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PLANT-MICROBE-ANIMAL INTERACTIONS – ORIGINAL RESEARCH

Plant induced defenses that promote cannibalism reduce herbivory as effectively as highly pathogenic herbivore pathogens

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Abstract

Plant induced defenses may benefit plants by increasing cannibalism among insect herbivores. However, the general efficacy of plant defenses that promote cannibalism remains unclear. Using a generalist Lepidopteran herbivore (*Helicoverpa zea*), we examined whether plant induced defenses in *Solanum lycopersicum* increased cannibalism among *H. zea* and whether defense-mediated cannibalism benefits both the plant and the cannibal. In a separate experiment, we also examined whether defense-mediated cannibalism has effects on *H. zea* herbivory that are comparable to the effects of pathogenic virus of *H. zea* (HzSNPV) and whether defense-mediated cannibalism modified pathogen efficacy. We found that both plant defenses and cannibalism decreased herbivory: *H. zea* consumed less plant material if plants were induced, if dead conspecifics were provided, or both. Cannibalism increased cannibal growth rate: cannibals effectively overcome the costs of plant defenses by eating conspecifics. Inoculating half of *H. zea* with virus strongly reduced caterpillar survival. Cannibalism occurred sooner among virus-inoculated groups of *H. zea*, and all caterpillars in virus-inoculated treatments died before the end of the 7-day experiment. Although the rise in mortality caused by HzSNPV occurred more rapidly than the rise in mortality due to defense-mediated cannibalism, overall *H. zea* mortality at the end of the experiment was equal among virus-inoculated and induced-defense groups. Defense-mediated cannibalism and viral inoculation equally reduced herbivory on *S. lycopersicum*. Our results provide evidence that defense-mediated increases in cannibalism can be as effective as other forms of classic herbivore population regulation, and that both viral pathogens and defense-induced cannibalism can have significant benefits for plants.

Keywords Cannibalism · Herbivory · Pathogens · Plant–herbivore interactions · Plant induced defenses

Introduction

Plants are not passive bystanders in plant–herbivore interactions. For example, plants may change their phenotype in response to information about herbivory risk in the environment (Karban 2020; Karban et al. 2016), generating induced defenses capable of reducing herbivore feeding, survival, and reproduction (Karban and Baldwin 1997). These defenses can lead to a significant reduction in herbivory, with substantial fitness benefits for plants (Karban and Baldwin 1997). Plant induced defenses also have broader community- and ecosystem-wide consequences, as they can alter herbivore population dynamics (Karban and Baldwin 1997; Lortzing and Steppuhn 2016), change herbivore communities (Lortzing and Steppuhn 2016; Poelman et al. 2008), and modify tritrophic interactions (Heil 2008; Kaplan et al. 2016; Orrock et al. 2018). Given the potential far-reaching impacts of plant induced defenses, understanding the ecological consequences of induced defenses is an important goal for plant–herbivore ecology (Agrawal 2011; Züst et al. 2012).

Recent work has illustrated one previously unrecognized mechanism by which plant induced defenses reduce

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herbivory: increasing cannibalism among herbivores. Induced plants not only have less plant tissue consumed, but cannibalism also reduces the number of herbivores and the amount of plant material herbivores consume (Orrock et al. 2017). Given the ubiquity of plant induced defenses (Karban and Baldwin 1997) and widespread observations of cannibalism among herbivores (Fox 1975; Polis 1981; Richardson et al. 2010), defense-mediated cannibalism is potentially an important component of plant–herbivore dynamics. However, it remains unclear how widespread and effective such defense-mediated cannibalism is, as few herbivore species have been examined in the existing studies (Elderd 2019; Orrock et al. 2017). Furthermore, although cannibalism can lead to significant changes in population size (Polis 1981), studies that compare the magnitude of cannibalism-mediated changes in herbivore abundance and herbivory to other factors that affect herbivores and herbivory (e.g., herbivore pathogens or predators) are rare (Chapman et al. 1999a; Chapman et al. 2000). Without this context, it may be difficult to fully appreciate the importance of plant defenses that increase cannibalism. Moreover, it is also unclear whether cannibalism could act to amplify or ameliorate other negative influences on herbivore populations (Sadeh et al. 2016; Van Allen et al. 2017). For example, induced defenses that modify cannibalism could increase or decrease pathogen spread among herbivores for some pathogens (Sadeh and Rosenheim 2016; Van Allen et al. 2017), such as nucleopolyhedroviruses (NPVs), that can be transmitted when herbivores consume living or dead infected conspecifics (Elvira et al. 2010; Moscardi 1999; Rebolledo et al. 2015).

