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Induced defences in plants reduce herbivory by increasing cannibalism

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Plant induced defenses reduce herbivory by increasing cannibalism

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Plants are under strong selection to defend themselves against herbivores. In two complimentary experiments with tomato, *Solanum lycopersicum*, we tested the hypothesis that induced defenses benefit plants by encouraging omnivorous insect herbivores (*Spodoptera exigua*) to eat other *S. exigua*. We show that promoting cannibalism by herbivores benefits plants in two ways: cannibals directly reduce herbivore abundance and cannibals eat significantly less plant material themselves.

Plants are not passive bystanders in their interactions with herbivores: plants alter their chemistry, morphology, and other components of their phenotype to reduce herbivory[1], often using cues from their environment to initiate these defenses before any actual attack occurs[2-4]. Because plant induced defenses are so ubiquitous[1] and may have important effects on herbivores[1,5] and other trophic levels[6,7], fundamental goals in plant-herbivore ecology are to understand the benefits of induced defenses to plants in terms of reduced herbivory[8], to describe how induced defenses operate (e.g., whether they reduce herbivore feeding, survival, or reproduction), and to characterize how plant induced defenses may affect other organisms[6,9]. We advance these goals by demonstrating a novel means whereby plant induced defenses reduce herbivory: via altering cannibalism among herbivores.

We found that induced defenses reduce damage to tomato (*Solanum lycopersicum*) plants by both 1) directly reducing herbivore feeding, and 2) by increasing rates of cannibalism among individual herbivores. We used a standard approach[10,11] to induce defenses in undamaged tomato plants according to one of four treatments (Fig. 1a). Plants that were strongly induced prior to exposure to generalist lepidopteran herbivores (*Spodoptera exigua*) caused insect herbivores to begin consuming each other earlier (Fig. 1a), leading to increased average rates of cannibalism (Fig. 1a inset), and a significant reduction in herbivory experienced by the strongly induced plants (Fig. 1b). Over five times as much plant biomass remained at the end of the experiment when plants received the high-induction treatment compared to the control treatment (Fig. 1b); the amount of plant biomass was also greater in the medium-induction treatment than the control treatment (Fig. 1b). Control plants were often completely defoliated (Supplementary Fig. S1).

This dynamic unfolded because, like many omnivorous herbivores[12,13], cannibalism in *S. exigua* occurs more readily when herbivores are nutritionally stressed[14]. Our work confirms that the low quality of induced plant tissue was responsible for this change in herbivore behavior: cannibalism happened sooner (Fig. 1a) and more conspecifics were consumed (Fig. 1a, inset; Fig. 1c) when herbivores were offered tissue from induced plants. Our findings also show that *S. exigua* can use cannibalism to compensate for the low quality of defended plant tissue: growth rates were significantly lower for *S. exigua* feeding on induced plants compared to control plants, but the growth rate of cannibals was not as compromised (Supplementary Fig. S2). Consistent with the hypothesis that plant induced defenses can moderate the initiation of cannibalism in herbivores, *S. exigua* that were fed control leaves exhibited lower rates of cannibalism (Fig. 1c) and *S. exigua* on whole plants with little or no induced defense only began to consume each other later (Fig. 1a), when the quantity of remaining plant material was minimal (or zero) and as the plant had sufficient time to mount chemical defenses[15].

Given that promoting cannibalism has considerable benefits for induced plants (Fig. 1b,d), and that facultative cannibalism is relatively common among herbivores[12,13,16,17], even in field and laboratory settings where herbivores can disperse[12,13], our work suggests that promoting cannibalism may be an important, but unappreciated, component of the evolution of plant induced defenses. Induced defenses that promote cannibalism provide several benefits for the plant. First, induced defenses directly reduce the consumption of plant tissue by individual herbivores[1] (Fig. 1d). Second, we have found that induced defenses reduce herbivory by triggering earlier cannibalistic behavior (Fig. 1a,c) that reduces the number of herbivores (Fig. 1a). Third, our results demonstrate that induced defenses reduce herbivory because cannibals consume less plant material (Fig. 1d). Encouraging cannibalism as a defensive strategy has similarities with plant defenses that attract natural enemies of herbivores[7,18], but also differs in an important way. Like predation and parasitism, cannibalism directly reduces herbivory because herbivores are killed and herbivore densities are lowered. However, unlike predation and parasitism, cannibalism also benefits plants because once an herbivore begins deriving nutrition from consuming other herbivores, it stops consuming as much plant biomass (Fig. 1d).

