Tetranychus urticae adaptation to phenylpropanoid defensive compounds in *Arabidopsis thaliana*

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BACKGROUND

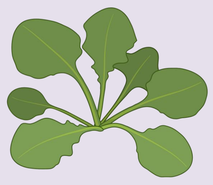
- The two-spotted spider mite (TSSM) - *Tetranychus urticae* (Koch) - is a global agricultural pest with a polyphagous diet extending over 1100 plant species[1]. Associated with this extreme polyphagy is the TSSM's capacity to rapidly develop pesticide resistance.
- Although *Arabidopsis thaliana* was an initially unfavorable host, an ancestral TSSM population reared on *Phaseoulus vulgaris* (bean-a) adapted to the Col-0 ecotype within 25 generations (Col-a). Mites adapted to Arabidopsis were found to use **metabolic detoxification** of some Arabidopsis defense compounds, such as indole glucosinolates, as a mechanism of adaptation[2,3].
- Phenylpropanoids are a large family of plant secondary metabolites associated with growth and structural support, as well as response to wounding and herbivory.
- Upon TSSM feeding, Arabidopsis phenylpropanoid biosynthetic genes are upregulated and many phenylpropanoid metabolites accumulate[4], implicating their potential role in Arabidopsis defense. Mutant Arabidopsis plants whose phenylpropanoid biosynthesis are disrupted, are more susceptible to mite herbivory (Grbic lab, unpublished). In addition, some phenylpropanoids induce mortality in bean-a mites (Grbic lab, unpublished). However, little is known about what individual phenylpropanoid metabolites are involved in Arabidopsis defense against TSSM herbivory, or if mites adapt to Arabidopsis phenylpropanoids through metabolic detoxification.

OBJECTIVES

1) Identify Arabidopsis phenylpropanoid compounds involved in defense against TSSM herbivory
2) Characterize bean-a and Col-a TSSM metabolic responses to phenylpropanoid exposure

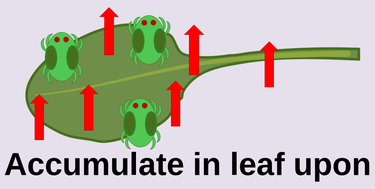
METHODS

Filter Phenylpropanoid Candidates



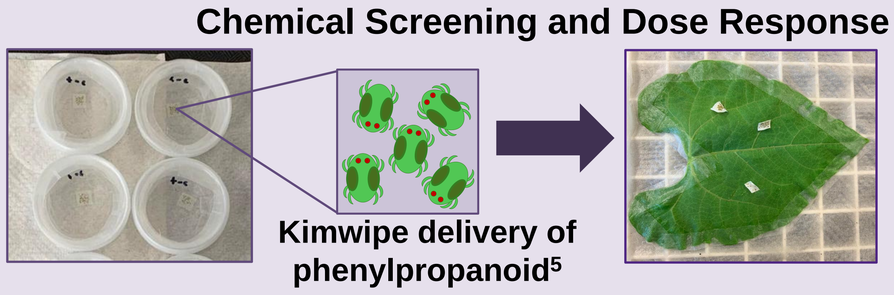
Arabidopsis specific

Associated with defense in literature



Accumulate in leaf upon mite feeding

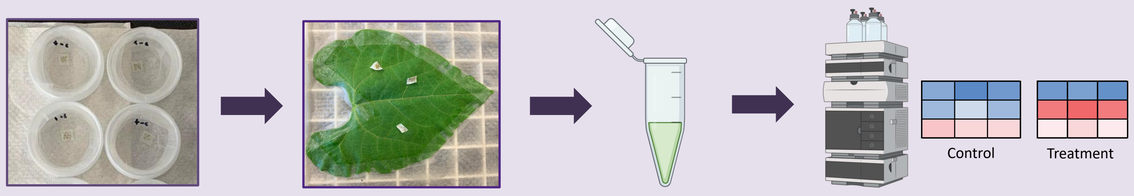
Chemical Screening and Dose Response



Kimwipe delivery of phenylpropanoid[5]

