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Diet-mediated immune response to parasitoid attacks on a caterpillar with a broad diet breadth

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Abstract

1. Bottom-up (plant) and top-down (natural enemy) trophic factors can interact to have significant influence on the diet breadth of herbivores. For herbivorous insects that are victim to parasitoid attacks, diet composition can modulate insect immune responses against the parasitoid. However, immune responses are costly and insect herbivores experience a trade-off between investment in immune defences and other physiological processes.
2. We used a split-brood laboratory experiment to explore how the diet of the fall webworm (*Hyphantria cunea*), a species that eats over 400 plant species, affects larval growth and fitness and cellular immune response to attacks from a parasitic wasp. We reared larvae on four different plant diets (apple, alder, chokecherry, cottonwood) and then exposed them to an immune challenge from a parasitoid attack.
3. We found that diet influenced larval development as well as parameters indicative of immune response. Larvae reared on the plant that led to the poorest development also had the fewest granulocytes and the highest odds of containing a parasitoid larva. However, larval growth was not a predictor of immune response.
4. Overall, we show that the bottom-up effect of diet variability has significant impacts on insect immune response such that larval fitness varies considerably when fed different dietary plant species. Broad diet ranges may offer herbivorous insects the opportunity to exploit a different set of resources depending on the severity of top-down pressures. Here, we show that this variability in plant quality also has significant impacts on larval immune response.

KEYWORDS
dietary generalism, ecoimmunology, fall webworm (*Hyphantria cunea*), haemocyte, natural enemy, plant–insect interactions

INTRODUCTION

Insect diet breadths are influenced by their nutritional needs but also by the enemies that threaten their survival (e.g., Singer & Stireman, 2005). Foraging herbivorous insects must select the most rewarding and least toxic plants, while simultaneously evading attacks from predators, parasitoids, and pathogens. Bottom-up (plant quality) and top-down (natural enemies) factors often interact to influence diet breadth, which has a significant impact on the fitness gains and costs experienced by herbivorous insects (Vidal & Murphy, 2018). In the face of significant top-down pressure, herbivorous insects may experience increased survival and performance on low-quality plants if the negative effect of natural enemies is lowered compared to high-quality plants (enemy-free space; Diamond & Kingsolver, 2010; Jeffries & Lawton, 1984; Meijer et al., 2016; Mulatu et al., 2004; Murphy, 2004; Torres-Vila & Rodríguez-Molina, 2013; Vosteen et al., 2016). Conversely, plants can also alter an herbivore's physiological or immune response to enemies (Carper et al., 2019; Ghosh &

Venkatesan, 2019; Singer et al., 2014; Smilanich, Dyer, Chambers, et al., 2009; Vogelweith, Dourneau, et al., 2013). Many insect herbivores rely on their immune systems for protection against parasitic enemies such as parasitoids (Carton et al., 2008), which are a significant source of insect mortality (Hawkins et al., 1997). Since diet can mediate insect immune response, immune function is an important link between bottom-up and top-down factors that affect an insect's survival and fitness.

Herbivorous insects use a diversity of strategies to defend against attacking enemies, including chemical, behavioural, or morphological defences (e.g., Bowers, 1990; Damman, 1986; Dyer, 1995; Grant, 2006; Grant, 2007; Lill et al., 2007; Murphy et al., 2010; Smilanich, Dyer, & Gentry, 2009; Stamp & Wilkens, 1993; Wagner, 2005). If an attacker bypasses behavioural and morphological defences, the host relies on humoral and cellular immune responses (Strand, 2008), with the cellular response used primarily against parasitoids, parasites, and pathogens (Beckage, 2008; Carton et al., 2008). Insect immune responses are led by cells called haemocytes that perform encapsulation, nodulation, or phagocytosis (Lavine & Strand, 2002; Schmidt et al., 2001; Strand & Pech, 1995). Although studies have demonstrated how haemocyte activation protects the host from parasitism (Smilanich, Dyer, & Gentry, 2009), as well as how plants augment insect immune responses against pathogens and artificial objects (Carper et al., 2019; Smilanich et al., 2018; Smilanich, Dyer, Chambers, et al., 2009; Vogelweith, Dourneau, et al., 2013), few studies have focused on immune responses to parasitoid attacks (Fors et al., 2014; Ghosh & Venkatesan, 2019).

