Received: 28 November 2023 | Accepted: 27 January 2024

DOI: 10.1111/afe.12616

ORIGINAL ARTICLE

Agricultural and Forest Entomology

Royal Entomological Society

Independent and interactive effects of diet and entomopathogenic microsporidia on an outbreaking forest insect defoliator

Leah Flaherty[1] | Flavio Preti[2] | Guncha Ishangulyyeva[3] | Nadir Erbilgin[3] | Taylar Whidden[1] | Maya Evenden[2]

[1]Department of Biological Sciences, MacEwan University, Edmonton, Alberta, Canada

[2]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

[3]Department of Renewable Resources, University of Alberta, Edmonton, Alberta, Canada

Correspondence
Leah Flaherty, Department of Biological Sciences, MacEwan University, Edmonton, Alberta, Canada.
Email: flahertyl@macewan.ca

Funding information
MacEwan University; Natural Sciences and Engineering Research Council of Canada

Abstract

1. Insect herbivore diet mediates interactions with entomopathogens, yet this is relatively unexplored for microsporidia. Here, we examine a diet-mediated tri-trophic interaction between an outbreaking forest defoliator, forest tent caterpillar (FTC) *Malacosoma disstria* Hübner and *Nosema* sp. microsporidia.
2. We conducted two experiments where diet quality was manipulated by incorporating lyophilized aspen (*Populus tremuloides* Michaux) foliage into an artificial diet, which was compared to a standard artificial diet. Diet quantity varied between fully fed and partially starved conditions, simulating an outbreak scenario. Microsporidia infection occurred naturally or was induced via experimental inoculation. We assessed FTC survival, microsporidia infection and load, and sublethal effects of treatments on FTC traits.
3. Plant secondary metabolite concentrations in aspen-augmented diets varied between experiments. In Experiment 1, the aspen-augmented diet contained lyophilized aspen foliage with low concentrations of secondary metabolites, which increased FTC survival and reduced microsporidia infection. Diet quality and infection load also interactively influenced adult wing traits in Experiment 1. In Experiment 2, the aspen-augmented diet contained lyophilized aspen foliage with higher concentrations of secondary metabolites, which negatively affected FTC. No diet-mediated interactions with microsporidia were observed in Experiment 2.
4. Diet quality (Experiments 1 and 2), diet quantity (Experiment 2) and microsporidia infection (Experiments 1 and 2) directly influenced FTC survival and/or had sublethal effects on FTC that may have cascading effects on population dynamics and dispersal.

5. We demonstrated that diet quality can mediate interactions between FTC and microsporidia, but these interactions depend on the defensive chemistry of the FTC diet. To our knowledge, this is the first study to report diet-mediated interactions between an outbreaking forest pest and microsporidia and one of only a few studies to examine this tri-trophic interaction among Lepidoptera.

KEYWORDS
diet quality, diet quantity, forest tent caterpillar, *Nosema*, tri-trophic interactions

INTRODUCTION

Tri-trophic interactions among plants, insect herbivores and their natural enemies can be mediated from the bottom up via a variety of mechanisms, including the quantity and quality of food consumed by the insect herbivore, that is, their diet (Cory & Hoover, 2006; Gassmann et al., 2010; Price et al., 1980; Shikano, 2017). The nutrition and defensive plant secondary metabolites in insect herbivore diet can directly impact fitness via alterations to survivorship, development and adult life history traits (Awmack & Leather, 2002; Colasurdo et al., 2009; Matsuki & MacLean, 1994), but it is also well established that diet can shape interactions between insect herbivores and entomopathogens or parasites (Cory & Hoover, 2006; Gassmann et al., 2010; Mason et al., 2023; Shikano, 2017; Sun et al., 2023). Most of this research, however, has focused on viral, bacterial and (non-microsporidian) fungal pathogens, with a comparably limited understanding of whether similar mechanisms operate in tri-trophic systems involving microsporidia. Here, we examined a diet-mediated tri-trophic interaction between an outbreaking forest insect pest (forest tent caterpillar [FTC] *Malacosoma disstria* Hübner [Lepidoptera: Lasiocampidae]) and *Nosema* sp. microsporidia (Dissociodihaplophasida: Nosematidae).

The forest tent caterpillar is a univoltine defoliator of deciduous hardwood forests in North America, with periodic outbreaks that cause stand-level defoliation (Fitzgerald, 1995; Sipell, 1962). Fluctuations in the population density of FTC and other forest Lepidoptera are associated with density-induced variation in food quality and quantity, alongside increased entomopathogen abundance (Fitzgerald, 1995; Myers & Cory, 2013; Parry et al., 2003; Thomson, 1960), but little is known about how these factors interact. Microsporidia, especially *Nosema disstriae* (Kyei-Poku & Sokolova, 2017; Thomson, 1959), are common in FTC populations (Thomson, 1959; G. G. Wilson, 1977), and infection rates in FTC populations are geographically variable (Jones & Evenden, 2008). Previous studies report the impacts of microsporidia infection on FTC mortality and pupal and adult traits (Preti et al., 2023; G. G. Wilson, 1977, 1984). Still, diet-mediated interactions with microsporidia are almost entirely unexplored for FTC and other major forest lepidopterans (but see Kononchuk et al., 2021; Preti et al., 2023; van Frankenhuyzen & Liu, 2016).

The primary host for FTC in western North America is trembling aspen, *Populus tremuloides* Michaux (Parry & Goyer, 2004). While foliage quantity and quality may vary throughout an outbreak, there is also genotypic and phenological variation in aspen foliage phytochemistry, which is well documented to impact FTC and other aspen-feeding Lepidoptera (Falk et al., 2018; Hwang & Lindroth, 1997; Jones & Despland, 2006; Parry et al., 1998). Correlative studies report that the phenolic glycoside content of foliage is the strongest phytochemical predictor of the performance of FTC and spongy moth, *Lymantria dispar dispar* L. (Lepidoptera: Erebidae) on aspen, with lesser effects of primary metabolites and condensed tannins (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). Tannins, however, also have antimicrobial properties (Scalbert, 1991) that may play a role in a tri-trophic context with entomopathogens. In this study, variation in diet quality primarily considers secondary metabolite concentrations, while quantitative food manipulation simulates density-induced partial starvation (Evenden et al., 2015) associated with the high FTC population densities experienced during outbreaks (Fitzgerald, 1995).

Plant secondary metabolites can alter the susceptibility of insect herbivores to entomopathogens and parasites (Cory & Hoover, 2006; Gassmann et al., 2010; Mason et al., 2023; Shikano, 2017; Sun et al., 2023). Dietary tannins and phenolic glycosides, for instance, reduce the effectiveness of *Bacillus thuringiensis kurstaki* to other outbreaking defoliators of aspen (Appel & Schultz, 1994; Hwang et al., 1995). These secondary metabolites also impact the susceptibility of forest Lepidoptera to nuclear polyhedrosis virus (Cook et al., 2003; Hunter & Schultz, 1993). There is some evidence that diets enriched with plant extracts or phytochemicals can inhibit microsporidia infection and delay mortality of microsporidia-infected lepidopteran larvae (Carloye et al., 1998; Smirnoff, 1967), but more research is needed to understand this tri-trophic phenomenon.