We conducted two experiments that examine how induced defenses in tomatoes (*Solanum lycopersicum*) alter survival, growth, and herbivory of *Helicoverpa zea*. *Solanum lycopersicum* is a common crop species grown across the world and a model used in plant defense research (Farmer and Ryan 1990; Orrock et al. 2017; Thaler et al. 1996). *Helicoverpa zea* is a widespread agricultural pest that causes substantial damage to corn, tomato, cotton, and other crops (Olmstead et al. 2016). In the field and in the lab, *H. zea* cannibalizes conspecifics (Chilcutt 2006). Like many Lepidopteran species, *H. zea* is also susceptible to attack by NPVs (Rowley et al. 2011). We tested (a) whether plant-induced defenses increase cannibalism and reduce herbivory, and (b) the relative effect of defense-mediated cannibalism vs. insect pathogens in reducing herbivore survival and plant herbivory.

Methods

Experiment 1: Does cannibalism by *H. zea* benefit plants and caterpillars?

In the first experiment, we focused on the potential for plant induced defenses to reduce herbivory by *H. zea* larvae, to alter cannibalism by *H. zea*, and for cannibalism to help *H. zea* maintain positive growth when confronted with induced plants. This experiment is a direct analog of experiments done with *S. exigua* on tomato (Orrock et al. 2017). Tomatoes (*Solanum lycopersicum* variety 'Moneymaker') were purchased from a commercial supplier and grown from seed in a Conviron S10H growth chamber (Conviron USA, Pembina, North Dakota) at 25 °C and a 12:12 photoperiod, and randomly assigned to either an induction or control treatment when they were 21 days old. These treatments were applied twice: once on March 6, 2017 and once on March 8, 2017. Plants receiving the induced treatment were sprayed twice with a 1.0 mM MeJA and 2% ethanol solution (both from Sigma-Aldrich, St. Louis, MO); plants receiving the control treatment were sprayed with a 2% ethanol solution. Previous studies have demonstrated that external application of exogenous MeJA at this dosage can induce systemic resistance in tomatoes via increased production of compounds associated with plant defense (Farmer and Ryan 1990; Orrock et al. 2018). On March 9, 2017, third-instar caterpillars obtained from Benzon Research (Carlisle, PA) were offered leaves from induced or control plants. Individual *H. zea* and *S. lycopersicum* leaves were separately weighed using an analytical balance (Fisher Scientific, Hampton, NH) and placed in a cup together (i.e., one living *H. zea* larva per cup). To understand the potential benefits of cannibalism when plants are well defended, half of the cups were randomly designated to receive two dead *H. zea* larvae to allow focal larvae to cannibalize conspecifics (Orrock et al. 2017).

Dead larvae were killed by freezing in a − 80 °C freezer (Fisher Scientific, Hampton, NH) for 2 min. This yielded a factorial experiment with four combinations of the two treatment levels (induced vs. control crossed with opportunities for cannibalism vs. no opportunity for cannibalism). Importantly, our use of dead larvae allowed us to estimate the potential benefits of cannibalism, but did not incorporate potential costs of cannibalism (e.g., costs of being wounded by attacking a living conspecific, costs of being infected by consuming infected conspecific). Each of the four combinations was replicated five times, yielding 20 total samples. After 2 days, we weighed all *H. zea* and *S. lycopersicum* leaf tissue.

To determine if the induction and cannibalism subsidy treatments altered *H. zea* growth and herbivory, we

constructed two linear models. The first modeled *H. zea* growth rate as a function of plant induction treatment (induced or uninduced control), opportunities for cannibalism (presence or absence of dead conspecifics), and the interaction between induction treatment and opportunities for cannibalism. Growth rate of *H. zea* was estimated as in the previous studies (Orrock et al. 2017; Thaler et al. 1996), as (mass at end of experiment − mass at beginning of experiment)/mean mass over the course of the experiment (Waldbauer 1968). The second linear model had an identical structure, but used the proportion of plant mass consumed during the course of the experiment as the response variable to quantify herbivory. All analyses, including those in experiment 2, were conducted in R 4.0.3 (R Core Team 2020). Unless otherwise specified, values represent least-square means with one standard error.