Parasitoids are a speciose group of insects that require a host for reproduction (Godfray, 1994) and therefore occupy higher trophic levels and exert important top-down pressure on herbivorous insects (Vidal & Murphy, 2018). Yet, an important relationship exists between parasitoids and the bottom-up effects of host diets because some parasitoids use herbivore-induced plant volatiles to locate suitable hosts (McCormick et al., 2012; Van Poecke et al., 2003). For parasitoids that develop inside a host, the parasitoid eggs and larvae are susceptible to attack from the host's haemocytes, which can encapsulate and kill the immature parasitoids (Strand, 2008). While parasitoids are under strong selection to overcome their hosts' immune defences, the hosts benefit from strategies that eliminate the immature parasitoids. A flexible diet breadth may enable insects to consume plants that enhance immune defences (Hansen et al., 2017; Mason et al., 2014) or to feed on noxious plants that decrease parasitoid survival inside the host (e.g., Singer et al., 2004, 2009). Launching an immune response is costly and can compete with other physiological processes (e.g., growth) for limited resources; therefore, herbivore hosts often trade-off investment in immune defences and development (Sheldon & Verhulst, 1996).

We examined how diet affects immune response against parasitoid attacks on the fall webworm (*Hyphantria cunea* Drury [Lepidoptera: Erebidae]; hereafter FW). FW is a wide-ranging polyphagous moth whose larvae feed on over 400 plant species (Greenblatt et al., 1978; Warren & Tadic, 1970). FW larvae are attacked by a diversity of parasitoids that target the egg, larval and pupal stages (Edosa et al., 2019; Nordin et al., 1972; Yang et al., 2008, 2015). Given its broad diet and susceptibility to parasitism (Murphy & Loewy, 2015 found conservatively that about a quarter of all field-collected larvae died from parasitism), FW is an ideal system for investigating how diet may mediate immune response. Our objective was to determine whether diet affected the performance of FW caterpillars as measured by their development and immune response to parasitoid attacks. We reared FW on four different plant diets and counted the number of haemocytes before and after parasitoid attacks to determine whether plant diet affected FW immune response. Our goals were to (1) measure how diet affected FW growth, (2) determine if haemocyte counts differed for larvae reared on different plants, (3) examine changes in haemocyte counts after parasitism and (4) determine if diet affected survival of the parasitoid.

MATERIALS AND METHODS

Study system

Fall webworm (FW) is a common moth species from North America and has been introduced to Europe and Asia (Wu et al., 2019). Female moths usually lay a single cluster of over 100 eggs (Loewy et al., 2013). Soon after hatching, the caterpillars spin a web in which siblings reside together until the final instar, after which larvae leave the natal tree and diapause as pupae under substrate. The adults eclose the following summer and males often disperse (Yamanaka et al., 2001), whereas adult females usually remain close their eclosion site (Masaka, 1975). FW is a polyphagous species with a diet range of over 400 deciduous trees (Schowalter & Ring, 2017; Warren & Tadic, 1970); however, an individual larva completes its development upon the natal tree on which it hatched. Depending on their geographic location, FW are either univoltine or multivoltine, and there are two caterpillar morphologies distinguished primarily by either a red or black head capsule (Oliver, 1964; Schowalter & Ring, 2017; Vidal et al., 2019). While red- and black-headed FW can co-occur in the same region, the red-headed morphotype is the only one documented in Colorado (Loewy et al., 2013). FW diet breadth is narrower in Colorado (CO) than that of conspecifics in other parts of North America with 10 tree species comprising most of the diet in CO (Murphy & Loewy, 2015). FW are attacked by a community of parasitic flies and wasps (Edosa et al., 2019; personal obs; Nordin et al., 1972; Yang et al., 2008, 2015), among which *Therion sassacus* Viereck (Hymenoptera: Ichneumonidae) is a larval-pupal ichneumonid wasp (Schaefer, 1977; Warren & Tadic, 1970).

