

RESEARCH ARTICLE

Functional Ecology



Top-down effects from parasitoids may mediate plant defence and plant fitness

Ching-Wen Tan¹ | Michelle L. Peiffer¹ | Jared G. Ali¹ | Dawn S. Luthe² | Gary W. Felton¹

¹Department of Entomology, Penn State University, University Park, PA, USA

²Department of Plant Science, Penn State University, University Park, PA, USA

Correspondence

Ching-Wen Tan

Email: czt5069@psu.edu

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Abstract

1. Plants face many environmental stresses that can impact their survival, development and fitness. Insects are the most diverse, abundant and threatening herbivores in nature. As a consequence, plants produce direct chemical and physical defences to reduce herbivory. They also release volatiles to recruit natural enemies that indirectly protect them from herbivory. The recruitment of parasitic wasps can benefit plant fitness because they ultimately kill their insect hosts.
2. Recently, studies showed that parasitoids can indirectly mediate plant defences by modulating herbivore oral secretions. In addition to the direct benefits of parasitoids in terms of reducing herbivore survival, we tested if the reduction in induced defences by parasitized caterpillars compared to non-parasitized caterpillars may reduce the costs associated with defence expression.
3. We provide evidence that tomato plants treated with saliva from parasitized caterpillars have significantly higher fitness parameters including increased flower numbers (16.3%) and heavier fruit weight (13.5%), compared to plants treated with saliva from non-parasitized caterpillars. Since plants were grown without actual herbivores, the higher values for these fitness parameters were due to lower costs of induced defences and not due to reduced herbivory by parasitized caterpillars. Furthermore, the resulting seed germination time was shorter and the germination rate was higher when the maternal plants were previously exposed to parasitized herbivore treatment compared to control (non-treated) plants.
4. Overall, application of saliva did not result in transgenerational priming of offspring defence responses. However, offspring of parents exposed to caterpillar saliva had lower constitutive levels and higher induced levels of trypsin inhibitor than offspring from unexposed parents.
5. This study shows that the saliva of parasitized caterpillars can modulate plant defences and further demonstrates that the lower induction of plant defences is associated with elevated plant fitness in the absence of herbivore feeding, suggesting that induced plant defences are costly.

KEYWORDS

parasitoid, plant defence, plant fitness, seed germination

1 | INTRODUCTION

Many biotic and abiotic environmental factors can influence plant development and reproduction. For instance, water (Aragón, Escudero, & Valladares, 2008), pathogens (Tian, Traw, Chen, Kreitman, & Bergelson, 2003), herbivores (Agrawal, 1999; Mothershead & Marquis, 2000; Pashalidou et al., 2015), plant genetic diversity (Johnson, Lajeunesse, & Agrawal, 2006), plant population size (Kolb, 2008) and soil microbial communities (Lau & Lennon, 2012) were all shown to severely impact plant fitness (fruit and seed production). Herbivorous insects are the most abundant and diverse herbivores that consume plant tissues (Howe & Jander, 2008) and can negatively affect plant fitness.

The negative effects of above- and below-ground herbivores on plant fitness can be caused by the loss of leaf tissues that reduce photosynthesis, changes in flower morphology and number that negatively affect pollinator preference, damage to reproductive organs (bud, flower and fruit) that limit seed production and/or increases in plant mortality (Barber et al., 2015; Juenger & Bergelson, 1998; Kessler & Baldwin, 2004; Maron, 1998; Mothershead & Marquis, 2000; Strauss, Conner, & Rush, 1996). In contrast, moderate herbivory may facilitate plants to produce higher above-/below-ground biomass and flower/fruit numbers than undamaged plants by overcompensation (Paige, 1999; Paige & Whitham, 1987; Poveda, Díaz, & Ramirez, 2018; Poveda, Jimenez, & Kessler, 2010).

Plants have evolved various strategies to protect themselves from intruders, including induced defences (Chen, 2008). It is often costly to produce and maintain plant defences. Therefore, induced plant defences, that are only activated when plants are directly under attack by herbivores, may be a cost-saving strategy (Huot, Yao, Montgomery, & He, 2014). Induced plant defences may protect plants from herbivores and a subsequent loss of plant fitness. For example, wild radish produces higher trichome densities in newly developed leaves after *Pieris rapae* larval feeding and reduces herbivory, which subsequently enhances plant fitness as measured by higher fruit and seed production (Agrawal, 1999). McArt, Halitschke, Salminen, and Thaler (2013) reported that plants (*Oenothera biennis*) fed on by Japanese beetle *Popillia japonica* had higher ellagitannin concentrations in flower buds, which suppressed ovipositional preference and amount of feeding by a seed herbivore, thus allowing plants to have higher seed yield. In addition, induced defences play an important role in transgenerational priming and have been shown to increase their offspring's ability to deal with stress in a faster and/or stronger manner (Holeski, Jander, & Agrawal, 2012; Rasman et al., 2012; van Hulten, Pelsner, van Loon, Pieterse, & Ton, 2006). For example, *Arabidopsis* plants showed higher defence-related gene expression and produced more indole glucosinolates when parental plants were exposed to *P. rapae* damage and then dramatically reduced subsequent herbivore growth performance (Rasman et al., 2012).

Besides direct effects, plants can indirectly reduce herbivore populations by recruiting natural enemies of these herbivores

(parasitoids and predators; Kessler & Baldwin, 2001; Mumm & Dicke, 2010), which may facilitate plant reproduction and fitness (Gómez & Zamora, 1994; Hoballah & Turlings, 2001; Kessler & Baldwin, 2004; Pashalidou et al., 2015; van Loon, de Boer, & Dicke, 2003). Recruitment of parasitoids by herbivore-damaged plants has been documented in many systems (Mumm & Dicke, 2010) and may benefit plant fitness due to their relatively low impacts on beneficial insects (e.g. pollinators and seed dispersers) and reduction of plant tissue damage by reducing herbivore populations (Gols et al., 2015; Gómez & Zamora, 1994; Romero & Koricheva, 2011; Turlings & Erb, 2018). However, only recently has research begun to address the consequences of parasitization on plant responses and the resulting effects on plant fitness.