Experiment 2: Placing induced defenses and cannibalism in the context of natural enemy interactions

In the second experiment, we examined whether changes in cannibalism due to plant induced defenses cause changes in host–pathogen dynamics and whether this creates different outcomes in *H. zea* survival and different benefits for plants. *Solanum lycopersicum* (variety E6203) were seeded on March 7, 2018 and transplanted into an agricultural field at Davis, CA (38.5332°N, 121.7847°W) on May 7, 2018. On July 2, 2018, we selected 44 tomato plants in the center of the field and randomly assigned them to one of two groups: induced or uninduced (control). Plants receiving the induced treatment were sprayed with a 1.0 mM MeJA and 2% ethanol solution (both from Sigma-Aldrich, St. Louis, MO); plants receiving the uninduced control treatment were sprayed with a 2% ethanol solution. All plants were sprayed twice on 2 consecutive days. The solutions were sprayed on the plants from above until all the leaves were thoroughly covered until runoff. A three-sided cardboard spray barrier was used during spray application to avoid treatment sprays from affecting neighboring plants. A few hours after the second spraying session on July 3, we clipped one stem consisting of three to five compound leaves from each plant. These stems were then weighed using a balance (Mettler Toledo, Columbus, OH) and kept hydrated by placing each stem in individual floral foam cubes; because they were well hydrated by floral foam cubes, these stems continued to grow and gain mass over the course of our experiment.

First, we prepared caterpillars that were either inoculated with virus or not. First-instar *H. zea* raised on laboratory diet were obtained from Benzon Research (Carlisle, PA) on June 29, 2018. Individual caterpillars were kept at room temperature before each *H. zea* was assigned in blocks to receive either a virus treatment, a control sham treatment, or no treatment. We exposed the caterpillars in the virus treatment group (four individuals per replicate) to a liquid suspension of *Helicoverpa zea* Single Capsid Nucleopolyhedrovirus (HzSNPV; Gemstar LC; Certis USA, L.L.C., Columbia, MD, USA). Nucleopolyhedroviruses have been isolated from many insect species, and viruses are typically restricted in the number of hosts they can infect (Moscardi 1999; Shikano et al. 2017). Gemstar contains virion-containing occlusion bodies (2 billion per ml) of a naturally occurring HzSNPV that infects larval *Helicoverpa* spp. and *Heliothis* spp.; the efficacy of the HzSNPV used in Gemstar has been verified in field trials (see references summarized in Moscardi 1999). Our own experiments also confirm that this HzSNPV is highly effective, as infection caused significantly reduced growth of *H. zea* within 24 h of infection (see "Results") and resulted in 100% mortality within 4 days (Supplementary Electronic Material). The HzNPV is also readily transmitted by the consumption of infected conspecifics (e.g., 26 cannibals that consumed infected conspecifics all died within 4 days, while 30 cannibals that consumed non-infected conspecifics all survived to pupation, Supplementary Electronic Material). Inoculation treatments were initiated on the afternoon of July 2; individuals had grown to be second-instar *H. zea* by this time. Caterpillars in the viral treatment group were exposed by painting the surface of *H. zea* laboratory diet with a paint brush dipped in the liquid suspension containing virus. Caterpillars that were not inoculated were reared on diet that had received an identical brushing treatment with sterile deionized water. These experiments revealed that method of inoculation was highly effective, and the control treatments had no effect on herbivore feeding behavior or growth (Supplementary Electronic Material). The remaining four caterpillars per replicate were not treated with viral suspension or water. All caterpillars were then kept in a non-illuminated incubation chamber (Acme Laboratory Equipment, New York, NY) at 25 °C for 24 h before the start of the experiment. After 24 h, we weighed all second-instar *H. zea*. This allowed us to determine whether virus inoculation had immediate effects on *H. zea* growth.

Next, we randomly assigned each of the clipped branches of *S. lycopersicum* to receive either virus infected or sham uninfected caterpillars, and placed four treated second-instar caterpillars of the corresponding virus treatment and four untreated second-instar *H. zea* in individual plastic arenas (clear plastic deli containers; approximately 14 cm tall × 11.4 cm diameter) that included a branch of *S. lycopersicum*; each arena was sealed with a perforated lid. Each arena thus had eight total *H. zea* at the start of the experiment. All the arenas were kept at room temperature next to a window. We created 11 replicates of each treatment combination (44 arenas in total).

To quantify changes in the number of *H. zea* alive, we checked arenas daily for 7 days, a duration that provides enough time for *H. zea* larvae to reach the fourth-instar stage under normal growing conditions, i.e., uninfected individuals at ~ 22 °C on high-quality diet (Butler 1976). During daily checks, we noted the number of caterpillars that were alive, dead, missing, or partially consumed, as well as visually estimated the proportion of leaf damage on each branch of *S. lycopersicum.* During checks of herbivory and caterpillar status, arenas were carefully handled to avoid cross contamination. During the experiment, a small number of caterpillars (8 total individuals, < 3% of all individuals) were accidentally crushed during daily checks, and these caterpillars were excluded from further analysis. On the final day of the experiment, we measured the weight of each living *H. zea* remaining caterpillar and the fresh mass of the *S. lycopersicum*. Two *S. lycopersicum* exhibited clear signs of tissue degradation and rot, presumably due to phytopathogen attack, and data from these plants were removed from our final analyses. As in experiment 1, we quantified herbivory as the proportion of plant mass remaining at the end of the experiment. This estimate of plant mass change was significantly correlated with our visual estimate of the percentage of leaf area removed, suggesting that it accurately captures the effect of *H. zea* on *S. lycopersicum* (Pearson correlation, $r = 0.50$, 40 d.f., $P < 0.001$).