We started a laboratory colony of FW in 2019 by collecting wild larvae from field sites in Boulder, Chaffee, Jefferson and Larimer counties in CO. We reared these larvae at the University of Denver following protocols outlined in Robinson-Castillo et al. (2021), and once they pupated and entered diapause in fall 2019, we placed pupae in growth chambers to overwinter. In May 2020, we ended diapause and mated adult moths with unrelated individuals (following Robinson-Castillo et al., 2021). After the female FW laid egg clusters,

we divided each egg cluster into four sections once head capsules were observed beneath the chorion. Each quartered section of eggs was placed on a leaf from one of four plant species used in our experiment and are all common food plants of FW in CO (Murphy & Loewy, 2015): thinleaf alder (*Alnus tenuifolia*), apple (*Malus* sp.), chokecherry (*Prunus virginiana*) or narrowleaf cottonwood (*Populus angustifolia*). We chose these four plant species because over the 10 years of collecting FW from the wild, we have found that generally thinleaf alder and apple are low-quality plants for FW with low levels of parasitism whereas chokecherry and narrowleaf cottonwood are high-quality plants with higher levels of parasitism (Murphy & Loewy, 2015; Vidal & Murphy, 2018). We reared these subgroups of FW larvae at a maximum density of 10 caterpillars per 1 L rearing container, and we replenished leaves as required. Sibling groups from each diet were haphazardly assigned to the control (unparasitized but with a pin prick) and experimental (pin prick and then parasitized) treatments.

We started a colony of *T. sassacus* parasitoids in August 2020 by collecting adult wasps that eclosed from FW pupae that had been collected as larvae from the field in 2019. We maintained adult *T. sassacus* at ambient room temperatures in 1 L plastic deli containers and provisioned with honey and a moist wick submerged in water. We housed newly eclosed female wasps with males for 48 h and then used them for experimental trials.

Experimental design

We conducted all experimental trials between 20 and 26 August 2020, in our laboratory at the University of Denver, CO. We used FW larvae from 10 randomly selected matrilines to measure the impact of diet on larval immune response to parasitoid attacks. Since *T. sassacus* began enclosing in August 2020, we were limited to using FW that were in the middle of their larval development. We used larvae that were 32–37 days old with head capsule widths between 1.47–2.77 mm, which is approximately within the fifth and sixth instars (Morris & Fulton, 1970). Digital callipers were used to measure head capsule width, which was the distance between the lateral sides of the head. While the preferred larval host size for *T. sassacus* is unknown, the wasps used in this study readily attacked each host regardless of its age or size. We used a complete factorial design with diet (thinleaf alder, apple, chokecherry, narrowleaf cottonwood), immune challenge (pin prick only [control] or pin prick then *T. sassacus* parasitism), and time (baseline, after immune challenge) as predictor variables. For each of the 10 matrilines, 10 larvae from each of the four diets were haphazardly selected and evenly divided into control ($n = 5$) and parasitism ($n = 5$) groups for a total of 40 siblings per matriline across the four diets (see Table S1 for exact sample sizes). In addition to these larvae, we measured the mass of FW ($n = 440$) that had pupated on each of the four plants from our colony. We calculated lifetime fitness of female FW by assigning each female pupa a fitness score, which was used by Murphy and Loewy (2015) as a proxy for FW lifetime fitness and for assessing plant quality for FW. Specifically, the lifetime fitness score is a measure of pupal mass divided by the number of days it took for larvae to pupate; large values indicate higher fitness (i.e., greater mass in a shorter development time) and low values indicate lower fitness.

At the start of each trial, we collected a baseline haemolymph sample from each larva by inserting a disinfected (with 70% alcohol) #0 size insect pin (pin prick) above the penultimate proleg; after withdrawing the pin, 0.2 μl of haemolymph was collected using a 10 μl pipette (the site of the puncture heals within 24 h without affecting the caterpillar's survival, therefore no adhesive or otherwise was required to seal the wound). We immediately transferred haemolymph samples to a 1.7 ml centrifuge tube containing 0.4 μl of anticoagulant stored in an ice bath; an anticoagulant was created following Smilanich et al. (2018) with 180 ml of phosphate-buffered saline, 346 mg of citric acid and 684 mg of EDTA. After mixing the haemolymph and anticoagulant, we pipetted 0.6 μl of this solution into a Neubauer Bright-Line haemocytometer. We used a compound microscope at ×400 magnification to count the total number of granulocytes and plasmatocytes, the two main haemocytes involved in lepidopteran immune defences (Lavine & Strand, 2002; Strand, 2008), within the haemocytometer's central square. We distinguished granulocytes from plasmatocytes based on the round spherical appearance of granulocytes and the small granules in their cytoplasm, whereas plasmatocytes had an elliptical shape with pointed ends and no granules in the cytoplasm (pers. observation). We then housed each larva individually in a 1.7 ml centrifuge tube for 24 h after which the procedures described above were used again to extract and assess an additional haemolymph sample from these same larvae.