Here, we conducted two experiments to examine interactions among FTC, diet quantity and quality, and disease-causing microsporidia. We evaluated FTC survival, infection rates and sublethal effects of the infection on larval development and adult and pupal traits. Microsporidia infection was induced either naturally or via experimental inoculation. Diet quality was manipulated by adding lyophilized trembling aspen foliage with known concentrations of secondary metabolites to an artificial diet. Food quantity was manipulated by varying the amount of food provided to FTC so that larvae experienced fully fed or partially starved conditions that simulated a high FTC density or outbreak conditions.

METHODS

We conducted two controlled experiments in Edmonton, Alberta, Canada, beginning in May 2018 (Experiment 1) and May 2019 (Experiment 2). For Experiment 1, egg masses were collected near Timmins, Ontario (49°42′45.7″ N 82°18′45.7″ W), in the second week of May 2018. For Experiment 2, egg masses were collected near Lac La Biche, Alberta (54°50′50.9″ N 112°13′03.8″ W), in February 2019 and Sault Ste. Marie, Ontario (46°31′17.6″ N 84°00′21.1″ W), in November 2018. In both experiments, egg masses were collected from infested aspen stands and were stored in paper bags at ~4°C until use. Egg masses in Experiment 2 were soaked in bleach for 2 min, rinsed for 5 min in tap water and rewashed in a 1% bleach solution for 30 s to remove the spumaline coating.

Diet preparation and chemical analysis of foliage powder

Each experiment contained two diet quality treatments: a standard artificial diet and an aspen-augmented diet. The standard diet used in both experiments was a modified Addy diet (Addy, 1969; Ebling & Dedes, 2015). This was compared to an aspen-augmented diet in each experiment, where 1% dry weight of lyophilized aspen foliage powder was added to the standard diet. To prepare foliage powder, new (unexpanded) aspen foliage was collected from the Edmonton area in 2018 (Experiment 1) and 2019 (Experiment 2). The foliage was cleansed in a 1% bleach solution for 5 min, rinsed with tap water and UV-treated on both sides for 10 min. The foliage was removed from petioles and lyophilized for 48 h (Labconco Corp., Kansas City, MO, USA) before grinding to a fine powder using a metal sieve and pestle.

Lyophilized foliage powder from both experiments was analysed for phenolic glycoside and condensed tannin content since these are the most significant defensive secondary metabolites of trembling aspen foliage against FTC (Bryant et al., 1987; Hemming & Lindroth, 1995; Palo, 1984). Three separate samples were analysed for each source of powder. Extraction included mixing 30 ± 5 mg of foliage powder with 1.5 mL HPLC grade methanol. This mixture was vortexed for 30 s, sonicated for 30 min at 4°C and centrifuged at 18,213 rcf for 5 min at 4°C. The supernatant was collected and transferred into gas chromatography vials using glass Pasteur tips fitted with glass wool to filter the extract. The samples were stored at −20°C until analysis (Najar et al., 2014).

For analysis of the phenolic glycosides, a 2 μL extract volume was analysed using an ultrahigh-performance liquid chromatography (UHPLC, 1290 Infinity Agilent Tech., Santa Clara, CA, USA) equipped with Diode Array Detector (DAD, 1290 Infinity Agilent Tech.). A column, InfinityLab Poroshell 120 EC-C18, with dimensions 2.1 × 150 mm 2.7 μm (part number 693775-902, Agilent Tech.), was fitted to identify phenolic glycosides at 50°C (column temperature). The mobile phase consisted of 0.1% formic acid in distilled deionized water (A, v/v) and 0.1% formic acid in methanol (B, v/v, HPLC grade). For the first 15 min, the gradient was 5%–60% (B), until 16 min 60% (B) was held, 16–18 min 60%–80% (B), 18–21 min 80%–50% (B), 21–22 min 50%–5% (B) and 22–23 min 5% (B). The run time per sample was 23 min with a flow rate of 0.250 mL min^{-1}. The phenolic glycosides salicin, tremulacin and salicortin were purchased from APIN Chemicals Ltd. (Oxfordshire, UK), and tremuloidin was received from Dr. Richard Lindroth (University of Wisconsin-Madison), which were applied to build calibration curves for sample quantification (Najar et al., 2014).

For analysis of condensed tannins, 10 μL extract was analysed using the UHPLC-DAD mentioned above, and an InfinityLab Poroshell 120 EC-C18 column with dimensions of 2.1 × 150 mm 1.9 μm (part number 693675-902, Agilent Tech) at 52°C. The binary mobile phase was the same as per phenolic glycosides, which contained 0.1% formic acid in distilled deionized water (A, v/v) and 0.1% formic acid in methanol (B, v/v, HPLC grade). The gradient was 40% (B) for the first 4 min, until 5 min 80% (B), 5–6 min 80%–100% (B), 6–6.50 min 100% (B) and 6.50–8 min 100%–0% (B). The run time per sample was 8 min with a flow rate of 0.50 mL min^{-1}. Condensed tannin standards were used to build calibration curves for quantification in samples; these standards were obtained by collecting trembling aspen leaves from the University of Alberta Campus and purifying them following the procedure in Hagerman and Butler (1980).

FTC rearing and treatments

In Experiment 1, 30 egg masses were placed in individual Petri dishes (100 × 15 mm) in a growth chamber at 22°C, 55% relative humidity, and LD 16:8 in late May 2018. After emerging, larvae from each egg mass were placed in 177 mL plastic cups with paper lids and given one of two diets (standard or aspen augmented). Larvae were culled to 20 ± 1 larvae per cup at the second instar, then split into two cups (10 ± 1 larvae per cup) at the fourth instar. Diet was replaced weekly. Pupae were moved to individual 22 mL cups and monitored daily, and adults were frozen after eclosion. Larvae were reared from 19 egg masses on the standard diet and 11 egg masses on the aspen-augmented diet. The full dataset was used to estimate FTC survivorship, while a reduced sample included surviving adults from the 11 egg masses reared on an aspen-augmented diet ($n = 91$) and from 12 randomly selected egg masses reared on a standard diet ($n = 92$).