Recover and assess mortality

HPLC-MS/MS of mite extracts



Kimwipe delivery of phenylpropanoid

Recover and harvest mites

Extract mite metabolites

Run extracts via HPLC-MS/MS

Enzyme Inhibition

Inhibit mite enzyme class

Kimwipe delivery of phenylpropanoid

Recover and assess mortality

RESULTS

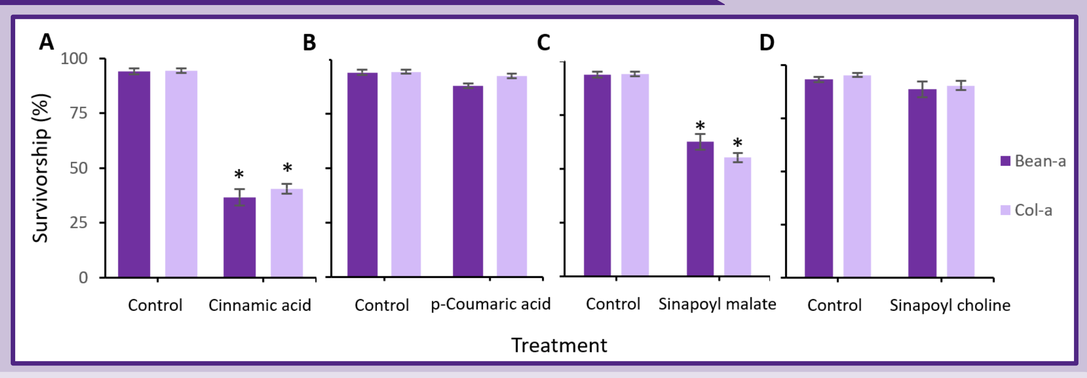


Figure 1: Survivorship of bean-a and Col-a mites treated with solvent (control), **(A)** *p*-coumaric acid, **(B)** cinnamic acid, **(C)** sinapoyl malate, and **(D)** sinapoyl choline (all at 10mM concentrations). Experiments were performed in four biological replicates per trial and in three independent trials (*n* = 12). Data represent the mean ± SE. Significantly different ($p<0.05$) treatment means (*) compared to the control within mite populations were determined using a two-way ANOVA, followed by a Dunnett's post hoc test.