Less than 30 min after the baseline haemolymph extraction, FW larvae in the parasitism treatment were individually presented to *T. sassacus* females for parasitism. We placed each larva in a glass Petri dish (9.6 cm diameter) containing a single wasp. In order to ensure that each larva was attacked at an equal frequency, we monitored interactions and removed the larva after a single *T. sassacus* attack. We were able to determine when the larva was successfully parasitized if the larva quickly swung its head towards the site of oviposition and excreted regurgitant. Vyas dissected each larval host 72 h after parasitism, at which time, the parasitoid larva was easily and reliably observable (Figure S1). We initially sought to record encapsulation and melanization, two measurable artefacts of a successful immune response against parasitoids (Smilanich, Dyer, & Gentry, 2009; Strand, 2008). However, the *T. sassacus* eggs adhered to the hosts' inner cuticle, making the eggs difficult to consistently observe during dissections when the cuticle was cut open. Instead, we recorded whether the *T. sassacus* larva was present and alive, indicating a successful parasitism and thus a failed immune defence.

Statistical analyses

We examined whether our data met normality and equality of variance assumptions and used a natural log transformation as necessary. All analyses were performed in JMP PRO 14.2.0. Our predictor variables were larval diet (alder, apple, cherry, cottonwood), immune challenge

(pin prick only [control] or pin prick then *T. sassacus* parasitism), head capsule width (mm) and baseline granulocyte or plasmatocyte counts. Baseline granulocyte and plasmatocyte values were continuous predictor variables because we wanted to examine if an abundance or paucity of these cells before immune challenge would affect their values after immune challenge. Our response variables were granulocytes and plasmatocytes (counts) after immune challenge. We used a linear mixed model to analyse the relationship between our predictor and response variables with matriline as a random effect.

FIGURE 1 Mean (±SE) head capsule width of fall webworm larvae feeding on four different diets. Different letters on top of the bars indicate significant differences (Tukey HSD $p < 0.01$) between diets. See supporting information (Figure S3) for additional data on head capsule widths

For assessing larval performance, we used a linear mixed model to analyse how head capsule width (mm) was affected by the four diets with matriline as a random effect. Head capsule width was treated as a continuous variable. Successful parasitism was defined as observing the *T. sassacus* larva alive inside the host 24 h after the parasitoid attack. A live *T. sassacus* larva indicates that the parasitoid egg escaped or overcome the FW immune response. The presence of the *T. sassacus* larva was treated as a binary response (present or absent) and analysed with a logistic regression using a binomial distribution and a log link function. Fitness score (pupal mass [mg]/days to pupate) was our measure of potential lifetime fitness (Murphy & Loewy, 2015), and it was treated as a continuous variable. We analysed the effect of diet and sex on pupal mass using a linear mixed model with the random effect of matriline.

RESULTS

FW development

FW head capsule widths differed based on their diet (diet: $F_{3,387} = 214.34$, $p < 0.0001$). Larvae fed alder had the smallest head capsules, while larvae fed chokecherry had the largest head capsules (Figure 1). In contrast with the large amount of variation in head capsule width explained by host plant, matriline was estimated to explain only 10.80% ($p = 0.08$) of the variance in head capsule width. We found that the fitness score (pupal mass/days to pupate) was dependent on the sex of FW and the diet eaten (sex × diet: $F_{3,410.7} = 2.78$,