Our results also demonstrate the value that plants can receive by inducing defenses prior to an actual attack[3,4,19]. Although plants in control- and low-MeJA treatments would have induced defense once attack was underway [15], this defense comes too late (Fig. 1b). At the end of the experiment, many of these plants were entirely consumed, and those that remained had very little biomass (Fig. 1b). For plants that had higher levels of induced defense at the start of herbivore exposure, increased levels of early cannibalism (Fig. 1a,c), reduced the potential for extreme levels of biomass loss (Fig. b,d).

An important question is the degree to which the plant-mediated cannibalism revealed in our lab-based study provides insight into dynamics in natural systems. Given that induced defenses are common in many plant species[1] and that cannibalism has been observed in a large number of primarily herbivorous organisms[12,13,16,17], even in field and laboratory settings where herbivores can disperse[12,13], changes in cannibalism caused by plant defenses could be an unevaluated mechanism contributing to the dynamics of plant and herbivore populations, as well as the dynamics of predators and pathogens[5-7,20-22]. For example, plant defenses that promote cannibalism might alter pathogen dynamics within herbivore populations by changing the frequency and/or density of herbivores[17]; which might arise if cannibals consume conspecifics, if cannibals have different dispersal capacity, if cannibals alter the dispersal of conspecifics, or some combination of all of these. Such behaviorally-mediated shifts in herbivore density might be a particularly important way that increased cannibalism alters dynamics in field settings where movement is less constrained. Increased cannibalism might also alter the nature of pathogen transmission if infected individuals are more or less likely to be cannibalized[21,23]. As such, plant induced defense may also accelerate the spread of viruses by increasing cannibalistic behavior. Such a dynamic would be beneficial for the plant as well as serve to increase the efficacy of strategies to control herbivorous pests in agricultural settings via induction of plant defense with exogenous JA[10], via the introduction of herbivore viruses[23], or both.

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Author contributions

JO conceived the study; JO and BC designed the study; JO, AK and BC conducted the experiment, JO performed all analyses and led manuscript preparation; BC and AK contributed to manuscript revision.

Additional Information

Supplementary Information is available for this paper.

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Competing interests

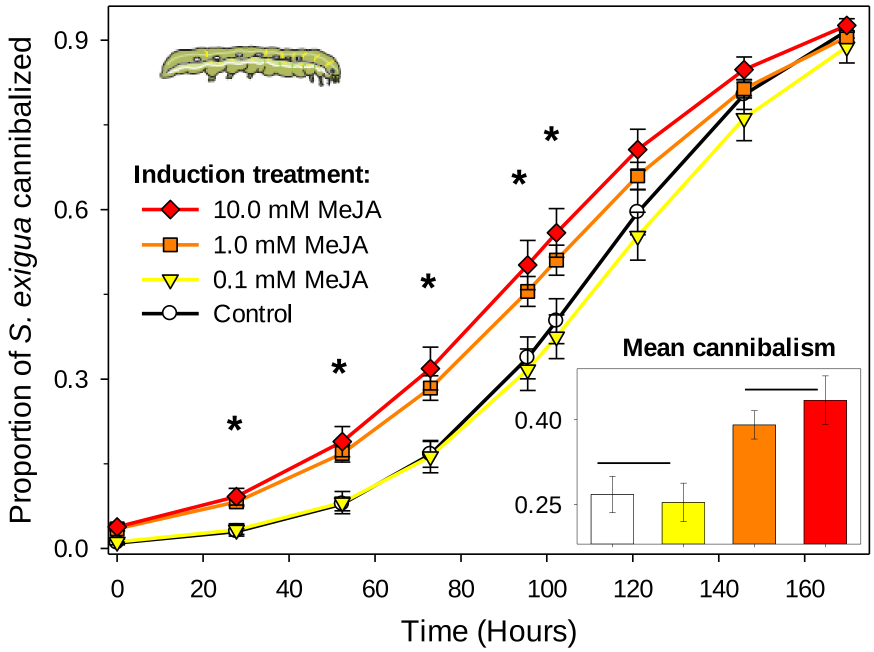
The authors declare no competing financial interests.