To determine if the induction and virus treatments altered the proportion of *H. zea* alive, we used a linear mixed model (LMM); we applied the logit transformation (Warton and Hui 2011) to our response variable. We modeled covariation between arenas measured on subsequent days using an autoregressive covariance structure with a lag of 1 time unit (i.e., AR(1)). Using an LMM with a transformed response variable (instead of a generalized LMM with a binomial response distribution) allowed us to incorporate the time-dependent covariance structure, which dramatically improved model fit and reflects the repeated-measures nature of our design. However, we note that the choice of LMM vs GLMM did not change the qualitative outcome we observed. We opted for LMM over approaches that explicitly incorporate time (i.e., survival analysis), because it allowed us to focus on the fraction of living *H. zea*, which we felt was the most relevant for understanding both herbivore survival as well as total herbivory on focal plants (although we note that our results are not qualitatively different if survival analysis is used). Our LMM modeled induction treatment (MeJA treated or control) and virus treatment (virus or sham) as fixed effects, and the day of the experiment was treated as a covariate. We also evaluated all possible interactions between induction treatment, virus treatment, and day of the experiment. To understand how rates of herbivory were affected by induction and virus treatments, we modeled plant growth rate as a function of induction treatment, virus treatment, and an induction × virus interaction.

To determine whether the time course of cannibalism was affected by plant defenses or viral treatment, we analyzed the first observation of cannibalism with Cox proportional hazards models. For this analysis, we only used observations where signs of cannibalism were directly observed (i.e., if dead or living conspecifics were found with parts consumed); this is likely a conservative estimate, since cannibalism often leads to complete disappearance of a consumed individuals. We do not evaluate cannibalism as a cause of death in virus-treated arenas, because *H. zea* consumed living or dead individuals; finding a consumed individual only indicated that cannibalism occurred, but does not allow us to infer the cause of death, which may be viral infection, cannibalism, or some other cause (e.g., starvation). We noted observations of liquefied individuals, as liquefaction is indicative of death by viral infection (Rowley et al. 2011). However, because liquefied individuals may be difficult to detect or may disappear because they are consumed, we felt that it was not prudent to use disappearance as indicative of cannibalism-caused death in virus-treated arenas.

Results

Experiment 1: Does cannibalism by *H. zea* benefit plants and caterpillars?

Cannibalism benefited plants and surviving caterpillars; plant induced defenses and opportunities for cannibalism reduced herbivory by *H. zea* and *H. zea* growth (Fig. 1). The proportion of plant tissue remaining was affected by an interaction between plant defenses and opportunities for cannibalism (interaction term $F_{1,16} = 5.30$, $P = 0.035$, main effect of plant induction $F_{1,16} = 4.86$, $P = 0.043$; main effect of opportunities for cannibalism $F_{1,16} = 9.38$, $P < 0.01$): herbivory was greatest on control (uninduced) plants where *H. zea* had no opportunities for cannibalism (Fig. 1A). Growth of surviving *H. zea* was significantly greater when plants were not induced (main effect of plant induction $F_{1,16} = 41.92$, $P < 0.001$; Fig. 1B) and when there were opportunities to cannibalize conspecifics (main effect of opportunities for cannibalism $F_{1,16} = 70.77$, $P < 0.001$). There was a marginally significant induction by cannibalism interaction ($F_{1,16} = 3.05$, $P = 0.09$), such that induced defenses reduced *H. zea* mass gain less when there were opportunities for cannibalism (Fig. 1A). Although plant induced defenses reduced *H. zea* mass in the absence of opportunities for cannibalism (Fig. 1A), there were no differences in survival among the four treatment combinations: no focal individuals died over the course of the experiment.