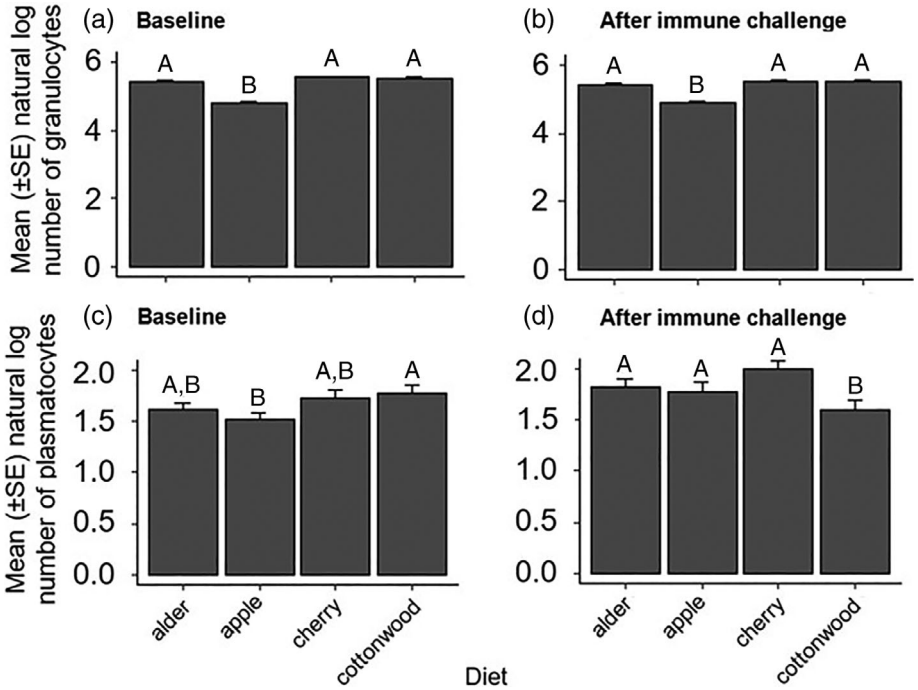

FIGURE 2 Baseline granulocyte (a) and plasmatocyte (c) values measured at the start of the experiment. Response granulocyte (b) and plasmatocyte (d) values were measured after immune challenge (pin prick control and *Therion sassacus* parasitism shown together because they did not differ statistically). Bars represent mean ± SE. Treatments with the same letter are not significantly different

$p = 0.04$). Female pupae from larvae reared on alder and cottonwood were heavier than male pupae from the same diet (female FW vs. male FW on alder: $t = 3.78$, $p = 0.004$; female FW vs. male FW on cottonwood: $t = 3.82$, $p = 0.004$) (Figure S2). In addition to diet and sex, fitness score was affected by the random effect of matriline, which explained 16.48% ($p = 0.01$) of the variance in FW fitness scores.

Baseline haemocyte values

Baseline granulocyte and plasmatocyte values were measured at the start of the experiment to determine whether FW diet caused these two haemocytes to differ prior to the immune challenge. Diet affected granulocytes ($F_{3,387} = 97.33$, $p < 0.001$) with the fewest number of granulocytes found in FW that fed on apple (Figure 2a). Diet had a slight effect on plasmatocytes ($F_{3,387} = 2.60$, $p = 0.05$) with differences found only between FW that ate apple and cottonwood (Figure 2b). Matriline explained 0% ($p = 0.88$) and 4.76% ($p = 0.16$) of the variance in granulocyte and plasmatocyte values, respectively.

Haemocyte response to immune challenge

Haemocyte values were assessed after immune challenge (pin prick only [control] or pin prick then *T. sassacus* parasitism) to determine whether *T. sassacus* parasitism and diet influenced FW immune responses. For granulocytes, the number of cells was dependent on the plant eaten by FW larvae (diet: $F_{3,372.8} = 11.06$, $p < 0.0001$) (Figure 2c). Similar to the granulocyte values at baseline, FW that fed on apple had the fewest granulocytes. However, *T. sassacus* parasitism failed to cause an increase in granulocytes (immune challenge: $F_{1,372.2} = 0.19$, $p = 0.66$) compared to FW in the control group. As a covariate, baseline granulocyte values were significantly related to granulocyte values after immune challenge (baseline granulocytes: $F_{1,381} = 16.83$, $p < 0.001$). For plasmatocytes, *T. sassacus* parasitism again failed to cause an increase in plasmatocytes (immune challenge: $F_{1,372.3} = 0.47$, $p = 0.49$) compared to FW in the control group. Plasmatocyte values differed across diets (diet: $F_{3,376.2} = 3.35$, $p = 0.02$) but only between FW reared on alder compared to larvae reared on cottonwood (Figure 2d). In addition to the effect of diet, plasmatocyte counts after immune challenge were dependent on baseline