In Experiment 2, 60 egg masses from each of Alberta and Ontario were individually placed in Petri dishes at room temperature with access to natural light in early June 2019. First-instar larvae received 1 cm^3 cubes of standard diet ad libitum. Five randomly selected second-instar larvae from each family group were screened for *Nosema* sp. infection (see methods below) and scored as naturally infected ($n = 7$ egg masses) or uninfected ($n = 90$ egg masses). We excluded 23 egg masses with insufficient second instars. The remaining larvae were split into groups of 10 in 177 mL plastic cups (with needle holes on the sides and lid) and randomly assigned the following experimental treatments: (1) diet quality (standard diet or aspen-augmented diet), (2) diet quantity (fully fed or partially starved), and (3) microsporidian infection (infected or uninfected).

Each cup was provided two cubes (1 cm^3) of the assigned diet quality once (partially starved) or three times (fully fed) per week. Larvae in the partially starved treatments were also provided two cubes of agar twice per week to maintain similar moisture across treatments (Evenden et al., 2015). Uninfected larvae assigned the infected treatment were manually inoculated with microsporidia. Naturally infected egg masses were assigned to the infected treatment, but we noted the mode of infection. Pupated larvae were moved to individual cups (100 mL) and monitored daily until eclosion. The dataset included 90 egg masses (48 from Alberta, 42 from Ontario) with surviving FTC.

L2 microsporidia screening and larval microsporidia inoculation (experiment 2)

The microsporidia infection we observed was overwhelmingly (>99%) Nosema sp., based on the oblong shape and size of spores (4.3 × 2.2 μm) and the regularity of infection (Thomson, 1959; G. G. Wilson, 1984). Most of the spores we observed were likely *N. disstriae* (Kyei-Poku & Sokolova, 2017; Thomson, 1959).

The five randomly selected second-instar larvae per egg mass that were selected for *Nosema* sp. screening were homogenized into solution in dH_2O, filtered through cheesecloth and allowed to rest for 12 h at 4°C to separate spores from liquid. After discarding the supernatant, spore concentration in the solution was determined using a compound microscope (400×) and haemocytometer. Uninfected and naturally infected egg masses were noted at this stage. Infected samples were used to make a spore solution (Cole, 1970), which was diluted to a sub-lethal concentration (1×10^5 spores/μL) (G. G. Wilson, 1977, 1984); 5 μL of the solution (5×10^5 spores) was pipetted onto diet cubes. Third instar larvae from uninfected egg masses were starved for 24 h and then exposed to inoculated diet cubes for 48 h. Larvae in the uninfected treatment and those from naturally infected egg masses were provided 5 μL of dH_2O on their diet as a control.

Adult and pupal microsporidia assessment

Adult abdomens (Experiments 1 and 2) and whole pupae (Experiment 2) were assessed for *Nosema* sp. infection. These were homogenized with 500 μL dH_2O using a pestle and vortexed (5 s), and 25 μL was allowed to rest for 2–3 min on a microscope slide. Ten fields of view were examined at 400×, and the count of *Nosema* sp. spores was recorded. In Experiment 1, spore counts were adjusted to adult weights (spore load = # spores/weight [mg]*56.4 mg, where 56.4 mg represents mean adult weight). Additionally, adults were categorized as infected or uninfected. In Experiment 2, infection levels were classified based on spore counts (0 = None, 1–100 = Low, 100+ = High). Individuals whose infection status conflicted with their original status assigned from larval infection assessments were excluded from the data.

Adult trait measurement

In Experiment 1, adults and associated cocoons were weighed to the nearest 0.01 mg following storage at −20°C for ∼36 months. For Experiment 2, cocoons and adults were weighed within 24 h of adult eclosion. Development time was measured as the number of days between egg hatch and adult eclosion.

Wings were removed from adults, glued to white paper and scanned at 118.1 pixels/cm (300 PPI) in JPEG format. To measure wing area, ImageJ software (Bethesda, MD, USA, https://imagej.net/ij/index.html) processed the image as black and white, and the area of all four wings was determined. To measure wing melanization (Experiment 1 only), we used greyscale 8-bit (0 = black, 255 = white) images to determine the mean grey value of two 30 × 30 pixel boxes on each forewing (one proximal and one distal to wing attachment, avoiding the dark bands/stripes, if present). To estimate mean wing pigmentation (melanization), mean grey values of the four selected areas were averaged for each individual and subtracted from 255.

Statistical analysis

All analyses were conducted in R (R Core Team, 2022), and Table 1 summarises the final statistical models. Interactions among all fixed effects were evaluated in full models, which were compared to reduced models using loglikelihood ratio tests. We dropped non-significant interactions from the final models when they did not improve model fit. Analysis of variance (ANOVA) Type II Wald chi-square tests or ANOVA Type III using Satterthwaite's method was used to estimate the significance of fixed effects. When applicable, estimated marginal means with a Tukey *p*-value adjustment were used to identify significant differences among treatments (models 2.1, 2.3a and 2.4 in Table 1). For generalized linear models (GLM) and generalized linear mixed-effects models (GLMM) using the lme4 library (Bates et al., 2015), we assessed model fit using Pearson chi-square goodness of fit, overdispersion tests and residual plots. For linear mixed models (LMM), residual normality and homoscedasticity were evaluated using residual and Q–Q plots and Shapiro–Wilk and Levene's tests.

In Experiment 1, microsporidian infection was incorporated into statistical models in two ways: (i) as a continuous spore load or (ii) as a binomial factor (infection status: infected or uninfected). We ran separate models for binomial and continuous treatment of infection, and when results were qualitatively similar, only results from the model that included spore load were reported. When results differed between the models, both were reported. To determine the error distribution for the model evaluating factors influencing spore load (model 1.3, Table 1), we compared Gaussian, negative binomial and Poisson models, selecting a negative binomial model of best fit based on AIC, residual plots and Pearson chi-square goodness of fit and overdispersion tests. For cocoon weight (model 1.6, Table 1), only non-zero values were included (models 1.4–1.5, Table 1). We separated analyses by sex when evaluating wing area, wing melanization

TABLE 1 Summary of final statistical models for Experiments 1 and 2.