Parasitism of caterpillars may suppress or enhance plant defences in a species-specific manner (Tan, Peiffer, Hoover, Rosa, & Felton, 2019). Several studies have shown that parasitoids can indirectly influence plant defences through changing oral secretions and feeding behaviour of host caterpillars. The colour of oral regurgitant of parasitized *P. rapae* caterpillars was lighter and induced higher defence responses in cabbage plants (Poelman et al., 2011). In addition, cabbage plants showed enhanced expression of glucosinolate metabolic genes and emitted distinct volatile compounds when being fed on by parasitized compared to non-parasitized *Pieris* caterpillars (Zhu et al., 2015). Ode, Harvey, Reichelt, Gershenzon, and Gols (2016) showed that parasitism increases leaf consumption of host caterpillars *Trichoplusia ni*, thereby increasing indole glucosinolates production in cabbage plants. Recent studies revealed that tomato plants showed lower defence responses when fed on by parasitized compared to non-parasitized caterpillars. *Microplitis croceipes*-parasitized corn earworm *Helicoverpa zea* had lower elicitor activity in their saliva (i.e. glucose oxidase, GOX) and significantly downregulated tomato defence-related gene expression and defence protein activities, which increased parasitized caterpillar growth and thus parasitoid fitness (Tan et al., 2018). In this case, the parasitoid's obligate mutualistic polydnvirus (PDVs) suppresses GOX gene expression and enzyme activity in labial glands of parasitized caterpillars. The downregulation of insect saliva elicitors was also found in *Cotesia glomerata*-parasitized *P. rapae* larvae (Cusumano et al., 2018). In that case, the PDVs and parasitoid venom suppressed elicitor gene (glucose dehydrogenase, GDH) expression in the caterpillar's salivary glands. Such linkages to defence attenuation may have important implications for trade-offs with plant growth.

Plants possess limited energy resources and have to allocate it to growth and defence (Huot et al., 2014). Trade-offs between defence and growth are common in plants (Fine et al., 2006) and are often mediated by crosstalk between phytohormones, where upregulation of one hormone pathway leads to the attenuation of another (e.g. jasmonates, salicylic acid, gibberellin and auxin). This can lead to the reallocation of resources towards either primary or secondary metabolisms, often carrying ecological consequences (Huot et al., 2014; Karasov, Chae, Herman, & Bergelson, 2017). The lower induced responses elicited by

parasitized caterpillars may reduce defence energy costs compared to responses to non-parasitized caterpillars. Therefore, understanding potential factors that could drive the mechanism of plant defence and growth/fitness, particularly those mediated by the third trophic level on plant fitness is essential. To date, no studies have examined the effects of parasitized caterpillars' saliva on plant defence and growth/fitness trade-offs, let alone downstream transgenerational consequences. In this research, we isolate one factor that may contribute to effects of parasitism on plant defence and fitness, namely the changes that occur in salivary elicitors in an insect herbivore as a consequence of parasitism. Thus, we hypothesize that the lower salivary elicitor activities of parasitized caterpillars and subsequent reduced levels of induced plant defences will enhance plant fitness relative to treating plants with saliva from non-parasitized caterpillars. Second, we determined how parasitoids indirectly influence plant seed germination and induced defence responses in the plants from the next generation.

2 | MATERIALS AND METHODS

2.1 | Insects

Helicoverpa zea eggs were purchased from Benzon Research (Carlisle, PA). Larvae were fed on artificial diet (Peiffer & Felton, 2005) and reared individually until pupal formation. Pupae were placed in a container [15 (diameter) cm × 28 (height)] and 10% sugar solution was provided as food for adult moths. Eggs were collected daily for the experiments. The colony has been kept in our laboratory for several generations.

Microplitis croceipes pupae were kindly provided by Dr Henry Fadamiro (Auburn University, Auburn, AL) and a colony was established and maintained in our laboratory. Briefly, 10 caterpillars (second and/or third instars) were exposed to one female parasitoid for 1 hr in a 9-cm diameter Petri dish. Usually, 20 female parasitoids were used in each round of parasitization of hosts. After being parasitized, caterpillars were transferred to cups with artificial diet and reared individually. Pupal cocoons were collected, and emerged adults were kept in a container (27 cm × 15 cm × 11 cm) provided with a 20% honey solution. Insect colonies were reared in a growth incubator (25 ± 2°C, 16L:8D).

2.2 | Plants

Tomato seeds (*Solanum lycopersicum* cv. Microtom) were sown in potting soil (Sunshine Mix4 Aggregate Plus, SunGrow Horticulture) in a greenhouse (16L:8D) at Pennsylvania State University. Microtom is a small tomato variety, which is ideal for plant fitness tests due to its overall small size and faster generation time than many cultivars (Lima, Carvalho, Neto, Figueira, & Peres, 2004; Martí, Gisbert, Bishop, Dixon, & García-Martínez, 2006) and it is self-pollinated

(Medina et al., 2013; Ueta et al., 2017). Two weeks after germination, seedlings were transferred to pots (10 cm × 10 cm × 9 cm) with potting soil and 3 g of fertilizer (Osmocote, 15-9-12) was applied. Plants were watered daily. Plants with three fully expanded leaves (5–6 weeks old) were used in the following experiments.

2.3 | Caterpillar GOX enzyme activities in labial glands

To evaluate the effects of parasitism on insect oral secretions, GOX activities were measured in labial glands from parasitized (P) and non-parasitized (NP) caterpillars. GOX is the most abundant enzyme in *H. zea* salivary labial glands and can be recognized and triggers defence responses in tomato plants (Tian et al., 2012).