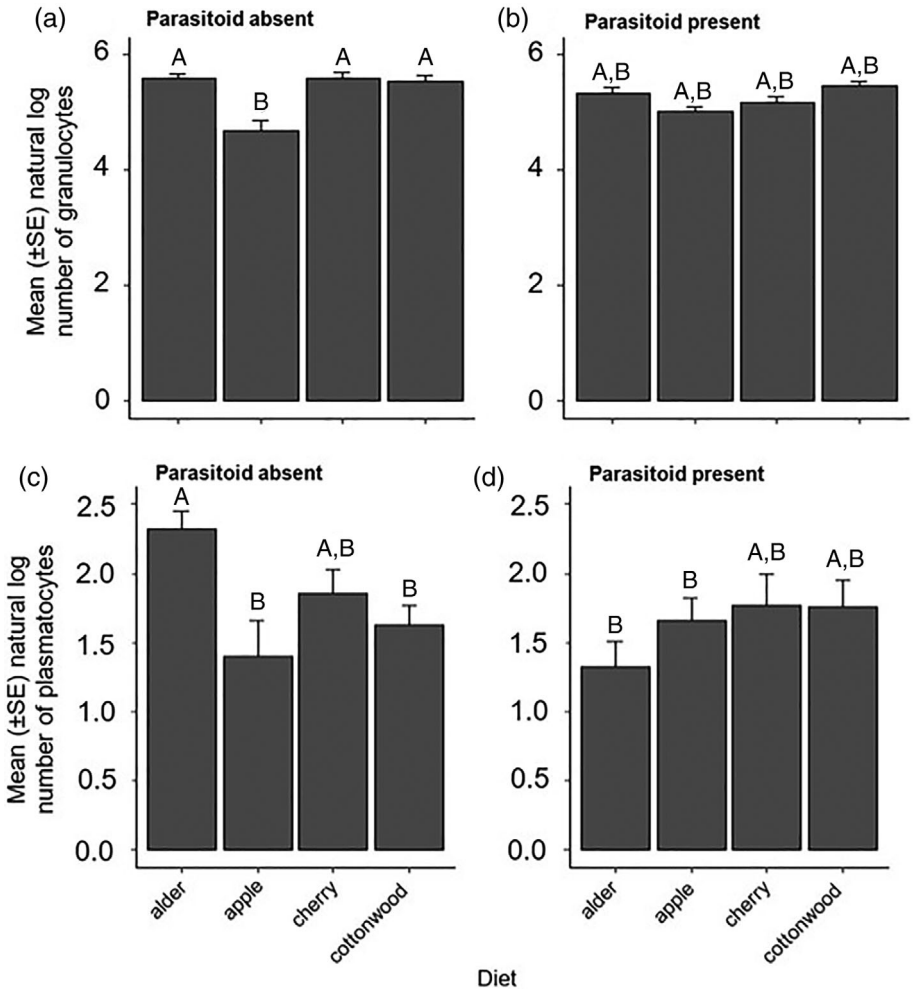

FIGURE 3 Granulocyte (a,b) and plasmatocyte (c,d) values depending on the FW diet and whether *Therion sassacus* was found alive inside the host. Bars represent mean ± SE. Treatments with the same letter are not significantly different

plasmatocyte values (baseline plasmatocytes: $F_{1,380.9} = 8.32$, $p = 0.004$). Matriline was an insignificant factor as it contributed to 0% ($p = 0.98$) and 4.35% ($p = 0.18$) of the variance observed in granulocyte and plasmatocyte values, respectively.

Our dissections revealed that of the FW hosts that were attacked by *T. sassacus* ($n = 194$) in the parasitism treatment, 55% of these hosts did not contain the *T. sassacus* larva. Since the absence of the parasitoid could have affected the host's immune response, we examined if granulocyte and plasmatocyte values differed across diets based on whether *T. sassacus* was present. We found that for both granulocyte and plasmatocyte values, the effect of diet depended on whether *T. sassacus* was inside the host (*T. sassacus* presence × diet: $F_{3,181.7} = 4.10$, $p = 0.008$ for granulocytes; $F_{3,181} = 4.77$, $p = 0.003$ for plasmatocytes). Granulocytes were fewest for FW that ate apple, but only when *T. sassacus* was absent (Figure 3a). Furthermore, for hosts lacking *T. sassacus*, FW that fed on alder had greater plasmatocytes than FW that ate apple or cottonwood (Figure 3c). The presence of the parasitoid led to a decrease in plasmatocytes among the FW feeding on alder (Figure 3c,d), whereas parasitism changed neither granulocyte nor plasmatocyte values of FW fed the other diets. For FW hosts inside which *T. sassacus* was observed, diet did not affect granulocyte or plasmatocyte values (Figure 3b,d).

Parasitoid survival

A successful *T. sassacus* parasitism was defined by finding the *T. sassacus* larva inside the host after the parasitoid attack. We found that successful parasitism was dependent on the FW host's diet (Pearson $\chi^2 = 15.61$, $df = 3$, $p = 0.001$; Figure 4). The odds of a parasitized FW host harbouring a *T. sassacus* larva were the highest for FW that fed on apple, which had greater odds of successful parasitism at 2.26 (95% CI: 0.99, 5.14) times the odds of FW fed cottonwood (Pearson $\chi^2 = 3.84$, $p = 0.05$), 3.33 (95% CI: 1.44, 7.70) times those of FW fed alder (Pearson $\chi^2 = 8.18$, $p = 0.004$), and 5.00 (95% CI: 2.11, 11.85) times the odds of FW fed chokecherry (Pearson $\chi^2 = 14.11$, $p = 0.0002$).