Experiment model	Dependent variable(s)	Independent variable(s)	Model type	Error distribution
1.1	Survival L2—adult	Diet quality (fixed)	GLM	Binomial
1.2	Adult infection status	Diet quality (fixed) Sex (fixed) Egg mass (random)	GLMM	Binomial
1.3	Adult spore load	Diet quality (fixed) Sex (fixed) Egg mass (random)	GLMM	Negative binomial
1.4	Cocoon production	Diet quality (fixed) Spore load (fixed) Sex (fixed) Egg mass (random)	GLMM	Binomial
1.5	Cocoon production	Diet quality (fixed) Infection status (fixed) Sex (fixed) Egg mass (random)	GLMM	Binomial
1.6	Cocoon weight	Diet quality (fixed) Spore load (fixed) Sex (fixed) Egg mass (random)	LMM	Normal
1.7a 1.7b 1.7c 1.7d	Female adult weight Male Adult weight Female wing area Male wing area	Diet quality (fixed) Spore load (fixed) Egg mass (random)	LMM	Normal
1.8	Male adult weight	Diet quality (fixed) Infection status (fixed) Egg mass (random)	LMM	Normal
1.9	Wing malformation	Diet quality (fixed) Spore load (fixed) Sex (fixed) Egg mass (random)	GLMM	Binomial
1.10a 1.10b	Female wing melanization Male wing melanization	Diet quality (fixed) Spore load (fixed) Diet quality × Spore load (fixed) Egg mass (random)	LMM	Normal
1.11a 1.11b	Female wing melanization Male wing melanization	Diet quality (fixed) Infection status (fixed) Diet quality × infection status (fixed) Egg mass (random)	LMM	Normal
2.1	Survival L2-adult[a]	Diet quality (fixed) Diet quantity (fixed) Origin (fixed) L2 infection status (fixed)[a] Origin/egg mass/cup (random)	GLMM	Binomial
2.2	Adult/pupal Infection level	Diet quality (fixed) Diet quantity (fixed) Origin (fixed) Infection mode (fixed) Origin/egg mass/cup (random)	GLMM	Binomial
2.3a 2.3b	Cocoon production[a] Wing malformation	Diet quality (fixed) Diet quantity (fixed) Origin (fixed) Adult/pupal infection level (fixed)[a] Origin/egg mass/cup (random)	GLMM	Binomial
2.4	Cocoon production[a]	Diet quality (fixed) Diet quantity (fixed) Origin (fixed)	GLMM	Binomial

(Continues)

TABLE 1 (Continued)

Experiment model	Dependent variable(s)	Independent variable(s)	Model type	Error distribution
		Infection mode (fixed)[a]		
		Origin/egg mass/cup (random)		
2.5a	Log_{10}(female cocoon weight)	Diet quality (fixed)	LMM	Normal
2.5b	Female adult weight$^{0.586}$	Origin (fixed)		
2.5c	Female wing area	Adult/pupal infection level (fixed)		
		Origin/egg mass/cup (random)		
2.6a	Log_{10}(male cocoon weight)	Diet quality (fixed)	LMM	Normal
2.6b	Male adult weight$^{-0.747}$	Origin (fixed)		
2.6c	Male wing area	Diet quantity (fixed)		
		Adult/pupal infection level (fixed)		
		Origin/egg mass/cup (random)		
2.7	Development time$^{-0.424}$	Diet quality (fixed)	LMM	Normal
		Diet quantity (fixed)		
		Origin (fixed)		
		Sex (fixed)		
		Adult/pupal infection level (fixed)		
		Origin/egg mass/cup (random)		

Note: Separate models were run for each dependent variable. Interactions among all fixed effects were evaluated in preliminary full models, but non-significant interactions that did not improve model fit were dropped from the final models reported here. Transformations of dependent variables are indicated, and superscripts represent lambda values in Box-Cox transformations. Nesting of independent variables is shown using '/'.
Abbreviations: GLM, generalized linear model; GLMM, generalized linear mixed-effects model; LMM, linear mixed-effects model.
[a]Models and variables where for which estimated marginal means with a Tukey *p*-value adjustment were used to identify significant differences among treatments.

and body weight (models 1.7–1.8 and 1.10–1.11, Table 1). As some adults eclosed with malformed wings, we evaluated wing area frequency distributions and visually inspected wings to determine if they were malformed (Preti et al., 2023). Malformed wings were defined as <180 mm^2 and <130 mm^2 for females and males, respectively, and were excluded from wing area models (models 1.7cd, Table 1).

In Experiment 2, cocoon weight was log_{10}-transformed before analysis, and Box-Cox transformations were applied to adult body weight (males: $\lambda = -0.747$, females: $\lambda = 0.586$) and development time ($\lambda = -0.424$) to satisfy model assumptions (models 2.5ab and 2.6ab, Table 1). Males and females were analysed separately in models for cocoon weight, adult body weight and wing area (models 2.5–2.6, Table 1). Due to the low survival ($n = 3$) of females in the partially starved treatment, they were removed from the analysis. Due to the low survival of naturally infected individuals, the influence of infection mode could not be estimated. Adult wings <190 mm^2 and <100 mm^2 were considered malformed for females and males, respectively, and were excluded from wing area models (models 2.5c and 2.6c, Table 1).

We used a two-tailed two-sample *t*-test assuming equal variances to compare the concentration of total condensed tannins, total phenolic glycosides and individual phenolic glycosides in the lyophilized aspen foliage powder used to make diets in Experiments 1 and 2.

RESULTS

Chemical analysis of foliage powder

The concentration of total condensed tannins ($t_4 = 8.1$, $p = 0.001$) and phenolic glycosides ($t_4 = 25.6$, $p < 0.0001$) was approximately two times higher in the lyophilized aspen foliage powder used to make aspen-augmented diet in Experiment 2 than Experiment 1 (Table 2). Similarly, the individual phenolic glycosides salicortin ($t_4 = 41.9$, $p < 0.0001$) and tremulacin ($t_4 = 43.9$, $p < 0.0001$) were 2–3× higher in the foliage powder used in Experiment 2 compared with Experiment 1 (Table 2). The concentration of tremuloidin was statistically greater in foliage powder used in Experiment 1 than Experiment 2 ($t_4 = 29.9$, $p < 0.0001$), but the concentration was low in both experiments. The salicin concentration of foliage powder was similar between experiments ($t_4 = 1.0$, $p = 0.35$) (Table 2).

TABLE 2 Phenolic glycoside and condensed tannin concentration (μg/mg) in lyophilized trembling aspen (*Populus tremuloides*) foliage powder used in Experiments 1 and 2.

Experiment	Salicin	Salicortin	Tremuloidin	Tremulacin	Total phenolic glycosides	Total condensed tannins
1	6.5 ± 0.09[a]	1.1 ± 0.01[a]	1.9 ± 0.02[a]	4.3 ± 0.06[a]	13.7 ± 0.18[a]	24.8 ± 1.0[a]
2	6.3 ± 0.09[a]	4.6 ± 0.08[b]	1.1 ± 0.02[b]	10.9 ± 0.14[b]	23.0 ± 0.32[b]	48.8 ± 2.8[b]

Note: Different superscript letters in each column represent significant differences ($p < 0.01$) in concentration between experiments based on two-tailed two-sample *t*-tests assuming equal variance.

Experiment 1

Interactions among fixed effects that did not improve model fit were removed from the final models (1.2–1.9, Table 1). The only statistical models where interaction was retained were those with adult wing melanization as a response, where diet quality by spore load or infection status interactions improved model fit (1.10–1.11, Table 1).