Figure Legend

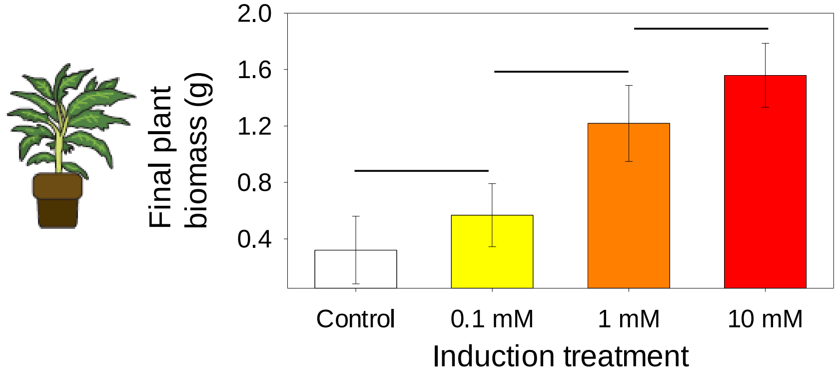
Figure 1 | Plant induced defenses lead to increased rates of cannibalism among herbivores which result in significant decreases in losses of plant biomass. a, Estimates (±SE) of *Spodoptera exigua* mortality due to intraspecific predation as a function of plant induced defense. Plant induction treatment levels spanned an order of magnitude in concentration (sprayed with 0.1 mM, 1.0 mM, and 10.0 mM Methyl Jasmonate [MeJA]) and also included spraying with a control solution. There was a significant effect of the induction treatment ($F_{3,36} = 4.95$, $P = 0.006$) on the proportion of *S. exigua* consumed by other *S. exigua*, a significant effect of the time that had elapsed during the experiment ($F_{1,316} = 552.97$, $P < 0.001$), and no interaction between treatment and time elapsed ($F_{3,316} = 1.66$, $P = 0.18$). Significant differences ($P < 0.05$) in comparisons between high- and medium-induction treatments compared to low-induction and control treatments are indicated with an asterisk. Inset: Mean (±SE) cannibalism across the entire trial; bars that do not share a vertical line are significantly different ($P < 0.02$). **b**, Plant induced defenses that promoted early cannibalism led to significant increases in the amount of plant tissue remaining at the end of the experiment ($F_{3,34} = 7.04$, $P < 0.001$); bars that do not share a horizontal line are significantly different ($P < 0.02$). **c**, In a second experiment where we manipulated the presence or absence of dead conspecifics, individual *S. exigua* demonstrated greater levels of cannibalism when housed with leaves of induced plants (1.0 mM MeJA) vs. control plants ($t_{20} = 3.67$, $P = 0.002$). **d**, This experiment also demonstrates that both induced defenses ($F_{1,37} = 23.41$, $P < 0.001$) and cannibalism ($F_{1,37} = 36.94$, $P < 0.001$) led to reduced herbivory by individual *S. exigua*, and these effects were additive (induction × cannibalism interaction term: $F_{1,37} = 0.31$, $P = 0.58$); bars that do not share a horizontal line are significantly different ($P < 0.004$).

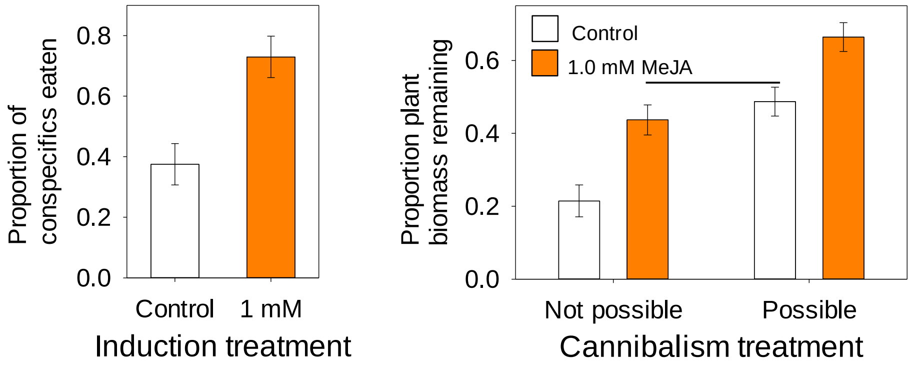
Orrock et al., Figure 1.

a. Plant induced defenses cause earlier herbivore cannibalism



b. Plants with defenses induced prior to attack lose less biomass





c. Plant defenses cause herbivores to eat more herbivores

d. Induced defenses and increased cannibalism both reduce herbivory

Methods

Experiment 1: Examining how induced defenses alter cannibalism and herbivory when herbivores access entire plants. The effects of plant induction on rates of intraspecific predation were evaluated with tomato (*Solanum lycopersicum* L., var. 'Moneymaker') plants assigned to one of four induction levels following exposure to one of four concentrations of an aerosolized volatile chemical: control (0.125% Triton-X), low (0.1 mM Methyl Jasmonate [MeJA] + 0.125% Triton-X), medium (1.0 mM MeJA + 0.125% Triton-X), and high (10 mM MeJA + 0.125% Triton-X). We used 10 plants for each treatment level, for a total of 40 plants. Concentrations of tomato plant proteins associated with herbivore deterrence (i.e., polyphenol oxidase, proteinase inhibitors, peroxidase) significantly increase following exposure to methyl jasmonate, and concentrations of jasmonic acid (a derivative of methyl jasmonate) identical to those used in this study generate increases in the production of these same proteins[10]. 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. Tomato plants were grown for 21 days under a 12-12 hour light-dark photoperiod (~220 μmol quanta/m^2 sec) at 23°C, at which point every plant had three or four true leaves. Treatments were applied on two consecutive days (see Supporting Methods and Supporting Results for additional information).