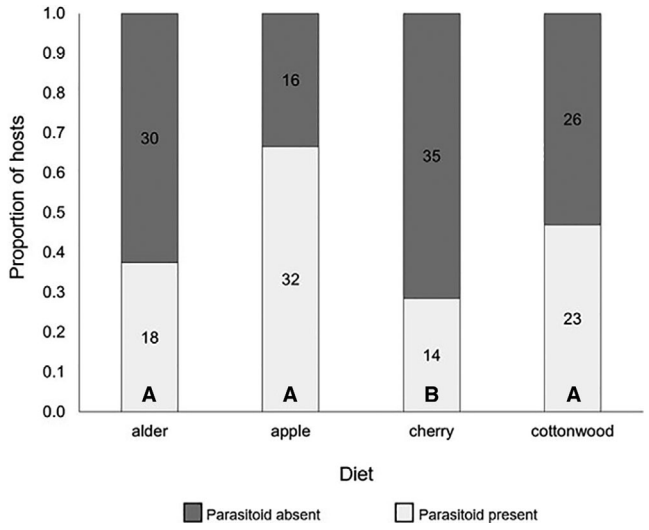

FIGURE 4 Survival of *Therion sassacus* parasitoid larva in fall webworm hosts reared on four different diets: Alder, apple, cherry and cottonwood. The numbers inside the bars represent the number of hosts with or without *T. sassacus* larvae. Treatments with the same letter are not significantly different

DISCUSSION

We found that diet significantly affected FW growth as measured by head capsule width and also that immune response differed for larvae reared on different host plants, but FW growth did not predict immune response. At the time that we measured immune response, FW larvae reared on alder and apple were generally smaller than larvae reared on cherry and cottonwood, which follows results we have found previously in this system (Murphy & Loewy, 2015; Vidal & Murphy, 2018). However, body size did not predict immune response. We found that larvae reared on apple had the lowest baseline granulocyte values and these values remained the lowest after an immune challenge. However, the FW reared on alder were small, yet had high granulocytes similar to the larger FW fed cherry and cottonwood. Interestingly, the greatest odds of finding a live parasitoid (*T. sassacus*) larva were in FW that were reared on apple, the diet with the lowest number of granulocytes.

We showed that diet affects FW immune function, but the mechanism is unknown. In other systems where diet-mediated immune responses occur, researchers find that plant nutrition and secondary metabolites affect the quality and quantity of immune defences among herbivorous insects. For example, Singer et al. (2014) found that woolly bear caterpillars (*Grammia incorrupta* Edwards [Lepidoptera: Erebidae]) increased consumption of pyrrolizidine alkaloids to increase resistance to parasitic flies. Haemocyte activity requires significant amounts of amino acids, thus dietary protein levels influence insect response (Lee et al., 2006; Raubenheimer & Simpson, 2009). As a polyphagous herbivore, populations of FW can process a large diversity of plant nutrients and metabolites. Additional studies are required to identify the biochemical underpinnings of a diet-mediated response in FW.

Murphy and Loewy (2015) suggested that immune function may have a negative relationship with diet quality, perhaps explaining why FW eating high-quality diets succumb to the highest parasitism. We were unable to test this hypothesis directly because we did not find significant variation in host plant quality as measured by FW fitness scores across multiple host plants (only larvae reared on alder and apple differed significantly at the end of development). Our results were inconclusive regarding any trade-offs between diet and immune response because apple and alder produced the smallest larvae, yet had divergent immune responses. FW larvae reared on apple had low granulocyte counts and were most likely to contain live immature *T. sassacus*, whereas larvae reared on alder did not differ in immune response from larvae reared on cherry or cottonwood.