Survival

Survival of FTC reared on the aspen-augmented diet (51.4 ± 0.07%) was higher than that on the standard diet (41.8 ± 0.07%) ($\chi^2 = 5.0$; $p = 0.026$). We could not directly evaluate the impact of microsporidia infection on survival because the infection status of larvae that died was unknown.

Infection status and spore load

Adults reared on a standard diet were more likely to be infected with microsporidia (57.6%, $n = 92$) than adults reared on the aspen-augmented diet (42.9%, $n = 91$) ($\chi^2 = 15.1$; $p < 0.001$), but there was no effect of sex ($\chi^2 = 2.8$; $p = 0.091$) on infection status. In a separate model evaluating spore load as a response, neither diet quality ($\chi^2 = 1.5$; $p = 0.21$) nor sex ($\chi^2 = 0.42$; $p = 0.51$) was significant.

Cocoon production and weight

An increase in spore load reduced the likelihood of cocoon production ($\chi^2 = 15.8$; $p < 0.0001$; Figure 1), but neither sex ($\chi^2 = 0.29$; $p = 0.59$) or diet quality ($\chi^2 = 0.22$; $p = 0.64$) were significant. When infection was included as a binomial variable in the model, uninfected FTC were twice as likely to produce a cocoon (64.4%, $n = 90$) than infected FTC (32.6%; $n = 92$) ($\chi^2 = 11.7$; $p < 0.001$). The weight of cocoons produced by prepupal FTC was not influenced by sex ($F_{1,80.7} = 2.1$; $p = 0.15$), diet quality ($F_{1,16.78} = 1.4$; $p = 0.25$), or spore load ($F_{1,40.2} = 0.37$; $p = 0.55$).

Adult weight

The weight of female and male FTC adults was not influenced by diet quality (female: $F_{1,17.8} = 0.75$, $p = 0.40$; male: $F_{1,15.7} = 0.002$, $p = 0.96$) or spore load (female: $F_{1,43.2} = 0.44$, $p = 0.51$; male: $F_{1,43.2} = 0.44$, $p = 0.51$). Infected males (37.9 ± 1.3 mg), however, were heavier than uninfected males (33.48 ± 1.5 mg) ($F_{1,21.3} = 5.0$; $p = 0.036$).

Wing area, malformation and melanization

Neither female nor male wing area was influenced by diet quality (female: $F_{1,18.7} = 2.5$, $p = 0.13$; male: $F_{1,3.1} = 5.0$, $p = 0.11$) or spore load (female: $F_{1,69.7} = 0.01$, $p = 0.91$; male: $F_{1,9.3} = 0.08$, $p = 0.79$). Similarly, there was no effect of sex ($\chi^2 = 0.04$; $p = 0.84$), diet ($\chi^2 = 0.51$; $p = 0.48$), or spore load ($\chi^2 = 1.5$; $p = 0.23$) on the likelihood of wing malformation.

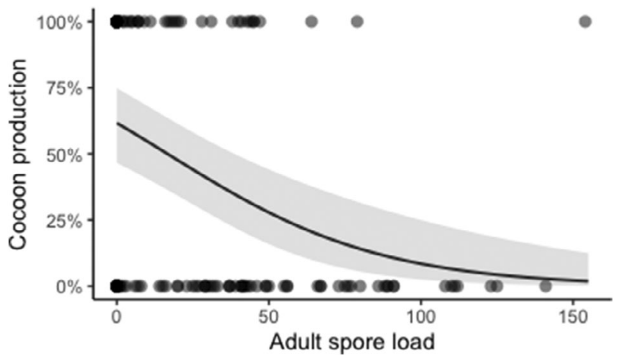

FIGURE 1 Predicted likelihood (solid line) and associated 95% confidence intervals (grey shaded area) of forest tent caterpillar cocoon production with varying adult microsporidia spore loads in Experiment 1 (model 1.4, Table 1). Raw data are shown as points. Adult spore load was determined by homogenizing adult abdomens and 500 μL dH_2O and pipetting 25 μL of the solution onto a microscope slide. We counted the number of *Nosema disstriae* spores across 10 fields of view at 400×. Adult spore load was corrected for differences in adult weight (adult spore load = spore count/adult weight [mg]*56.4, where 56.4 mg is a constant representing mean adult weight).

Adult female wing melanization was influenced by diet quality ($F_{1,21.9} = 6.5$; $p = 0.018$) and by the interaction between diet quality and spore load ($F_{1,66.0} = 8.7$; $p = 0.0042$) but not by the main effect of spore load ($F_{1,66.0} = 0.010$; $p = 0.92$). Melanization increased with spore load for adult females fed the standard diet, but the relationship between spore load and melanization was not significant for females fed the aspen-augmented diet (Figure 2). There was no effect of diet quality ($F_{1,20.5} = 2.29$; $p = 0.15$), spore load ($F_{1,69.6} = 0.189$; $p = 0.67$), or their interaction ($F_{1,69.6} = 1.74$; $p = 0.19$) on male wing melanization. Wings of male moths were darker (133.7 ± 1.6 grey units) than females (143.6 ± 1.5 grey units). In models with infection status (binomial), infection status, diet quality or their interaction was predictors of male or female wing melanization ($p > 0.10$).

Experiment 2

Interactions among fixed effects did not improve model fit for any statistical models and were removed from final models (2.1–2.7, Table 1).

Survival (L2—Adult)

There was a significant effect of diet quality ($\chi^2 = 14.3$, $p < 0.001$), diet quantity ($\chi^2 = 7.35$, $p = 0.007$), second instar infection status ($\chi^2 = 19.5$, $p < 0.0001$) but not egg mass origin ($\chi^2 = 0.96$, $p = 0.33$) on larval survival from second instar to the adult stage. Survival was higher on the standard diet compared with the aspen-augmented diet,

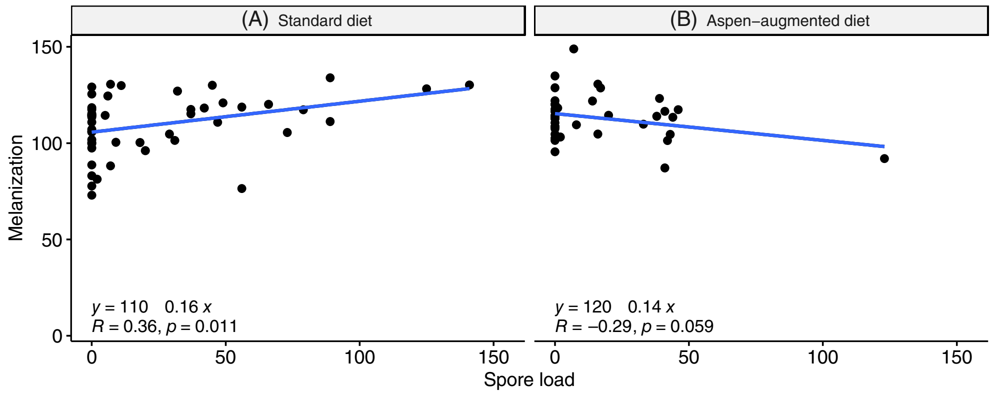

FIGURE 2 Linear relationship and associated 95% confidence intervals (model 1.10a, Table 1) between microsporidia spore load and wing melanization for adult female forest tent caterpillar (FTC) fed a (a) standard diet or (b) aspen-augmented diet in Experiment 1. We used greyscale 8-bit (0 = black, 255 = white) images and forewings to determine the mean grey value of two 30 × 30 pixel boxes on each wing (proximal and distal to wing attachment). Average mean grey values per adult were subtracted from 255 to estimate mean wing pigmentation or darkness (i.e. melanization).