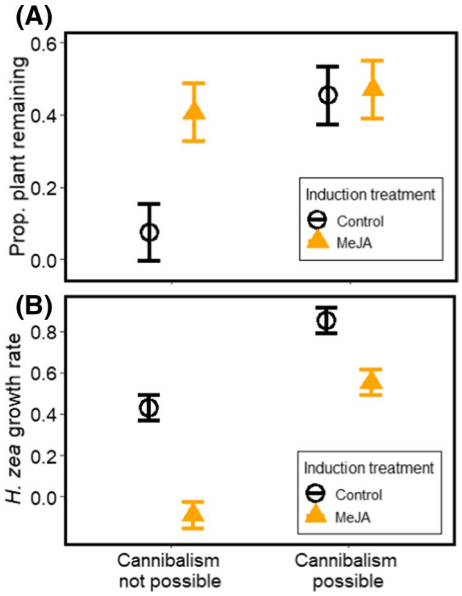

Fig. 1 **A** Herbivory experienced by *Solanum lycopersicum* exposed to *H. zea* in experiment 1 depended upon whether *S. lycopersicum* had induced defenses as well as whether *H. zea* had opportunities for cannibalism. Herbivory is measured as the proportion of plant material remaining at the end of the herbivory trial, such that smaller values represent greater herbivory. **B** The growth rate of *H. zea* is greater when *S. lycopersicum* plants do not have induced defenses and is also greater when *H. zea* has opportunities to cannibalize conspecifics. Response variables in both plots do not have units because they are proportions. Points and bars represent least-squared means ± 1 SE

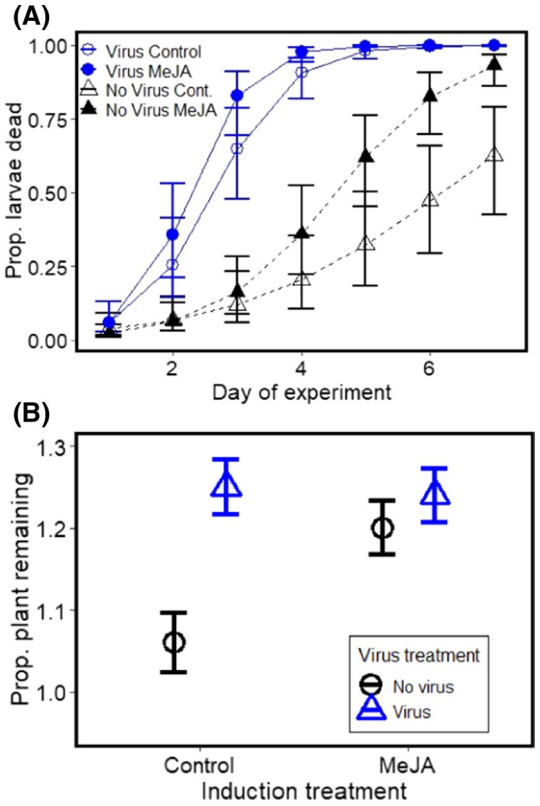

Fig. 2 **A** Proportion of *Helicoverpa zea* caterpillars that died over the course of experiment 2. Caterpillars were housed with *Solanum lycopersicum* plants induced with methyl jasmonate (MeJA; closed symbols) or with uninduced plants (control; open symbols). Blue symbols indicate that individuals were exposed to two conspecifics infected with a virus, whereas black symbols indicate that caterpillars were exposed to a sham non-virus control treatment. Points represent least-square means, and error bars indicate 95% confidence limits to facilitate visual comparison of mean values. **B** Herbivory experienced by *Solanum lycopersicum* over the 7-day exposure to *H. zea* in experiment 2. Herbivory is measured as the proportion of plant material remaining at the end of the herbivory trial, such that smaller values represent greater herbivory. Points and bars represent least-squared means ± 1 SE

Experiment 2: Placing induced defenses and cannibalism in the context of natural enemy interactions

Preliminary experiments revealed that the virus acts quickly and is highly pathogenic: individuals died within 4 days of initial infection and infected individuals consumed much less diet over that period (Supplementary Electronic Material). Inoculation with virus led to a rapid reduction in *H. zea* growth: after 24 h, mass of inoculated individuals (2.98 ± 0.03 mg SE) was significantly less than mass of uninoculated *H. zea* (4.35 ± 0.03 mg; $t_{20} = 3.16$, $P < 0.01$).

The proportion of *H. zea* alive decreased over the course of the experiment (Fig. 2A; main effect of time: $F_{1, 248} = 38.99$, $P < 0.001$). Virus effects on *H. zea* mortality were generally strong: all *H. zea* in the virus treatment were dead by the fifth day of the 7-day experiment (Fig. 2A). Importantly, the proportion of *H. zea* surviving over the course of the experiment depended upon the interaction between the virus treatment and the induction treatment (time x induction treatment x virus treatment interaction; $F_{1,248} = 3.85$, $P = 0.05$). This interaction arose because the virus treatment caused rapid mortality of *H. zea* regardless of induction treatment, but in the absence of virus, plant induced defenses led to significantly quicker mortality than uninduced controls (Fig. 2A). For example, the proportion of caterpillars remaining in virus-treated groups was significantly lower than in non-treated groups by day 2, whereas decreases in survival caused by plant induced defenses (and concomitant increases in cannibalism among *H. zea*) only became apparent at day 5 (Fig. 2A). Two-way interactions were also significant between induction and time ($F_{1, 248} = 6.61$, $P = 0.01$) and virus treatment and time ($F_{1, 248} = 15.17$, $P < 0.01$). There was no significant interaction between induction treatment and virus treatment ($F_{1, 38} = 0.71$, $P = 0.40$), no significant main effect of induction treatment ($F_{1, 38} = 0.50$, $P = 0.49$), and no significant main effect of virus treatment ($F_{1, 38} = 0.71$, $P = 0.41$). We are confident that *H. zea* death observed in virus-treated

