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SUPPLEMENTARY INFORMATION

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Induced defences in plants reduce herbivory by increasing cannibalism

John Orrock*, Brian Connolly and Anthony Kitchen

Department of Integrative Biology, University of Wisconsin, Madison, WI 53704, USA. *e-mail: jorrock@wisc.edu

Supplementary Materials

Plant induced defenses reduce herbivory by increasing cannibalism

John Orrock[1], B. Connolly[1], and A. Kitchen[1]
[1]Department of Zoology, University of Wisconsin, Madison, WI 53704.

Supplementary Methods

Additional information for Experiment 1:

Tomatoes were grown in individual clear plastic containers (9 x 12 cm circular cups with dome lids) filled with ~382 cm^3 of RediEarth Sunshine Professional Growing Mix; four holes were punctured in the bottom of each cup and containers were placed in individual plastic trays to 1) eliminate interplant signaling via a shared water source and 2) provide a refillable water supply that limited physical disturbance to plant tissue during watering.

We used spray application of methyl jasmonate (MeJA) to induce changes in plant chemistry, as MeJA application is known to cause changes in plant defensive chemistry in the absence of any actual herbivory[1-5]. To apply MeJA treatments, each plant and its corresponding plastic dish were covered with a hollow, rigid green plastic cylinder (37 cm tall; diameter 14 cm). The open end of the cylinder was covered by a thin film of plastic to ensure MeJA spray did not volatilize and cross contaminate neighboring plants. For each treatment application, the plastic film was removed from the top of the cylinder, the nozzle of a spray bottle was inserted into the opening (~22 cm from the plant) and two sprays (~6.00 mL) were applied to each plant. Immediately after spray treatments, plastic covering was replaced over the top of the cylinder. Plants were left covered for a minimum of 1 hour before being moved. We measured plant height and the number of true leaves on each plant prior to the initiation of the herbivory trial.

One hour after the second MeJA treatment, eight randomly selected third-instar *Spodopetera exigua* larvae were sealed into each plastic container, yielding 8 larvae for each of 40 replicate plants. We selected 8 larvae per plant based on previous studies that manipulate *S. exigua* density to examine plant induced defense[6] as well as on field studies that suggest the potential for high densities of *S. exigua* in some settings[7,8], including densities used in our study (see Figure 1 in Kolodny-Hirsch et al. 1993[8]).

To assess cannibalism, the number of *S. exigua* was counted daily. When counting *S. exigua* during these monitoring events, the top of the soil was also searched to ensure that no individuals were in the soil. During both sessions, we assumed that any *S. exigua* that disappeared were consumed. This assumption was supported by direct observations of cannibalism and no indication that any *S. exigua* ever escaped from their holding container. At the end of the experiment, the entire soil column in each pot was also checked for any living larvae; none were found. At the end of the experiment, we also noted whether plants were completely defoliated (visually assessed as having less than 10% of leaf biomass remaining).

The experiment was conducted in two sessions (8-15 April 2016 and 15-22 May 2016) with 6 replicates of each treatment (24 plants; 192 *S. exigua*) during the first session, and 4 replicates of each treatment (16 plants; 128 *S. exigua*) during the second session.

Additional information for Experiment 2:
Application of treatments to plants: On day 21, each potted plant was placed within a larger 16-ounce plastic cup. A smaller translucent 9 ounce plastic cup was affixed to the top of the bottom cup with two pieces of masking tape, effectively enclosing each plant and preventing the potential for cross-contamination. Spray application of treatments were performed in a fume hood to avoid potential contamination between treatments during application. Between sprays, plants were returned to the growth chamber.

One hour after the second MeJA treatment, eight randomly selected third-instar *Spodopetera exigua* larvae were sealed into each plastic container, yielding 8 larvae for each of 40 replicate plants. We selected 8 larvae per plant based on previous studies that manipulate *S. exigua* density to examine plant induced defense[6] as well as on field studies that suggest the potential for high densities of *S. exigua* in some settings[7].

To estimate rates of mass loss in tomato leaves due to water loss over the two-day feeding trial, we weighed a single, fresh tomato leaf and placed it in a plastic cup with a lid (identical to those used in feeding trials). This was replicated for leaves from ten individual tomato plants. The cups were placed on the same area where herbivory trials were conducted and weighed after two days. After two days, the mean proportion of leaf mass lost due to water loss was 0.265 ± 0.016. As expected, this value was smaller than the proportion of leaf mass lost for leaves in the feeding trial (Fig. 1d). However, it was similar to the mean leaf mass observed when herbivores were fed leaves with induced defenses and herbivores had conspecifics available to consume (on average, the proportion of biomass lost by leaves in this treatment combination was 0.336 ± 0.039), suggesting that herbivores in this situation consumed very little plant material.