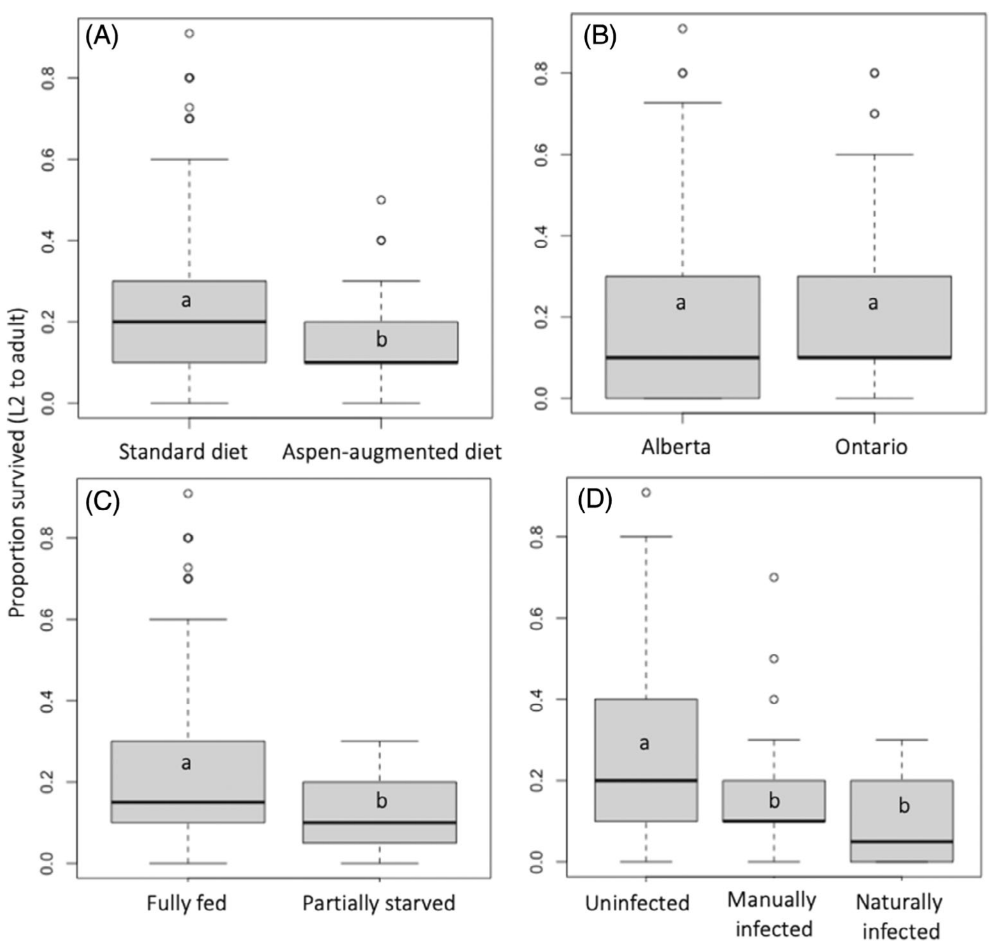

FIGURE 3 Boxplot illustrating survival of forest tent caterpillar from second instar (L2) to adult eclosion in Experiment 2 when (a) fed a standard diet versus an aspen-augmented diet, (b) egg masses originated from Alberta versus Ontario, (c) larvae were fed ad libitum (fully fed) versus a partially starved regime, (d) when infection status and mode varied. Different letters within boxes indicate significant differences ($p < 0.05$) between treatments within a plot.

in the fully fed versus the partially starved treatment and for uninfected FTC compared with infected FTC (Figure 3). Survival of uninfected FTC was higher than those naturally infected ($Z = 2.41$, $p = 0.043$) and manually infected ($Z = 3.92$, $p < 0.001$), but there was no difference in survival for larvae that were manually versus naturally infected ($Z = 0.99$, $p = 0.58$) (Figure 3).

TABLE 3 Mean (±SE) cocoon weight, adult weight, wing area, and development time of forest tent caterpillar adults in Experiment 2.

Predictor	Treatment	Female cocoon weight (mg)	Male cocoon weight (mg)	Female adult weight (mg)	Male adult weight (mg)	Female wing area (mm^2)	Male wing area (mm^2)	Development time (days)
Diet quality	Standard diet	37.1 ± 1.7	26.6 ± 1.1*	83.4 ± 3.6	19.7 ± 0.7*	3.19 ± 0.05*	2.25 ± 0.04	40.4 ± 0.4**
	Aspen-augmented diet	36.5 ± 3.4	21.1 ± 1.6*	76.7 ± 10.7	22.1 ± 1.2*	2.91 ± 0.25*	2.07 ± 0.13	44.2 ± 1.4**
Diet quantity	Fully fed	-	26.9 ± 1.0***	-	20.5 ± 0.7*	-	2.26 ± 0.04**	40.3 ± 0.35***
	Partially starved		16.2 ± 1.2***		14.9 ± 1.0*		1.92 ± 0.10**	52.1 ± 1.7***
Egg mass origin	Ontario	37.8 ± 1.9	25.0 ± 1.1	93.2 ± 4.7**	21.1 ± 0.8*	3.26 ± 0.07	2.24 ± 0.05	42.3 ± 0.58**
	Alberta	36.4 ± 2.4	26.6 ± 1.7	73.4 ± 4.5**	18.9 ± 0.9*	3.09 ± 0.06	2.22 ± 0.06	39.5 ± 0.45**
Infection level	None	36.2 ± 1.6	26.0 ± 1.1	82.5 ± 3.8	19.6 ± 7.6	3.17 ± 0.05	2.20 ± 0.05	40.8 ± 0.45
	Low	45.5 ± 10.0	28.1 ± 3.5	79.6 ± 14.7	20.8 ± 5.9	3.30 ± 0.19	2.51 ± 0.10	40.2 ± 1.40
	High	37.4 ± 3.8	22.2 ± 1.8	84.0 ± 8.9	22.5 ± 11.2	3.14 ± 0.12	2.21 ± 0.09	41.7 ± 0.66
Sex	Male	-	-	-	-	-	-	40.6 ± 0.51**
	Female							41.4 ± 0.57**
Mode of infection	Natural	-	-	-	-	-	-	43.8 ± 2.00
	Manual							41.0 ± 0.64

Note: Asterisks indicate significance levels, where *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Blank cells indicate insufficient sample size for analysis or for the predictor 'sex' when males and females were analysed separately.

