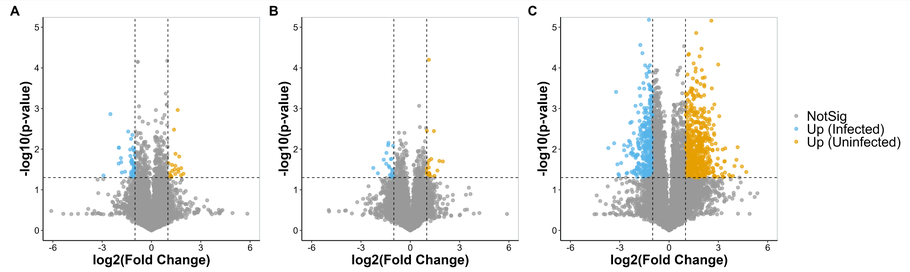

Fig. 2 "Volcano" plot of negative mode metabolites. The graph shows the calculated fold change (x-axis) and significance of that change (y-axis) of individual metabolites in the roots of infected vs. uninfected 'Désirée' (A), 'Red Polenta' (B) and *S. sisymbriifolium* (C). ***S. sisymbriifolium* shows a significant change in its metabolome after infection, with altered levels of many metabolites and a broader range of modulated compounds compared to potatoes.**

Metabolite	Red Polenta		Désirée		*S. sisymbriifolium*	
	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
2-Furoic acid	2 [a]	1 [a]	1 [a]	1 [a]	39 [b]	18 [b]
Aconitic acid	664 [a]	637 [a]	477 [a]	667 [a]	2036 [b]	2379 [b]
Quinic acid	555 [a]	886 [ab]	593 [ab]	1191 [b]	4825 [c]	13006 [d]

Table 1. Mean concentrations (mg/L) of cis-aconitic acid, 2-furoic acid, and quinic acid in *M. incognita*-infected and uninfected roots of 'Red Polenta', 'Désirée', and *S. sisymbriifolium*. Different letters show significant within-row differences (Sidak-adjusted CLDs). **These compounds accumulate constitutively in S. sisymbriifolium, suggesting a built-in chemical defense.**

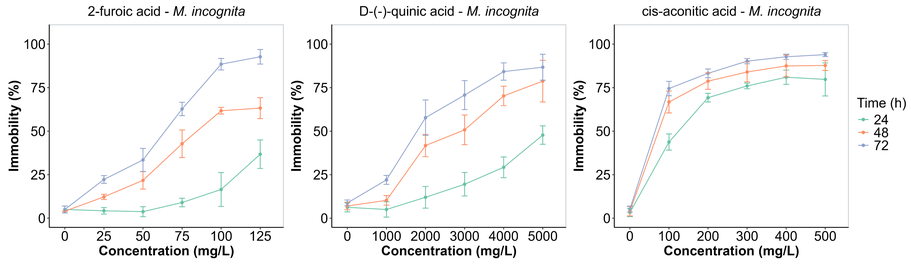

Fig. 3 Cumulative immobility (%) of *M. incognita* J2 larvae to 2-furoic, cis-aconitic, and quinic acids. Each value represents the average of four replicates and their standard error. Similar results were obtained with *M. hapla* and *M. chitwoodi*. **Selected organic acids reach LC50 after 48 hours at concentrations similar to those found in the resistant *S. sisymbriifolium*.**

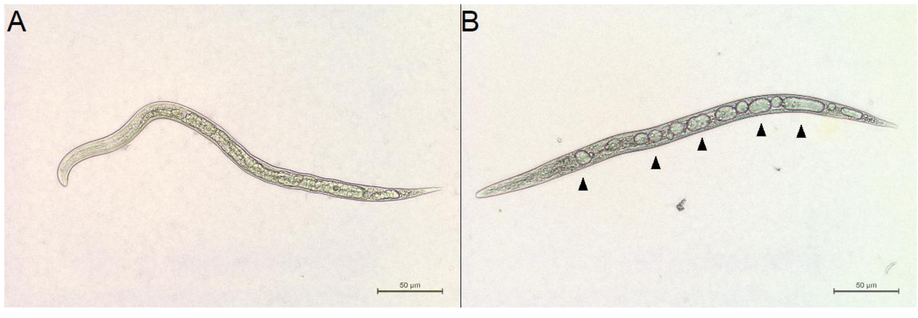

Fig. 4 Morphological responses of *M. hapla* to treatments: A) untreated live nematode B) treated nematode with numerous vesicle-like structures (arrows). Scale bars = 50 µm**. Treatment immobilizes nematodes and causes clear structural disruption.**

pH	Water adjusted with HCl	Citric acid (1921 mg/L)	2-Furoic acid (100 mg/L)
3	9 ± 2 [a/A]	10 ± 4 [a/A]	87 ± 3 [b/C]
4	4 ± 2 [a/A]	4 ± 2 [a/A]	14 ± 8 [b/B]
5	1 ± 1 [a/A]	3 ± 2 [a/A]	13 ± 3 [b/B]
6	2 ± 2 [a/A]	2 ± 2 [a/A]	2 ± 2 [a/A]
7	2 ± 2 [a/A]	1 ± 1 [a/A]	1 ± 2 [a/A]

Table 2. Mean immobility (%) of *M. hapla* juveniles after 24 h exposure to citric, 2-furoic, and hydrochloric acids at different pH levels (mean ± SD, n = 3). Lowercase letter = within-pH differences; uppercase letter = across-pH differences (Sidak-adjusted CLDs). **2-Furoic acid proves far more toxic than citric acid, indicating its nematicidal effect is not solely due to acidity.**

Conclusions

- The metabolomic analysis highlighted a set of organic acids that show nematicidal properties at concentrations found *in planta,* **implying that secondary metabolites can contribute to the defense that *S. sisymbriifolium* shows against nematodes.** Other chemicals that participate in nematode resistance have also been identified (Schulz *et al.,* 2024).
- *S. sisymbriifolium* maintains nematicidal metabolite levels even without infection, **enabling pre-emptive defense.**
- The nematicidal activity likely results from **the additive effects of chemical structure and acidic pH.**
- This work contributes new insights into an underexplored aspect of plant defenses and provides **one of the first reports of 2-furoic, quinic, and cis-aconitic acids present in plants at nematicidal levels.**
- Further work is needed to explore additional metabolites in the chemical 'cocktail', clarify the precise mechanisms by which the assayed metabolites mediate the anti-nematode response, and investigate how nematodes uptake these compounds.

References

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Acknowledgments

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