Statistical analyses:
The rate and severity of intraspecific predation in *S. exigua* was assessed with a generalized linear mixed effects model (generalized LMM) with a binomial response distribution [9]. The experimental induction treatment was evaluated as a fixed effect with four levels: control, low induction, medium induction, and high induction. The time interval at which censuses were conducted was treated as a continuous covariate and we also evaluated the interaction between treatment and census period. Session (first or second) and shelf where the trial was conducted (top or bottom) was included as random model effect. Repeated measures were modelled using a first-order autoregressive covariance structure; other covariance structures were evaluated (e.g., compound symmetry), but none yielded an improved fit based on AIC values. To evaluate plant height and leaf number prior to the start of herbivory trials, general linear mixed models were used that also included session and shelf as random effects. All analyses conducted for experiment 2 utilized general linear models.

Supporting Results

Experiment 1
Prior to the initiation of experimental treatments, there were no significant differences among treatments for plant height (general linear mixed model (GLMM), $F_{3,34} = 0.41$, $P = 0.75$), plant width (GLMM, $F_{3,34} = 2.03$, $P = 0.13$), or the number of leaves (GLMM, $F_{3,34} = 1.27$, $P = 0.30$).

Experiment 2

Plant induction and no-choice feeding assays: These trials indicate that there was a strong interaction between plant induced defense and opportunities for cannibalism in affecting *S. exigua* growth rate (Supplementary Fig. S2; general linear model (GLM), $F_{1,37} = 46.00$, $P < 0.001$), as well as significant main effects of induced defenses (GLM, $F_{1,37} = 96.27$, $P < 0.001$) and cannibalism (GLM, $F_{1,37} = 125.72$, $P < 0.001$). This interaction arose because there was a significant reduction in *S. exigua* growth rate when individuals were reared for 48 hours on induced leaves, but only when those individuals did not have the opportunity to cannibalize dead conspecifics (Supplementary Fig. S2). Although the amount of leaf material consumed varied greatly, leaf material was only totally consumed in 1 of the 41 trials (i.e., 2%).

After weighing, plant samples were placed in a drying oven at 50°C for 48 hours to ascertain the relationship between wet and dry leaf biomass; this relationship was highly significant (GLM, $r^2 = 0.96$, $F_{1,40} = 1088$, $P < 0.001$).

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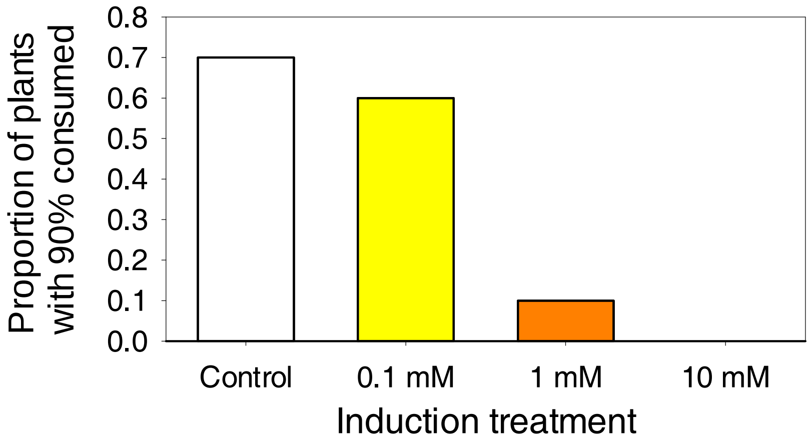
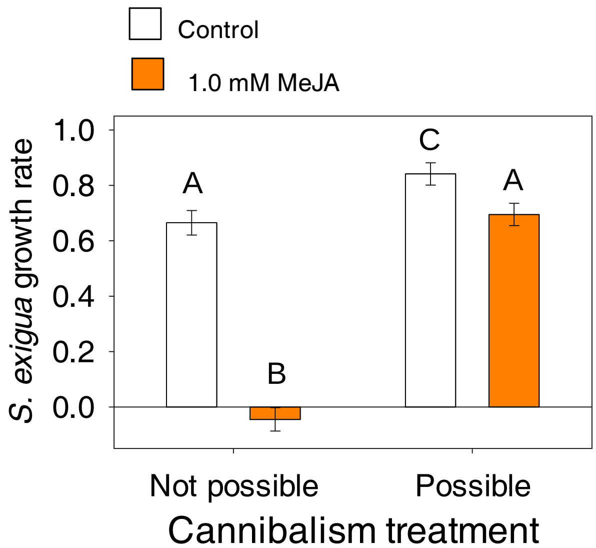
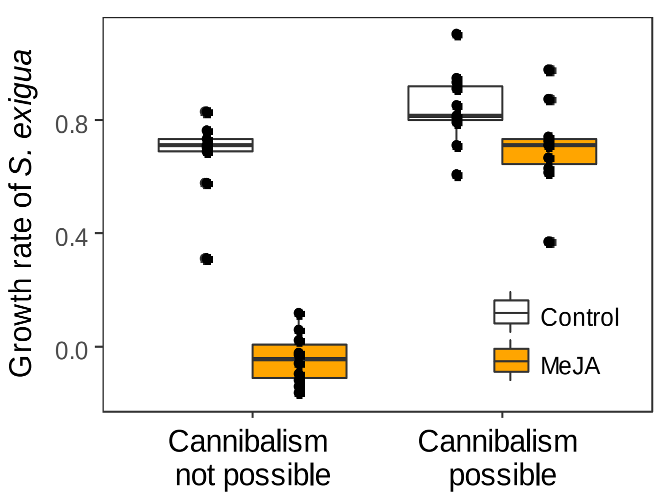
Supplementary Figure S1. Plant induced defenses affected the likelihood that plants would have at least 10% of their biomass remaining after the conclusion of the herbivory trial. Data presented are pooled across sessions of the experiment and across shelves where trials were conducted. Differences in the frequency of plants experiencing 90% loss of biomass are significantly different among treatments (X^2 = 16.26, 3 d.f., P = 0.001).