Adult/pupal infection level

Naturally infected FTC were more likely to be heavily infected as pupae or adults (95.5%, $N = 22$) than FTC inoculated at third instar (67.0%, $N = 103$) ($\chi^2 = 4.9$, $p = 0.037$), but infection level was not influenced by diet quality ($\chi^2 = 0.13$, $p = 0.91$), diet quantity ($\chi^2 = 0.002$, $p = 0.97$) or egg mass origin ($\chi^2 = 0.05$, $p = 0.83$).

Cocoon production and weight

Cocoon production was influenced by infection level ($\chi^2 = 7.9$, $p = 0.02$); highly infected FTC (48.4%, $N = 91$) were less likely to produce a cocoon than uninfected individuals (66.8%, $N = 397$) ($Z = 2.7$, $p = 0.02$). Cocoon production by FTC with low levels of infection (65.7%, $N = 35$) did not differ from that of uninfected ($Z = 0.085$, $p = 0.99$) or highly infected FTC ($Z = 1.7$, $p = 0.20$). There was no effect of diet quality ($\chi^2 = 0.02$, $p = 0.88$), diet quantity ($\chi^2 = 1.9$, $p = 0.17$) or egg mass origin ($\chi^2 = 0.003$, $p = 0.96$) on cocoon production by FTC. The mode of microsporidia infection also impacted cocoon production ($\chi^2 = 13.9$, $p < 0.001$). Naturally infected individuals were less likely to produce a cocoon (6.3%, $N = 16$) than those manually inoculated with microsporidia (55.3%, $N = 47$) ($Z = 2.4$, $p = 0.004$) and uninfected individuals (65.6%, $N = 163$) ($Z = 3.7$, $p < 0.001$).

Male FTC fed the standard diet produced heavier cocoons than those fed the aspen-augmented diet ($F_{1,72.2} = 6.9$, $p = 0.01$), but there was no effect of diet quality on cocoon weight for female FTC ($F_{1,83.5} = 0.005$, $p = 0.95$) (Table 3). Cocoons were also heavier for males that were fully fed versus partially starved ($F_{1,78.7} = 20.8$, $p < 0.001$). The weight of female and male cocoons was not influenced by egg mass origin (female: $F_{1,43.4} = 1.4$, $p = 0.25$; male: $F_{1,52.0} = 0.11$, $p = 0.75$) or infection level (female: $F_{2,67.0} = 1.1$, $p = 0.34$; male: $F_{2,76.4} = 0.25$, $p = 0.78$) (Table 3).

Adult weight

Female moths from egg masses that originated in Ontario were heavier than those from Alberta ($F_{1,37.4} = 8.2$, $p = 0.007$), but there was no effect of diet quality ($F_{1,92.1} = 1.2$, $p = 0.28$) or infection level ($F_{2,81.5} = 0.09$, $p = 0.91$) on female adult weight (Table 3). The impact of diet quantity on female adult weight could not be evaluated since only three partially starved females survived. Male moths that were reared on the aspen-augmented diet were heavier than those reared on standard diet ($F_{1,160.7} = 5.8$, $p = 0.017$); fully fed male moths were heavier than those that were partially starved ($F_{1,125.0} = 6.4$, $p = 0.013$); adult males reared from egg masses that originated in Ontario were heavier than those from Alberta ($F_{1,52.6} = 4.0$, $p = 0.049$) (Table 3). There was no effect of infection level on male adult weight ($F_{2,155.4} = 2.2$, $p = 0.12$).

Development time

Development time was shorter for larvae reared on the standard diet (vs. aspen-augmented diet) ($F_{1,93.6} = 10.6$, $p = 0.002$), for fully fed (vs. partially starved) FTC ($F_{1,112.4} = 51.6$, $p < 0.001$), for FTC from Alberta (vs. Ontario) ($F_{1,66.3} = 8.3$ $p = 0.005$) and for males (vs. females) ($F_{1,266.5} = 8.9$, $p = 0.003$) (Table 3). Infection level did not influence development time ($F_{2,280.6} = 0.15$, $p = 0.86$).

Wing area and malformation

Female ($F_{1,100} = 4.7$, $p = 0.03$), but not male ($F_{1,126.2} = 2.2$, $p = 0.14$), FTC reared on the standard diet had significantly larger wings than those reared on the aspen-augmented diet (Table 3). Fully fed male FTC had larger wings than partially starved males ($F_{1,126.7} = 8.1$, $p = 0.005$) (Table 3). Neither egg mass origin (female: $F_{1,36.6} = 2.8$, $p = 0.10$; male: $F_{1,46.2} = 0.47$, $p = 0.50$) nor infection level (female: $F_{1,92.5} = 0.048$, $p = 0.83$; male: $F_{1,128.2} = 3.4$, $p = 0.074$) influenced adult wing area.

Larvae reared on the standard diet developed malformed wings less frequently (7.4%, $N = 229$) than larvae fed on the aspen-augmented diet (25.0%, $N = 32$) ($\chi^2 = 9.4$, $p = 0.0025$). Wing malformation was not influenced by infection level ($\chi^2 = 0.50$, $p = 0.48$), egg mass origin ($\chi^2 = 0.74$, $p = 0.39$), sex ($\chi^2 = 0.004$, $p = 0.95$) or diet quantity ($\chi^2 = 0.41$, $p = 0.52$).

DISCUSSION

We explored the effects of altering diet quality and quantity on interactions between FTC and *Nosema* sp. microsporidia. Our findings show that qualitative variation in diet can impact interactions between FTC and *Nosema* sp., with the nature of these tri-trophic interactions contingent on the concentration of secondary metabolites in the FTC diet. Additionally, diet quality, diet quantity and microsporidia infection independently influenced FTC survival and were associated with sublethal effects, which may affect the population dynamics and dispersal of this outbreaking forest defoliator.

Impact of diet quality

Diet quality treatments had different effects on FTC and FTC-microsporidia interactions between experiments. In Experiment 1, using the aspen-augmented diet with relatively low secondary metabolite concentrations led to reduced rates of natural microsporidia infection and increased survival among adults compared with the standard diet. In contrast, when using the aspen-augmented diet with high secondary metabolite concentrations (Experiment 2), FTC performed better on the standard diet, and we did not observe any diet-mediated interactions between FTC and microsporidia. Our findings revealed the potential for a novel dose-dependent effect of aspen secondary metabolites on FTC-microsporidia interactions.