During the last day of their second instar, *H. zea* caterpillars (with head capsule slippage) were exposed to *M. croceipes* females. Caterpillars were removed immediately once parasitized by a female parasitoid and then reared individually. Six days after being parasitized, labial glands were collected (Tan et al., 2018). Labial glands from non-parasitized caterpillars were collected at the same developmental stage. Parasitized and non-parasitized caterpillars were placed on ice for 20 min before dissection and labial glands were collected under a dissecting microscope. GOX activities were assessed as described by Eichenseer, Mathews, Bi, Murphy, and Felton (1999). Briefly, labial glands were homogenized (40 µl phosphate buffer, 0.1 M, pH 7) and supernatant was collected after centrifugation (4°C, 12,850 × g, 10 min). Each sample (5 µl) was mixed with 200 µl of substrate [1.3 mg dianisidine-HCl (Sigma D-3252), 2.5 ml of phosphate buffer (0.1 M, pH 7), 0.5 ml of D-glucose (100 mg/ml, Aldrich 253073) and 20 µl of horseradish peroxidase (1 mg/ml, Sigma P2088)]. The changes in absorbance values were recorded at 460 nm in a plate reader (Spectramax 190, Molecular Devices). Protein in each sample was quantified by Bradford assays using BSA (bovine serum albumin, Fraction V, Omnipur) as the protein standard (Bradford, 1976).

2.4 | Plant defence response

To evaluate how parasitized caterpillars influence plant defence responses, plant defence-related gene expression and enzyme activities were tested. There were three treatments: control plants with no treatment (C); plants treated with parasitized (P) caterpillar saliva; and plants treated with non-parasitized (NP) caterpillar saliva. The saliva treatment was used as a proxy for herbivory to standardize both the timing and amount of herbivory. This treatment produces a uniform herbivore phenotype and has previously been shown to elicit plant defence responses similar to those observed in response to caterpillar feeding (Tan et al., 2018, 2019). Thus we were able to isolate the effects of parasitoid-induced changes in salivary elicitors on plant defence and fitness apart from any changes in the amount and timing of herbivory.

Parasitized caterpillars were obtained as described above. Labial salivary glands were collected from parasitized (6 days after parasitism) and non-parasitized caterpillars and homogenized with phosphate buffer (0.1 M, pH 7.0). Supernatant was collected after centrifugation (4°C, 8,403 × g, 10 min). Protein in parasitized and non-parasitized insect saliva samples was quantified by Bradford assays (Bradford, 1976). A serrated wounding tool (Bosak, 2011) was used to wound the third terminal leaflet (counting from the bottom) and immediately 15 µl (1 µg/µl protein; Peiffer & Felton, 2005) of insect saliva from parasitized or non-parasitized caterpillars was applied with a pipette.

Twenty-four hours after saliva application, plant tissues (1 g) were collected from the treated leaflet. RNA extraction, cDNA synthesis and qRT-PCR analysis were processed as described (Tan et al., 2018). Reference genes (actin and ubiquitin) were used and the relative expression of target genes was compared with that of intact control (C) plants by using the 2-ΔΔct method (Livak & Schmittgen, 2001). Primers used in this assay are listed in Table 1.

Forty-eight hours after saliva application, plant tissues (50 mg) were collected from the third terminal leaflet for peroxidase (POD), polyphenol oxidase (PPO) and trypsin inhibitor (TI) assays. PPO and POD assays were performed as described by Felton, Donato, Del Vecchio, and Duffey (1989). Briefly, samples were powdered with a Genogrinder (Spex Sample Prep 2000) and a phosphate buffer (1.25 ml, 0.1 M, pH 7) with 5% polyvinylpyrrolidone (PVP) (Alfa Aesar 41631) was added to each sample. Samples were set on ice for 10 min. Supernatant was collected after centrifugation (4°C, 12,850 × g, 10 min). Five microliters of sample was added to 200 µl of caffeic acid (3 mM, Sigma C0625) for PPO activity; 5 µl of supernatant was mixed with 10 µl of hydrogen peroxide (3%, CareOne) and 190 µl of guaiacol (3 mM, Sigma G5502) for the POD assay. The change in absorbance at 450 nm was recorded in a plate reader (Spectramax 190, Molecular Devices) for both the PPO and POD assays. The protein concentration in each sample was quantified using the Bradford assay with BSA (Fraction V, Omnipur) as the standard (Bradford, 1976). For TI activity assays, samples were powdered as described above and 1.25 ml of assay buffer (0.046 M Tris and 0.0115 M CaCl₂, pH 8.1) with 5% PVP was added. Supernatant (4°C, 12,850 × g, 10 min) was collected for the assay. Ten microliters of each sample was mixed with 10 µl of Trypsin (20 µg/ml, Sigma T1426) and 80 µl of assay buffer. Ten minutes later, 100 µl of TAME (p-toluene-sulfonyl-L-arginine methyl

ester, 0.002 M, Sigma T4626) was added and the absorbance values were recorded at 247 nm in a plate reader. Percentage of inhibition in each sample was calculated by comparing to the activity of trypsin and assay buffer alone (without leaf supernatant). Protein concentration in each sample was quantified by Bradford assays as described above.

2.5 | Plant fitness

To determine if parasitized or non-parasitized caterpillar treatments differentially influence plant fitness, three treatments were used: control plants with no treatment (C); plants treated with parasitized (P) caterpillar saliva; and plants treated with non-parasitized (NP) caterpillar saliva.