replicates was due to viral infection, as our infection protocol was highly effective (Supplementary Electronic Material), there was no other difference between virus-treated and sham-treated replicates, and we directly observed virus-killed (i.e., liquefied) individuals in 100% of the replicates receiving inoculated individuals (22 of 22 replicates); virus-killed individuals were never observed in replicates that received the sham (i.e., non-inoculated) individuals (0 of 20 replicates).

Cannibalism was directly observed in 32 of the 42 arenas, and the frequency of observed cannibalism did not differ among induction or viral treatments ($X^2 = 2.35$, df = 3, $P = 0.50$). In arenas where cannibalism was directly observed, the onset of cannibalism occurred sooner in virus-treated arenas (main effect of virus treatment likelihood ratio test $X^2 = 4.35$, df = 1, $P < 0.04$; Fig. 3). The onset of cannibalism was not affected by the induction treatment (likelihood ratio test $X^2 = 0.29$, df = 1, $P = 0.60$) or the interaction between the virus treatment and induction treatment (likelihood ratio test $X^2 = 0.26$, df = 1, $P = 0.61$). In non-virus arenas where disappearance was likely a more reliable indicator of death by cannibalism, a greater proportion *H. zea* individuals disappeared from arenas with induced plants (0.70 ± 0.06 SE) compared to arenas with non-induced plants (0.48 ± 0.06; binomial GLMM treating arena as a random effect, $X^2 = 6.48$, df = 1, $P = 0.01$).

Virus and plant induced defenses also interacted to shape *H. zea* herbivory of *S. lycopersicum* (virus × induction interaction: $F_{1,38} = 5.12$, $P = 0.03$, virus main effect $F_{1,38} = 15.30$, $P < 0.01$; induction main effect $F_{1,38} = 9.46$, $P < 0.01$; Fig. 2B). The interaction was significant because induction or virus inoculation (or both) led to significantly less biomass loss to herbivory (and thus greater plant growth) compared to the control treatment that received no induction and no virus inoculation; application of either of these treatments led to effectively half of the herbivory experienced by control plants where caterpillars were not inoculated and plants were not induced. Despite significant differences in herbivory and *H. zea* survival, the final mass of surviving individual *H. zea* (when considering only non-inoculated arenas because there were no survivors in virus-inoculated groups) did not differ between induced and uninduced plants ($t_{12} = 1.18$, $P = 0.23$).

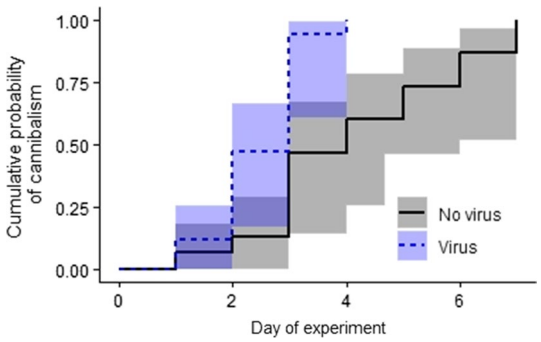

Fig. 3 Direct observation of cannibalism by *H. zea* occurred sooner in experiment 2 when caterpillars were in arenas that received individuals inoculated with virus. The main effect of virus treatment is plotted because there were no significant effects of induction treatment and no interaction between induction treatment and virus treatment on the time when the first cannibalism event was observed. Shaded areas represent 95% confidence limits

Discussion

While plant defenses can increase the predation risk of their herbivores (Heil 2008), the role of plant defenses in increasing risk of cannibalism has only recently been appreciated (Orrock et al. 2017). Our results extend our knowledge of the interplay between cannibalism and plant defenses in several ways. First, plant defenses that promote cannibalism led to reduced consumption of plant biomass by *H. zea* (Fig. 1A), demonstrating that this consequence of plant defense occurs for *H. zea* just as it does for *S. exigua* (see below). Second, our results suggest that surviving cannibals can circumvent plant defenses, maintaining their growth on well-defended plants (Fig. 1B). Third, our results help place the demographic effect of plant defenses in context relative to other factors that influence herbivore populations: although some individual caterpillars benefitted from cannibalism, plant induced defenses led to significantly higher overall rates of *H. zea* mortality (Fig. 2A); while this increased mortality did not occur as quickly as the effect of the extremely pathogenic virus, mortality caused by cannibalism rivaled mortality caused by the virus at the end of our experiment. Together, our results demonstrate that plant induced defenses operating via changes in herbivore behavior can provide benefits to plants similar in magnitude to those from herbivore populations being greatly reduced by a viral pathogen (Fig. 2B).