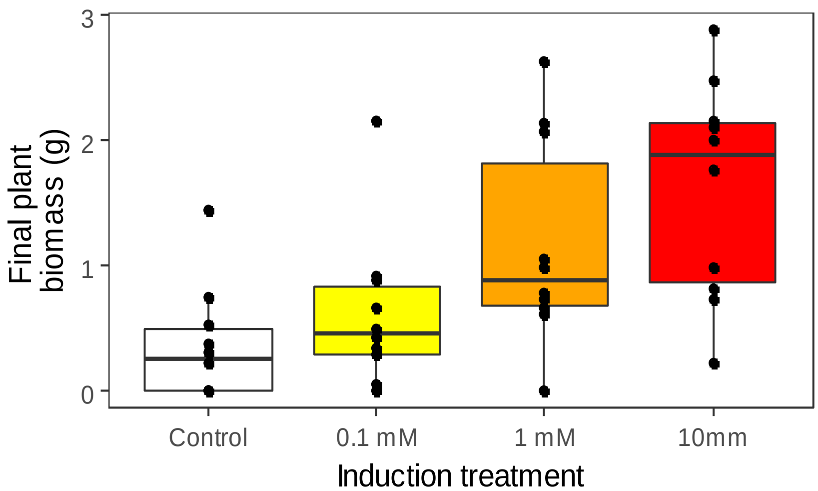


Supplementary Figure S2. Growth rate of *S. exigua* in the presence or absence of four dead conspecifics when offered a single leaf in a no-choice feeding trial for 48 hours; each leaf was obtained from a different tomato plant that was sprayed twice with one of two defense-induction treatments (Control or 1.0 mM MeJA). Top panel presents data as least-squared means with error bars that represent 1 SE. Bars that have a different letter are significantly different (linear contrasts following omnibus GLM, all $P < 0.02$). Lower panel presents same data as a box-and-whisker plot with individual data points plotted.

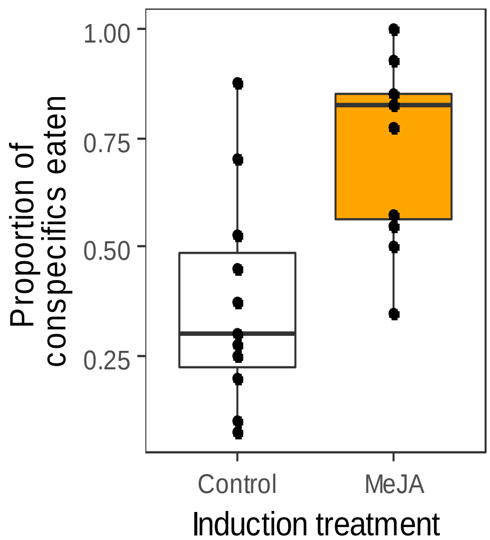




Supplementary Figure S3. A box-and-whisker plot of the data from Figure 1b. Plant induced defenses that promoted early cannibalism led to significant increases in the amount of plant tissue remaining at the end of the experiment (general linear model (GLM), $F_{3,34} = 7.04$, $P < 0.001$).



Supplementary Figure S4. A box-and-whisker plot of the data from Figure 1c. Individual *S. exigua* demonstrated greater levels of cannibalism when housed with leaves of induced plants (1.0 mM MeJA) vs. control plants (Welch's t-test, $t_{19.28} = 3.67$, $P = 0.002$).



Supplementary Figure S5. A box-and-whisker plot of the data from Figure 1d. Both induced defenses (GLM, $F_{1,37} = 23.41$, $P < 0.001$) and cannibalism (GLM, $F_{1,37} = 36.94$, $P < 0.001$) led to reduced herbivory by individual *S. exigua*, and these effects were additive (GLM, induction × cannibalism interaction term: $F_{1,37} = 0.31$, $P = 0.58$).