Labial salivary glands were collected and homogenized from parasitized and non-parasitized caterpillars as described above. Briefly, a serrated wounding tool was used to wound the third terminal leaflet of tomato plants (counting from the bottom, 37-day-old) and immediately 15 µl (1 µg/µl protein) of insect saliva from parasitized or non-parasitized caterpillars was applied with a pipette. Two days later, the same process was repeated on two leaflets of the fourth leaf. Four days after the first application, insect saliva was applied to two leaflets of the fifth leaf. In total, insect saliva was applied three times to five leaflets to simulate caterpillar feeding. Plants were watered as needed and pots were rotated randomly every week. Testing plants were excluded from other biotic and/or abiotic factors which might have varied with natural conditions.

The first flowering date, flower number, fruit weight, seed weight and seed number were recorded to represent plant fitness. The first flowering date is the number of days between the first saliva application and appearance of the first flower. The experiment was ended when plants were 133 days old. By that time, more than half of the leaves had turned brown and no green fruit ripened within 1 week.

2.6 | Second-generation performance

To determine if herbivore treatment of maternal plants influenced offspring performance, seed germination rate and plant defence

TABLE 1 Primer pairs used for tomato gene expression

Gene name	Description	Species	Forward	Reverse	Accession No.
<i>CysPI</i>	Cysteine proteinase inhibitor	Tomato	GGTGAAGGAATGGGAGGACTTCAA	GGAGGTTTGGGAATGGAACATTGG	AF198390
<i>PPOB</i>	Polyphenol oxidase B	Tomato	TTCGCGAGTGGGAATACCTCGTTT	AGTCAGGGACTGTTTGGACACGAA	Z12834
<i>UBI</i>	Ubiquitin	Tomato	GCCAAGATCCAGGACAAGGA	GCTGCTTCCGGCGAAA	X58253
<i>ACT</i>	Actin-7	Tomato	AGGTGTTATGGTCGGAATGG	TCATCCCAATTGCTGACTATACC	AB199316

responses were measured. To determine seed germination time and rates, seeds were collected from the plant fitness experiments described above. There were three treatments: seeds from maternal plants with no treatment (C), seeds from maternal plants treated with parasitized caterpillar saliva (P) and seeds from maternal plants treated with non-parasitized caterpillar saliva (NP). Thirty seeds from each maternal plant were sown in potting soil in the greenhouse and a total of 10 maternal plants of each treatment were used. Pots were placed on trays and water was added to the tray to maintain soil moisture. Seeds were observed daily for germination time. Germination rates of seeds were calculated at 11 days after sowing as follows: [germinated seed #/ total seed # (30 seeds)] $\times$ 100.

In the offspring defence response assay, there were three maternal plant treatments (C, P and NP) and two herbivore treatments (N: no herbivore treatment and H: herbivore treatment). Seeds were planted as described above. Plants with three fully expanded leaves (5–6 weeks old) were used for the experiment. Labial glands were collected and homogenized from non-parasitized caterpillars as described above. For the herbivore treatment (H), 15 μ l (1 μ g/ μ l protein) of insect saliva was applied after mechanical wounding of the third terminal leaflet (counting from the bottom) of tomato plants, while the other half plants were without herbivore (N) treatment.

Twenty-four and 48 hr after saliva application, plant tissues (50 mg) were collected from the third terminal leaflets for PPO, POD and TI assays as described above. Each plant was only used once.

2.7 | Statistical analyses

Data were transformed as needed to obtain a normal distribution and to address homogeneity of residual variances; SAS 9.4 (SAS Institute Inc) was used for data analyses. The GOX activities in labial glands were analysed by Student's *t* test.

Plant fitness was analysed by one-way ANOVA (Proc GLM), and plant defence enzyme activities and seed germination rate were analysed by two-way ANOVA (Proc GLM), followed by means comparisons using the Tukey's least significant difference (LSD) test (significance level, $p < 0.05$). Seed germination days were analysed by Kruskal–Wallis tests, followed by pairwise multiple comparison (DSCF) tests (significance level, $p < 0.05$).

3 | RESULTS

3.1 | Effects of parasitism on labial gland GOX activities

Microplitis croceipes-parasitized *H. zea* caterpillars showed significantly lower GOX activity (8.6 times) in the salivary labial glands than non-parasitized caterpillars (Figure 1).

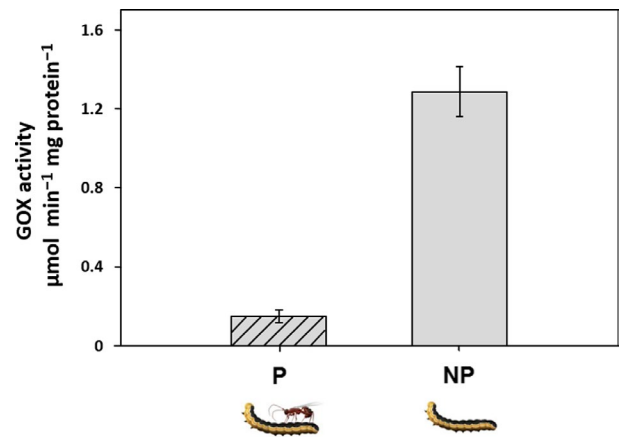


FIGURE 1 Effects of parasitism on labial gland glucose oxidase (GOX) activity in *Helicoverpa zea* caterpillar. Values are untransformed $M \pm SEM$. Different letters indicate significant differences between treatments: Student's *t* test, $n = 15$ –17, $F_{1,30} = 92.93$, $p < 0.0001$