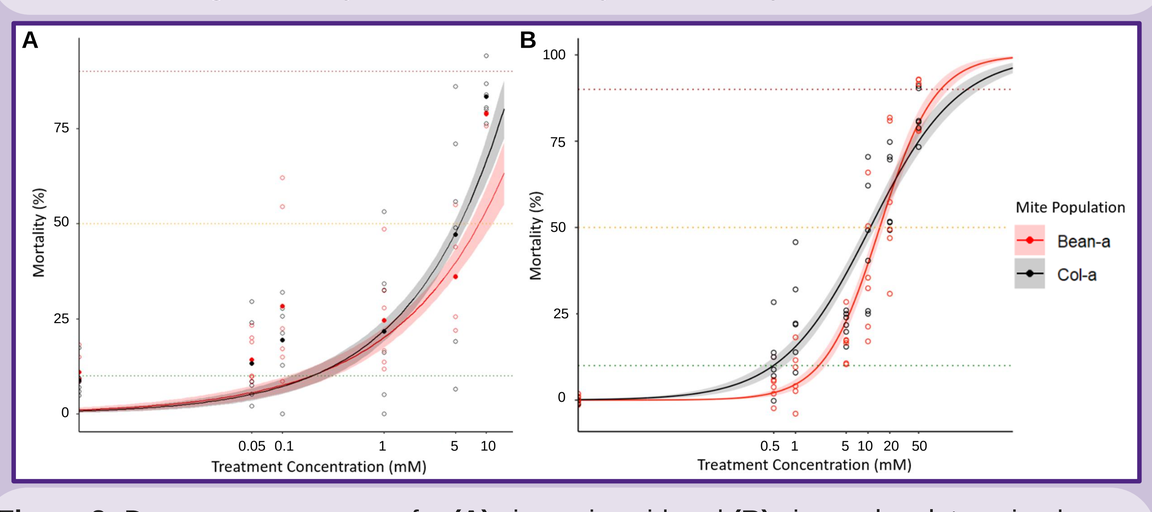


Figure 2: Dose response curves for **(A)** cinnamic acid and **(B)** sinapoyl malate using bean-a and Col-a mites. Treatments are represented by a range of phenylpropanoid concentrations from 0 mM (negative control) – 50 mM. Shaded regions represent 95% CI. Open dots represent individual data points. Solid dots represent means. The lethal concentration of cinnamic acid inducing 50% mortality (LC_{50}) in bean-a mites and col-a mites is 12 mM and 6 mM respectively, and 9 mM and 6 mM for sinapoyl malate treatment with bean-a and Col-a mites, respectively.

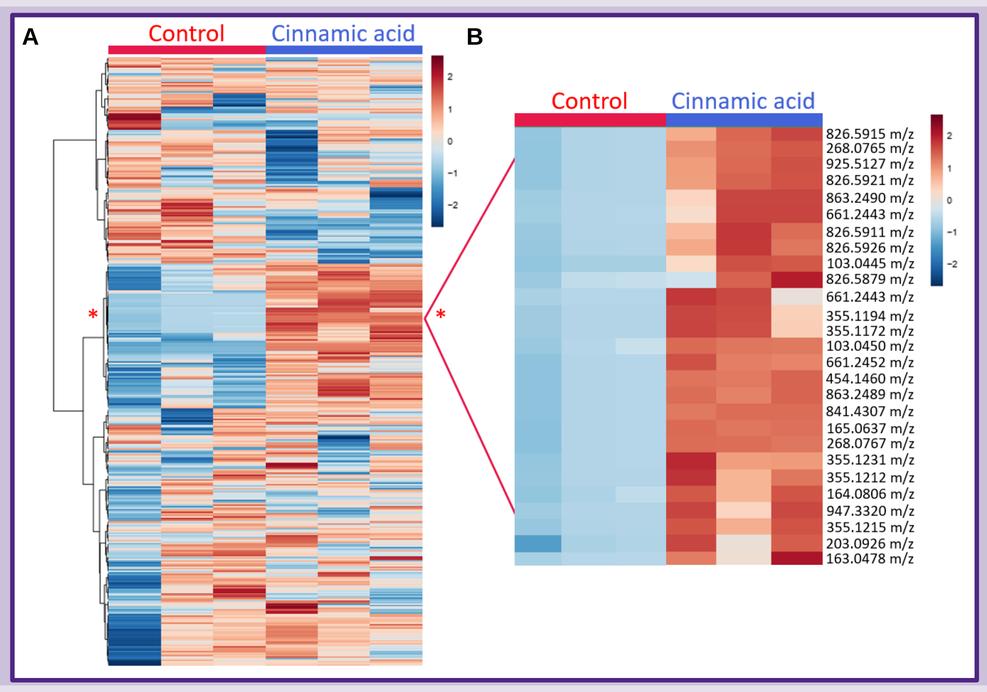


Figure 3: (A) An overview heatmap visualizing the relative abundances of 412 mite metabolites detected by HPLC-MS in mite extracts from bean-a mites treated with 10 mM cinnamic acid or solvent (control). **(B)** A cluster of metabolites exhibiting upregulation relative to control cohort provides a tentative shortlist of compounds requiring identification.

FUTURE STEPS

- Determine the physiological levels of phenylpropanoid candidates in Arabidopsis leaves following mite feeding via HPLC-MS
- Continue chemical screening of phenylpropanoid candidates using bean-a and Col-a mites
- Characterize the metabolic profiles of bean-a and Col-a mites following exposure to other phenylpropanoid candidates via HPLC-MS/MS
- Perform enzyme inhibition assays using bean-a and Col-a mites
- Identify mite enzymes required for the modification of Arabidopsis phenylpropanoids

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