Plant-defense-mediated cannibalism as a potential limiting factor for Lepidopteran herbivores

Plants defenses may limit herbivore populations by both bottom-up control (i.e., changing plant quality) and top-down control (i.e., by recruiting natural enemies of herbivores; Heil 2008). However, most natural enemies considered in this role are predators (Wetzel et al. 2018) or parasitoids (Kaplan et al. 2016), and the potential for plants to promote cannibalism has only recently been considered (Orrock et al. 2017). Recruiting predators and parasitoids might not always be a reliable form of plant defense, however. This strategy of recruiting arthropod natural enemies of herbivores depends on proximity to predators who can recognize and respond to

signals (Allison and Hare 2009) and may involve a time lag between the start of herbivory and the arrival of predators by dispersal or numerical responses (Kugimiya et al. 2010). Conversely, by turning herbivores into cannibals, plants may draw on a more immediate and consistent pool of natural enemies. Importantly, our work demonstrates that benefits of defense-mediated cannibalism accrue to plants (Fig. 1B) as well as to the insects who cannibalize. Individual *H. zea* reared on induced plants consumed less plant tissue compared to controls (Fig. 1B), as cannibalism helped *H. zea* sustain body mass despite a reduction in host plant quality (Fig. 1A); similar results have been documented for *S. exigua* (Orrock et al. 2017), as well as noted more broadly for *S. frugiperda* feeding on a high- vs. low-quality plant species (Raffa 1987).

A primary question arising from recent work demonstrating that induced defenses can benefit plants by increasing cannibalism among *Spodoptera exigua* (Orrock et al. 2017) is whether cannibalism induced by plant defenses occurs in multiple herbivore species. Our current study extends this previous work to show that cannibalism mediated by plant defenses can also occur in the widespread insect herbivore, *H. zea*. Because cannibalism is widespread among insects and often depends upon resource quality (Fox 1975; Polis 1981; Richardson et al. 2010), our findings suggest that increased cannibalism may be a widespread response to plant-induced defenses in Lepidopteran herbivores and other arthropods. Because cannibals may also act as intra-guild predators (Polis 1981; Richardson et al. 2010), the occurrence of defense-mediated cannibalism in multiple species could alter interspecific interactions among herbivores in field settings.

Plant induced defenses and the dynamics of herbivore disease

The benefits of cannibalism for herbivores (i.e., simultaneously gaining energy and nutrients while reducing the number of competitors) may be offset by significant costs, such as increased likelihood of injury (Richardson et al. 2010) and the cost of becoming infected by a pathogen (Williams and Hernández 2006), such that cannibalism may not always have fitness benefits (Chapman et al. 1999b). Indeed, since viruses may benefit from increased transmission when herbivores consume infected conspecifics, selection may favor viruses that increase the likelihood that infected hosts will be cannibalized (Sadeh et al. 2016; Sadeh and Rosenheim 2016; Van Allen et al. 2017). In demonstrating that cannibalism happened more quickly in the virus-inoculated group of *H. zea* (Fig. 3), our experiment suggests that, from the cannibal's perspective, viruses and plant defenses provide different motivations for cannibalistic behavior. Viral infection can increase cannibalism by making it easier for a cannibal to subdue an infected conspecific (Van Allen et al. 2017): in experiment 2, infected larvae were 46% smaller and also more lethargic (personal observation), making infected conspecifics very easy targets for attack. Our experiments on viral transmission also support this conclusion, as the proportion of infected individuals cannibalized was 0.72 after a single night, significantly greater than the proportion of uninfected individuals (0.42) that were cannibalized (Supplementary Electronic Material). These results with *H. zea* are consistent with studies in other species, as infected *S. exigua* larvae were more readily cannibalized either alive or dead (Elvira et al. 2010; Rebolledo et al. 2015). Plant induced defenses, on the other hand, are likely to increase cannibalism because attacking a conspecific, while risky, may be the best option when induced defenses make plant tissues of very low nutritional value. Stated another way, viruses may increase cannibalism by reducing the immediate risks of being injured while attacking an infected conspecific and plant defenses may increase cannibalism by making it profitable to attack a conspecific. The same necessity of attacking a healthy conspecific may also arise when less-virulent pathogens modify herbivore metabolism. For example, *Helicoverpa armigera* exposed to sublethal doses of NPVs had increased metabolic rate and preferred protein-rich diets over carbohydrate-rich diets (Bouwer et al. 2009).