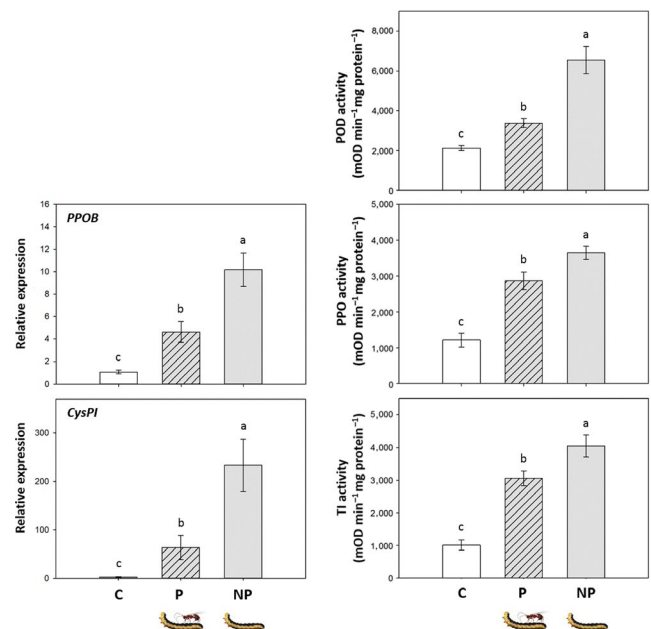


FIGURE 2 Effects of *Helicoverpa zea* caterpillar parasitism treatments on induction of tomato defensive responses. Values are untransformed $M \pm SEM$. Different letters indicate significant differences between treatments: ANOVA followed by least significant difference (LSD) test, $\alpha = 0.05$; PPOB, $n = 10$, $F_{2,27} = 25.98$, $p < 0.0001$; CysPI, $n = 10$, $F_{2,27} = 44.79$, $p < 0.0001$; POD, $n = 14$ –15, $F_{2,41} = 46.75$, $p < 0.0001$; PPO, $n = 14$ –15, $F_{2,41} = 33.78$, $p < 0.0001$; TI, $n = 14$, $F_{2,39} = 47.28$, $p < 0.0001$. C, intact control plant; P, plant treated with parasitized caterpillar saliva [15 μ l (1 μ g/ μ l protein)]; NP, plant treated with non-parasitized caterpillar saliva [15 μ l (1 μ g/ μ l protein)]

3.2 | Effects of parasitization on plant defences

Saliva from P caterpillars triggered significantly lower defence gene (PPOB and CysPI) expression and protein (PPO, POD and TI) activities in tomato plants than saliva from NP caterpillars (Figure 2).

3.3 | Effects of parasitization on plant fitness

Plants without caterpillar saliva treatment (C) showed the highest fitness, followed by the P caterpillar treatment and then the NP caterpillar treatment. There was no significant difference between C and P treatments in flower number, fruit weight, seed number and seed weight. However, C plants flowered 5 days earlier than plants treated with herbivore saliva (P, NP; Figure 3). Plants treated with P caterpillar saliva showed significantly higher flower numbers (16.3% more) and fruit weight (13.5% higher) compared to plants from the NP caterpillar treatment.

3.4 | Transgenerational effects of parasitization on plant fitness

Germination time and germination rates were significantly faster and higher for seeds from maternal plants that were exposed to parasitized herbivore treatments (P) compared to control (C) plants, with seed from NP-maternal plants falling in between (Figure 4).

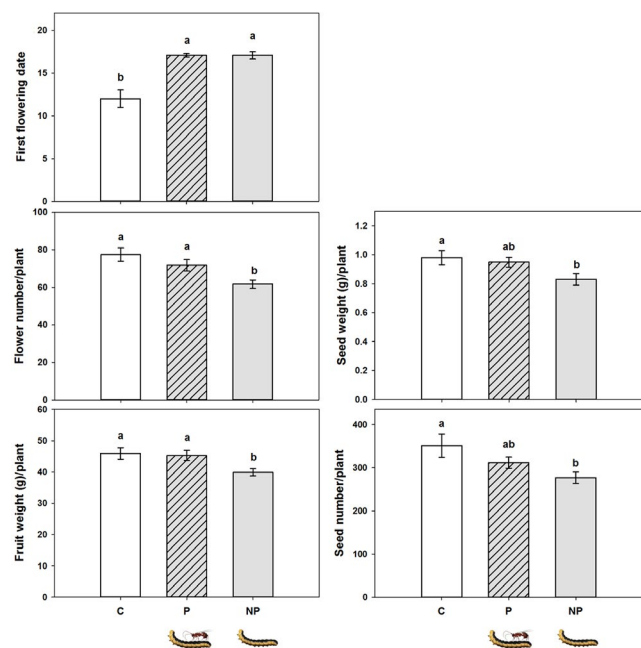


FIGURE 3 Tomato fitness in different treatments. First flowering date, flower number, fruit weight, seed number and seed weight were recorded to represent plant fitness. Values are untransformed $M \pm SEM$. Different letters indicate significant differences between treatments: ANOVA followed by least significant difference (LSD) test, $\alpha = 0.05$; first flowering date, $n = 13-15$, $F_{2,39} = 19.38$, $p < 0.0001$; flower number, $n = 13-15$, $F_{2,39} = 7.50$, $p = 0.0018$; fruit weight, $n = 13$, $F_{2,36} = 4.54$, $p = 0.0175$; seed number, $n = 13$, $F_{2,36} = 3.88$, $p = 0.0297$; seed weight, $n = 13$, $F_{2,36} = 3.62$, $p = 0.0371$. C, intact control plant; NP, plant treated with non-parasitized caterpillar saliva; P, plant treated with parasitized caterpillar saliva

3.5 | Transgenerational effects of parasitization on plant defences

Polyphenol oxidase, POD and TI activities were significantly induced after herbivore (H) treatment compared to no-herbivore control (N) plants (Figure 5). Among these three defence proteins, PPO activity was increased more strongly in offspring from maternal plants that were treated with P caterpillar (P) compared to NP plants 24 hr after saliva application (Figure 5A; Table 2). Overall, there was no maternal effect detected for POD and TI activities at 24 and 48 hr after herbivore saliva treatment.