One hour after the second MeJA treatment, eight randomly selected third-instar *Spodopetera exigua* larvae (25.96 ± 2.13 mg, mean starting mass ± SE of 1 randomly selected larvae per group of 8) 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[24] as well as on field studies that suggest the potential for high densities of *S. exigua* in some settings[25]. The number of *S. exigua* was monitored at least once daily for 8 consecutive days (see Supplemental Methods for additional information). During the experiment, we also noted whether plants were ever completely defoliated (visually assessed as having less than 10% of leaf biomass remaining). At the end of the experiment, plants were clipped at the ground level and immediately weighed to determine above-ground biomass.

Experiment 2: Using leaf-feeding trials to examine the unique contribution of induced defenses and cannibalism to plant defense. In this experiment, the same variety of tomato used in experiment 1 was grown for 21 days in a growth chamber at 25C with a 12:12 photoperiod. Plants were grown in standard potting mix in 6 x 6cm square plastic pots that were 9cm tall. Plants were sprayed with either a control solution of 0.125% Triton-X) or a medium-induction solution of 1.0 mM MeJA + 0.125% Triton-X (identical to the respective solutions used in experiment 1). Two identical sprays were given to each plant, one on day 22 (Feb. 19, 2017) and another on day 24 (Feb. 21); see Supplementary Methods for additional information. On Feb. 22, a leaflet from each plant was clipped and placed in a 2-oz plastic cup. A singe third-instar larva of *S. exigua* was weighed and placed in each cup. A cannibalism treatment with two levels (conspecifics added to cup, or no conspecifics added to cup) was randomly assigned to each cup (stratified by induction treatment). For cups assigned to receive conspecifics, we added a total of four third- and fourth-instar larvae of *S. exigua* that had been killed by briefly flash-freezing them in a -80 freezer. This resulted in 9 replicates of the control + no conspecific treatment, 10 replicates of the induced + no conspecific treatment, 11 replicates of the control + conspecific treatment, and 11 replicates of the induced + conspecific treatment.

Two dead larvae were added at the start of the trial on Feb. 22; another 2 dead larvae were added 24 hours later on Feb. 23. The trial was concluded after 48 hours, when the amount of dead larvae consumed was visually estimated and the living larvae and remaining plant material were weighed.

Data availability. The data from this study supporting our findings are available from the corresponding author upon reasonable request.

Supplementary Information

Supplementary Methods

Additional information for Experiment 1:

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.

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.

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.

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 with a binomial response distribution [26]. 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

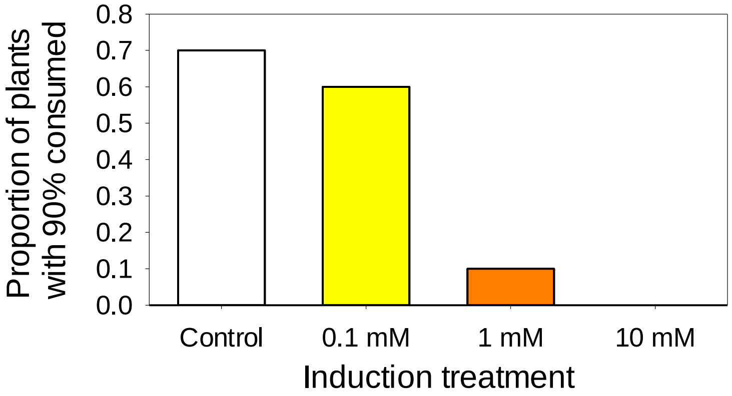
Prior to the initiation of experimental treatments, there were no significant differences among treatments for plant height ($F_{3,34} = 0.41$, $P = 0.75$), plant width ($F_{3,34} = 2.03$, $P = 0.13$), or the number of leaves ($F_{3,34} = 1.27$, $P = 0.30$).

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 ($F_{1,37} = 46.02$, $P < 0.001$), as well as significant main effects of induced defenses ($F_{1,37} = 96.27$, $P < 0.001$) and cannibalism ($F_{1,37} = 125.72$, $P < 0.001$). 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 Figure 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 ($r^2 = 0.96$, $F_{1,40} = 1088$, $P < 0.001$).

Supplementary Figure S1. Plant induced defenses affected the likelihood that plants would have at least 10% of their biomass remaining after herbivore attack. 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). Bars that have a different letter are significantly different (all $P < 0.02$).