Baseline granulocyte and plasmatocyte counts were similar to those following immune challenge, suggesting that neither type of

challenge (pin prick or parasitism) elicited measurable differences in immune response. Granulocytes and plasmatocytes values can change after invasion by a pathogen (Shikano et al., 2010; Vogelweith et al., 2014), parasitoid (Fors et al., 2014; Singer et al., 2014) or synthetic foreign body (Carper et al., 2019; Smilanich, Dyer, & Gentry, 2009). Only FW fed alder showed a significant decrease in plasmatocytes after parasitism when *T. sassacus* was confirmed alive inside the host. The reduction in plasmatocytes in hosts with *T. sassacus* could have resulted from venoms and viruses injected during oviposition; however, we only suppose that *T. sassacus* females inject these substances as part of their ovipositional fluid. From our dissections of female *T. sassacus*, we have observed glandular and tubular structures indicative of venom glands and ducts. The venoms and viruses found in parasitoid oviposition fluids can impair the host's immune function (Shelby & Webb, 1999; Strand & Pech, 1995). When we failed to find *T. sassacus* in hosts after they were attacked by a wasp, the FW plasmatocyte values may have remained high because oviposition failed to occur, thus precluding the injection of haemocyte inhibiting agents. While we ensured that each FW larva was attacked by a female *T. sassacus*, we could not control whether the parasitoid injected an egg. For FW that lacked *T. sassacus*, this absence could result from a failed attack (i.e., female did not inject an egg) or from a successful host immune response that killed the *T. sassacus* egg.

We found some evidence supporting a bottom-up and top-down interaction where FW diet influenced the herbivore's natural enemy. FW fed apple were most beneficial to the parasitoid because FW hosts fed this diet had the greatest odds of yielding a live *T. sassacus* larvae. Perhaps the immune system experiences deficiencies resulting from the small size of FW caterpillars fed apple. Immunocompetence increases with age in some lepidopteran species that are challenged with pathogenic bacteria (Stoepler et al., 2013; Vogelweith, Thiery, et al., 2013; However, a relationship between FW caterpillar size and immune response is unlikely since the caterpillars fed alder were the smallest yet had haemocyte values similar to larger siblings fed cherry and cottonwood. We consider a diet-mediated effect as a more salient explanatory factor as to why an apple diet was the best for *T. sassacus*.

Parasitoids face significant fitness costs when they invest eggs in herbivorous hosts that are successful at killing parasitoid offspring. In nature, hosts are often unevenly distributed and found on a diversity of plants that can affect the host's development and immune system (Hansen et al., 2017; Muller et al., 2015). Among this heterogeneity in host distribution, parasitoids experience selection pressure to find a host that increases offspring survival. Based on over 10 years of field collected FW samples in Colorado, we documented *T. sassacus* emerging from FW feeding on 1 apple tree, 6 chokecherries, 29 cottonwoods, 6 elms and 3 willows. We have never observed *T. sassacus* emerging from FW collected on alder and we can think of two possible mechanisms that may explain this. First, these parasitoids may not search alder for hosts, perhaps because they are not attracted to volatiles from alder, but this seems unlikely given how many unrelated host plants on which we have found *T. sassacus*. Second, our results show that hosts reared on alder have high granulocyte and plasmatocyte values, which may make these hosts inhospitable and risky for *T. sassacus*. Additional laboratory and field experiments are necessary to test whether the polyphagous FW diet contains plants that offer differential protection from FW natural enemies.

AUTHOR CONTRIBUTIONS

Dhaval K. Vyas: Conceptualization, Data Curation, Investigation, Methodology, Formal Analysis, Writing-Original draft preparation, Writing-Review & Editing, Writing-Revisions, Visualisation, Supervision, Funding Acquisition. **Shannon M. Murphy**: Writing-Original draft preparation, Writing-Review & Editing, Writing-Revisions, Supervision, Resources.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1. Sample sizes of larvae in each host plant and immune challenge treatment.

Figure S1. A *Therion sassacus* larva dissected 72 h after oviposition into the fall webworm host. Image taken from a dissecting microscope.

Figure S2. Larval performance measured by fitness score (pupal mass/days to pupae) on four host plants in our split-brood experiment for both males and females. Bars represent mean ± SE. Treatments with the same letter are not significantly different.

Figure S3. Mean (±SE) head capsule widths measured at five ages of fall webworm larvae that were reared on different plants: alder, apple, cherry and cottonwood. Different individuals were measured for each group (e.g. individuals from group A are different from those in group B), but siblings within matrilines were measured across plant species. Each group represents a FW measured at different ages (days old): A = 32 days, B = 34 days, C = 35 days, D = 36 days and E = 37 days. Different superscript letters indicate significant differences (Tukey HSD $p < 0.01$) between diets within the same group.

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