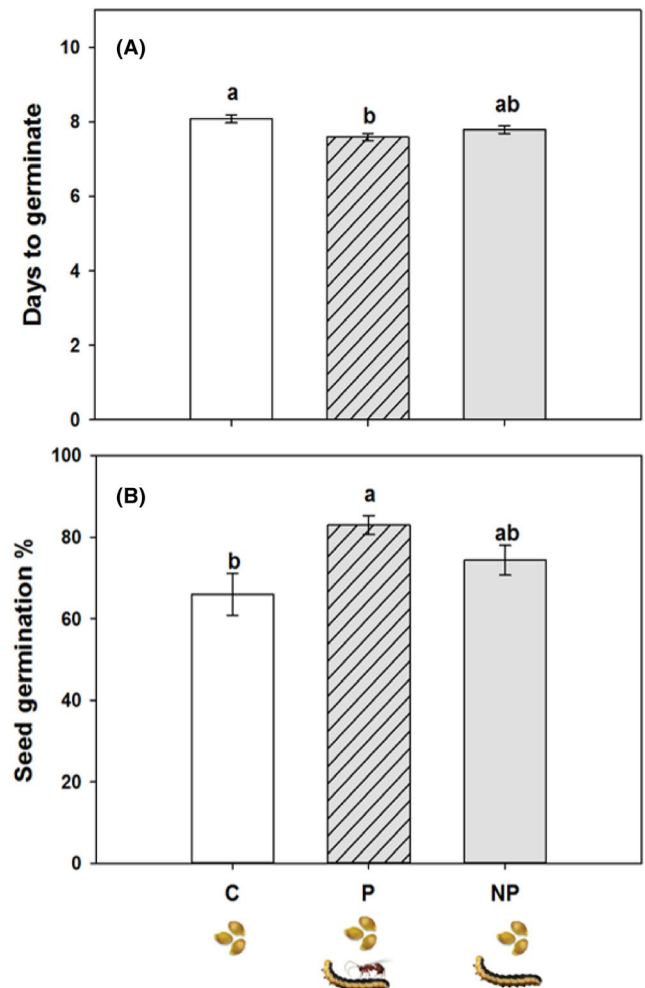


FIGURE 4 Maternal effects on time required for seed germination and germination rate. Values are untransformed $M \pm SEM$. Different letters indicate significant difference between treatments. (A) Days to germination, Kruskal–Wallis followed by DSCF test, $\alpha = 0.05$; $n = 198-249$, $F_{2,667} = 5.73$, $p = 0.0034$. (B) Seed germination rate, ANOVA followed by least significant difference (LSD) test, $\alpha = 0.05$; $n = 10$, $F_{2,27} = 4.92$, $p = 0.015$. C, seed from maternal plant without any treatment; P, seed from maternal plant treated with parasitized caterpillar saliva; NP, seed from maternal plant treated with non-parasitized caterpillar saliva

FIGURE 5 Induced plant defence responses in maternal offspring. Plant defence protein, POD, PPO and TI, activities were measured at 24 hr (A) and 48 hr (B) post-herbivore saliva application to wounded leaflets. Values are untransformed $M \pm SEM$. Different letters indicate significant difference between treatments: ANOVA followed by least significant difference (LSD) test, $\alpha = 0.05$; 24 hr POD, $n = 5-7$, $F_{5,30} = 4.55$, $p = 0.0033$; PPO, $n = 6$, $F_{5,30} = 14.11$, $p < 0.0001$; TI, $n = 6$, $F_{5,30} = 9.81$, $p < 0.0001$; 48 hr POD, $n = 4-7$, $F_{5,31} = 5.14$, $p = 0.0015$; PPO, $n = 4-7$, $F_{5,31} = 4.77$, $p = 0.0024$; TI, $n = 4-9$, $F_{5,30} = 4.16$, $p = 0.0055$. C, seeds from maternal plants with no treatment; P, seeds from maternal plants treated with parasitized caterpillar saliva; NP, seeds from maternal plants treated with non-parasitized caterpillar saliva; N, plants without herbivore treatment; H, plants treated with herbivore saliva. POD, peroxidase; PPO, polyphenol oxidase; TI, trypsin inhibitor