While the shift in the onset of cannibalism we observed (Fig. 3) was not affected by plant-induced defenses, the rapid action of the virus used in our study likely reduced the ability to detect subtle shifts in the initiation of cannibalism. Our results suggest that the rate at which caterpillars die due to viral pathogens is greater than the rate at which they die due to cannibalism on induced plants, although final mortality at the end of our experiment is similar (Fig. 2A). However, pathogens of Lepidoptera vary in their transmission and virulence. Variation in one or both of these pathogen characteristics may influence whether plant induced defenses alter the spread of disease (Van Allen et al. 2017). Cannibalism may be more likely to promote the spread of less-virulent Lepidopteran diseases, such as fungal infections (Boucias et al. 1984), as infected individuals have more time to interact with conspecifics before dying. Cannibalism may also serve to promote pathogen spread if cannibals are better hosts for the pathogen than the infected individuals that the cannibals consume. For example, if cannibals are larger than the infected individuals they consume (consistent with our results), pathogens that infect cannibals may benefit from increased resources and ultimately produce more pathogen propagules (Sadeh et al. 2016; Sadeh and Rosenheim 2016). Alternatively, cannibalism may decrease the spread of pathogens by reducing rates of feeding (lowering the likelihood of ingesting a virus; Elderd 2019), and/or by reducing contact among hosts through reduced activity, lower population density, or both (Van Allen et al. 2017). This may be particularly

likely in stage- and thus size-structured lepidopteran herbivore populations, as viral infection leads to a cessation of growth of infected individuals (Supplementary Electronic Material), rendering them smaller and more likely to be consumed by larger uninfected individuals (Sadeh et al. 2016; Van Allen et al. 2017). Thus, while plant induced defenses that promote cannibalism may be expected to promote the spread of diseases a priori, our results suggest that this effect will depend on the nature of the pathogen, how it is spread, how rapidly the pathogen kills its hosts, and the degree to which the pathogen benefits from reproducing within an infected non-cannibal vs. an infected cannibal. Future studies that use factorial manipulation of virus and plant induction with a variety of different pathogens would be extremely useful in building a general understanding of whether the ultimate effect of plant induced defense on herbivore-pathogen interactions can be predicted by thresholds in virus transmission or virulence.

Conclusions and future directions

Our results add to a growing body of evidence that plant induced defenses can promote cannibalism among herbivores. However, our experiments focused on cannibalism, herbivore survival, and herbivory in a setting with a fixed starting number of infected and uninfected individuals. Future studies that evaluate different starting conditions, such as different starting densities of herbivores (Andow et al. 2015), are needed to more fully explore how varying the number and fraction of infected vs. uninfected hosts will alter the dynamics of cannibalism and disease. An important avenue for future research is to evaluate the degree to which defense-mediated cannibalism occurs in field settings and document how realistic field conditions (e.g., varying densities of infected and uninfected individuals, the ability for herbivores to move among host plants) affect the potential for cannibalism to modify insect-pathogen interactions; see Elderd (2019) and Elderd and Dwyer (2019) for examples of field studies of cannibalism and transmission for *Spodoptera frugiperda*. Studies that examine plant species with different propensities for induced defenses may also be informative for predicting when defense-mediated cannibalism will be common and when it might interact with herbivore-pathogen dynamics. For example, adult insects may be less likely to oviposit on plants that are constitutively well-defended, opting instead to oviposit on plants with low levels of constitutive defense. However, plants with low levels of constitutive defense may be capable of strong induced defenses, creating a scenario where high rates of oviposition lead to large numbers of insects that then trigger strong plant induced defenses. In the field, we might therefore expect cannibalism to be low on constitutively defended plants, but highest on plants that exhibit low levels of constitutive defense but have the potential for strong induced defenses. An additional unexplored hypothesis is that variation in within-plant induced defenses may lead to spatial variation in cannibalism on the same plant, such that one benefit of producing some tissues of low inherent quality is that these tissues may more readily prompt herbivores to become cannibals that have benefits that extend to all leaves of the plant.

Our current experiments show that induced plant responses could encourage cannibalism under field conditions and that these two processes can interact to reduce populations of caterpillars and enhance plant performance. Future experiments that manipulate individual density, potential for pathogen transmission, and the potential for cannibalism will be essential for understanding how often plant defenses trigger cannibalism in field settings, the degree to which defense-mediated cannibalism will reduce herbivory, and the interplay of plant defenses, cannibalism, and insect pathogens.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

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