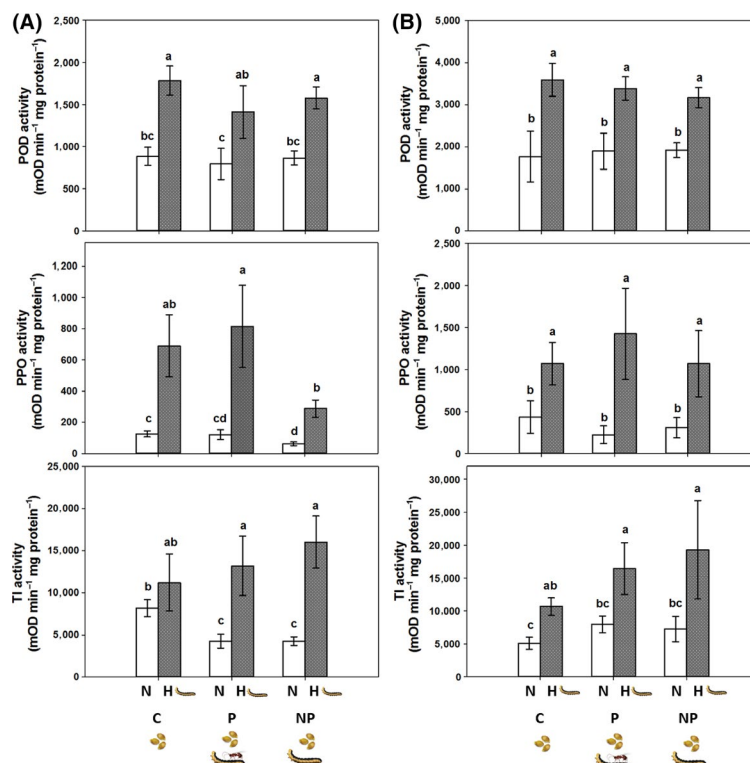


TABLE 2 Two-way ANOVA analysis of seedling induced defence responses

Time	Variable	df	p	F	N
24 hr	PPO		<0.0001	14.11	36
	Maternal plant	2	0.0093	5.50	
	Herbivore treatment	1	<0.0001	59.35	
	Maternal × herbivore	2	0.9077	0.10	
	POD		0.0033	4.55	36
	Maternal plant	2	0.4987	0.71	
	Herbivore treatment	1	<0.0001	21.09	
	Maternal × herbivore	2	0.7669	0.27	
	TI		<0.0001	9.81	36
	Maternal plant	2	0.1977	1.71	
	Herbivore treatment	1	<0.0001	34.67	
	Maternal × herbivore	2	0.0095	5.46	
48 hr	PPO		0.0038	4.38	38
	Maternal plant	2	0.8593	0.15	
	Herbivore treatment	1	0.0001	19.59	
	Maternal × herbivore	2	0.5439	0.62	
	POD		0.0015	5.14	37
	Maternal plant	2	0.9899	0.01	
	Herbivore treatment	1	<0.0001	24.01	
	Maternal × herbivore	2	0.7263	0.32	
	TI		0.0055	4.61	36
	Maternal plant	2	0.1340	2.81	
	Herbivore treatment	1	0.0003	16.79	
	Maternal × herbivore	2	0.9477	0.05	

Note: Values are untransformed $M \pm SEM$ (two-way ANOVA, GLM). Bold values indicate $p < 0.05$. Abbreviations: POD, peroxidase; PPO, polyphenol oxidase; TI, trypsin inhibitor.

4 | DISCUSSION

In natural populations, a large fraction of the larvae of insect herbivores can be parasitized by parasitoids. For example, parasitism rates of fall armyworm *Spodoptera frugiperda* on maize can exceed 70% (Ashley, 1986; Ashley, Barfield, Waddill, & Mitchell, 1983) and corn earworm *Helicoverpa zea* parasitization rates ranged from 50% to over 80% depending upon location and host plants (King & Coleman, 1989; Tipping, Holko, & Bean, 2005; Young & Price, 1975). High rates of parasitism may protect plants from damage and ultimately increase plant fitness due to the reduction of herbivore populations. This benefit of natural enemies on plant fitness has been documented in several systems. For instance, corn plants had significantly lower leaf damage (30%) and higher fitness (1.3–1.5-fold; seed number, ear number and seed biomass) when fed on by *Cotesia marginiventris*-parasitized *Spodoptera littoralis* larvae compared with non-parasitized caterpillars (Hoballah & Turlings, 2001). Charlock mustard *Sinapis arvensis* had more seed pods (>fourfold) and seed numbers when fed on by (*Hyposoter ebeninus* or *C. glomerata*) parasitized *Pieris brassicae* larvae compared with non-parasitized caterpillars, due to the lower percentage of seed pod damage (Gols et al., 2015). However, the fitness benefits have not always been documented especially in some cases of gregarious endoparasitoids which have more than one offspring that can fully develop from their host caterpillars. For example, *C. glomerata*-parasitized *P. brassicae* and *Copidosoma floridanum*-parasitized *Trichoplusia ni* host caterpillars consume more plant tissue and grow larger than non-parasitized ones, resulting in increased rather than decreased plant damage (Coleman, Barker, & Fenner, 1999; Ode et al., 2016). Although the effects of parasitization on the damage that caterpillars inflict on their individual host plants can thus be variable, it is important to realize that parasitization will eventually kill the caterpillars, resulting in a reduction in the population size of the herbivores that can cause ultimately attack on plants.

Parasitism may influence plant fitness by changing host herbivore physiology and/or feeding behaviour. Recently, two studies highlighted that parasitized *Spodoptera latifascia* caterpillars consume less lima bean leaf tissue and facilitate plant seed production (Bustos-Segura, Cuny, & Benrey, 2019; Cuny, Gendry, Hernández-Cumplido, & Benrey, 2018). It was the intent of our study to isolate the effects of parasitism on the ability of herbivores to induce plant defence and thus affect changes in plant fitness. Parasitized caterpillars induce lower plant defences during feeding than their non-parasitized counterparts (as observed in tomato, Tan et al., 2018, 2019 and cabbage, Cusumano et al., 2018), because parasitism lowers salivary enzyme activities that are responsible for reducing plant defence responses. We hypothesized that the reduction in defence-related costs caused by saliva of parasitized caterpillars may contribute to higher plant fitness of plants exposed to saliva from parasitized caterpillars compared to plants exposed to saliva from non-parasitized caterpillars.

Glucose oxidase is the most abundant protein in *H. zea* salivary labial glands and is an important elicitor that triggers tomato defence

expression in a dose-dependent manner (Tan et al., 2018; Tian et al., 2012). This study confirms that parasitization of *H. zea* by the solitary parasitoid *M. croceipes* reduces the levels of salivary GOX. Consequently, applying saliva from parasitized caterpillars elicited lower levels of expression of tomato plant defence-related genes and reduced activity of defence proteins (as observed in tomato cv. Betterboy in Tan et al., 2018, 2019; and in this study cv. Microtom), which further demonstrates that the lower induction of defence is associated with reduced fitness costs. Trade-offs between plant growth and defence have been demonstrated by metabolic allocation studies (Bekaert, Edger, Hudson, Piers, & Conant, 2012), functional analyses using transgenic plants (Zavala, Patankar, Gase, Hui, & Baldwin, 2004) and exogenous application of defence phytohormones (Redman, Cipollini, & Schultz, 2001). Trade-offs can be found in many systems, including pathogen-mediated plant defence (Heil, Hilpert, Kaiser, & Linsenmair, 2000; Karasov et al., 2017) and caterpillar defences against natural enemies (Higginson, Delf, Ruxton, & Speed, 2011). Our study demonstrates that similar defence-linked effects on growth/fitness can be mediated in a top-down fashion by parasitoids. Under these experimental conditions, our results reveal a negative correlation between plant-induced defence responses and plant growth (allocation costs). Tomato plants produced more flowers and yielded heavier fruit when treated with parasitized caterpillar saliva compared with non-parasitized ones. These results illustrate the possible trade-off between plant defence responses and plant growth/reproduction leading to a change in investments that affects their fitness (Herms & Mattson, 1992; Huot et al., 2014; Zangerl & Bazzaz, 1992). The higher fecundity seen in tomato plants treated with parasitized caterpillar saliva compared with the non-parasitized treatment was not caused by either the higher pest parasitism rate or lower levels of herbivore damage observed in lima bean system (Bustos-Segura et al., 2019; Cuny et al., 2018); instead, it was principally due to the downregulation of induced plant defence responses. By keeping the timing and the amount of plant damage standardized with salivary applications rather than insect feeding, we were able to isolate a potential additional benefit of parasitism for plants.

Flowering timing is crucial to the reproduction and offspring success of plants; altering the timing of flowering may affect plant fitness by changing interactions with mutualist (pollinators) and antagonist (seed predators) herbivores (Elzinga et al., 2007). Herbivore-treated plants showed a 5-day delay in the first flowering date compared with control plants. Previous studies also found that damage by vertebrate and invertebrate herbivores postponed plant flowering dates (Bustos-Segura et al., 2019; Juenger & Bergelson, 1998, 2000; Kettenring, Weekley, & Menges, 2009; McClay, 1992; Strauss et al., 1996; Tooker & Hanks, 2006). The delay of flowering may be due to the direct damage to vegetative or reproductive tissues. However, in this study, insect saliva was applied on plant leaves and there was no removal of plant tissue. Thus, in this study plants diverted energy to induced defences likely resulting in the delay in initial flowering time. Early flowering may promote higher fitness due to a longer reproductive period (Kelly & Levin, 2000). Although the first flowering date was delayed in P-treated plants, plants produced similar flower numbers

and fruit weight in the end compared with the control plants. Thus, the P-treated plants had the plasticity required to compensate for the delayed flowering time by accelerating flower and fruit production.

A meta-analysis of 55 studies suggested that early seed germination can benefit seedling growth and fertility (Verdú & Traveset, 2005). In this study, caterpillar saliva treatments resulted in faster and higher offspring seed germination rates compared with the non-damaged control seeds. Moreover, seeds from P-maternal plants germinated faster and had higher germination rates than NP seeds; consequently, it seems that moderately induced defence responses may not influence tomato fitness. This is in line with the observation that application of a low concentration of jasmonic acid did not negatively affect fruit number, seed numbers and fruit weight on tomato compared with untreated plants, whereas higher levels did (Redman et al., 2001; Thaler, 1999). Likewise, a lower level (15%–30%) of defoliation did not influence fruit weight in wild and cultivated tomato plants (Welter & Steggall, 1993). Thus, moderately induced responses may not have negative consequences for tomato fitness and our study shows that moderate damage (parasitized herbivores) can accelerate plant growth in the second generation.

Transgenerational priming may help offspring to deal with stress by triggering plant defence responses more rapidly and/or by producing a stronger response (Holeski et al., 2012; Rasmann et al., 2012; van Hulten et al., 2006); however, there was no transgenerational priming effect in plant defence-related protein activities found in herbivore-maternal plant seedlings (P and NP) when compared with the C treatment. It is interesting that we observed lower plant constitutive defence (TI) when maternal plants were exposed to herbivores (P and NP) compared to C treatment. This suggests that the magnitude of induced defences is higher in the P and NP offspring after induction. We observed a transgenerational effect in plant-induced defence responses (PPO activity) at 24 hr after herbivore treatment. P-maternal plant seedlings showed a significantly higher PPO activity compared with the NP treatment. It is possible that the NP-maternal plant seedlings produced lower PPO activity than the other two treatments because of differences in allocation costs.

In this study, we isolated the impact of parasitism on herbivore saliva and its effects on tomato plants, suggesting a possible mechanism by which parasitism of caterpillars may reduce plant defence costs. Previous work demonstrated that tomato plants showed a similar trend of induced responses when fed on by caterpillars or treated with herbivore saliva (Tan et al., 2018, 2019). Parasitism by *M. croceipes* not only reduces GOX activity in *H. zea* saliva (Tan et al., 2018, 2019) but also reduces the amount of herbivore consumption (Hopper & King, 1984). Therefore, we suggest that the impact of parasitism on plant fitness might be stronger than we observed in the study. Although, the isolated factor (changes in saliva) can apparently drive changes in plant defences and fitness, field studies conducted with caterpillar feeding and parasitism that may involve differences in the timing and amount of plant tissue loss are needed to determine the overall effects of caterpillar parasitism on plant fitness.

These results support our hypothesis that plants can distinguish damage between non-parasitized and parasitized caterpillars and alter their defence responses accordingly. Parasitism suppresses insect oral elicitor activity which triggers lower plant-induced defences and elevated plant fitness.

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AUTHORS' CONTRIBUTIONS

C.-W.T. and G.W.F. conceived the experiments; C.-W.T. and M.L.P. performed the experiments; J.G.A. and D.S.L. provided guidance on experiments; and C.-W.T., J.G.A. and G.W.F. wrote the paper.

DATA AVAILABILITY STATEMENT

Data were archived in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wwpzgmsgp> (Tan, Peiffer, Ali, Luthe, & Felton, 2020).

ORCID

Ching-Wen Tan  <https://orcid.org/0000-0001-5518-6955>

Jared G. Ali  <https://orcid.org/0000-0001-9870-0299>

Dawn S. Luthe  <https://orcid.org/0000-0002-1978-8825>

Gary W. Felton  <https://orcid.org/0000-0002-1076-1431>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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