We believe the varied responses to diet quality treatments within and between experiments are largely attributed to differences in secondary metabolite concentrations among treatments, consistent with the established correlation between aspen defoliator performance and secondary metabolite concentrations in foliage (Donaldson & Lindroth, 2007; Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). To further support this hypothesis, macronutrient impacts in our study were likely minimal; the foliage powder comprised only 1% of aspen-augmented diets. However, the potential influence of micronutrients on the observed differences in diet quality treatments should be considered, as they can influence immune responses or susceptibility to entomopathogens in other lepidopterans (Popham & Shelby, 2009; Pristavko & Dovzhenok, 1974).

The foliage powder used in diets contained secondary metabolites at concentrations within the lower reported range for aspen (Falk et al., 2018; Hwang & Lindroth, 1997). This suggests that FTC and FTC–microsporidia interactions might be sensitive to even modest shifts in secondary metabolite concentrations. In nature, diet-mediated interactions between FTC and microsporidia may be limited to tree genotypes, phenological stages and outbreak phases associated with lower secondary metabolite concentrations. Future research should explore FTC–microsporidia interactions using artificial diets with varying secondary metabolite concentrations within a single experiment to provide more robust evidence of the dose-dependent impact of secondary metabolites on FTC–microsporidia interactions.

While diet- or phytochemical-mediated variations in Lepidoptera-pathogen dynamics are common (Cory & Hoover, 2006; Shikano, 2017), this phenomenon remains underexplored for microsporidia. Smirnoff (1967), however, reported reduced *Nosema cerasivoranus* Thomson (Nosematidae) infection rates and increased survivorship and longevity for infected *Archips cerasivoranus* Fitch (Lepidoptera: Tortricidae)-fed foliage treated with onion (*Allium cepa* L.) extract. Similarly, longevity and survivorship of *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) infected with microsporidia vary when secondary metabolites are manipulated in artificial diets (Carloye et al., 1998). Conversely, Preti et al. (2023) and van Frankenhuyzen and Liu (2016) report only independent effects of diet quality and microsporidia infection on FTC and *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), respectively.

Unlike Experiment 1, the aspen-augmented diet did not benefit FTC in Experiment 2. Instead, this diet decreased FTC survival, cocoon weight and female wing area, while increasing wing malformation and development time. This aligns with existing literature on the negative impacts of aspen secondary metabolites on FTC performance (Hemming & Lindroth, 1995; Hwang & Lindroth, 1997). The impact of diet quality on wing traits in Experiment 2 is particularly significant, since reduced Lepidoptera wing size is associated with reduced fecundity (Evenden et al., 2006) and flight (Shi et al., 2015; van Hezewijk et al., 2018) with implications for dispersal and population dynamics. Preti et al. (2023) also reported the impacts of host quality on wing size and malformation in FTC. While the cause of the diet-induced wing malformation in our study and Preti et al. (2023) is unknown, diet-induced wing malformations have been reported for FTC (Barbehenn & Martin, 1994) and other Lepidoptera (Bracken, 1982; Chippendale et al., 1965). Surprisingly, male FTC reared on the aspen-augmented diet were heavier than those reared on the standard diet, possibly due to higher mortality rates among small FTC fed this diet.

Our results suggest a possible association between diet quality, wing melanization and microsporidia infection in FTC. While we observed a positive correlation between wing melanization and spore load in FTC females fed the standard diet, this link was not evident for males or females fed the aspen-augmented diet. Earlier studies report that low diet quality can reduce wing melanization in Lepidoptera (Ethier et al., 2015; Talloen et al., 2004), as well as links between insect diet quality, melanization, and disease resistance or immune function (Lee et al., 2008; Mikkola & Rantala, 2010). Despite some studies reporting that heightened melanization correlates with greater disease resistance or immunocompetence (Mikkola & Rantala, 2010; K. Wilson et al., 2001), others suggest it might lead to heightened susceptibility to pathogens (Goulson & Cory, 1995) or elevated microsporidia infection (Becnel & Andreadis, 2014). Due to the cost of melanization, heavily melanized moths reared on the standard diet in our study may have allocated fewer resources to pathogen resistance, leading to higher *Nosema* sp. spore loads. This trend was only conspicuous for FTC females on the standard diet, possibly due to the broader range of spore loads observed for FTC fed this diet or due to secondary metabolites in aspen-augmented diets altering this relationship. Additionally, this trend was specific to females, likely owing to their elevated resource requirements compared with males. Wing melanization in Lepidoptera impacts various ecological processes such as reproduction, dispersal and vulnerability to natural enemies (Majerus, 1998; Roland, 1982; Rosa & Saastamoinen, 2020), thus advocating for further exploration of the broader implications of the interactions among infection, diet quality and melanization.

Impact of diet quantity

In Experiment 2, partially starved FTC exhibited reduced survival and adult performance, but we found no evidence of an interaction with microsporidia. This suggests these factors work additively, but not interactively to reduce FTC populations at peak densities. Evenden et al. (2015) similarly reported negative effects of reduced food quantity on FTC larvae and pupae but did not report effects on adult traits, as we observed. .

Influence of microsporidia infection

Microsporidia infection interacted with diet in Experiment 1, as discussed above. However, we saw only independent effects of microsporidia infection on FTC in Experiment 2. Uninfected FTC had higher survival and cocoon production rates, consistent with earlier findings (G. G. Wilson, 1977), where *N. disstriae* infection in FTC leads to spore concentration elevation in the labial silk glands, causing the absence of a pupal cocoon (G. G. Wilson, 1977). Interestingly, microsporidia infection did not affect cocoon weight, possibly due to the additional weight of spores in the silk (Thomson, 1959). Infected males were also heavier than uninfected males in Experiment 1, which could again be due to the weight of the *Nosema* sp. spores.

Experiment 2 revealed that the mode of infection can influence microsporidia spore load and cocoon production, with naturally infected FTC exhibiting higher spore loads and a lower probability of cocoon production relative to those manually inoculated at the third instar. This underscores the significance of timing for microsporidia pathogenicity in FTC, aligning with earlier reports (G. G. Wilson, 1984). Differences in infection mode may also contribute to varied outcomes between experiments and should be considered in future studies.

Future studies should also investigate the potential implications of high microsporidian infection in silk glands for FTC larvae since they rely on silk trails and pheromones for gregarious movement in search of food (Fitzgerald, 1995; Fitzgerald & Costa III, 1986). Disruption of silk production may affect larval foraging efficiency. Additionally, pupae with smaller or no cocoons could face increased risk from natural enemies or unfavourable environmental conditions.

Influence of egg mass origin

In Experiment 2, adult FTC from Ontario were heavier and took longer to develop than those from Alberta. Since the Alberta egg masses were collected in an area with high defoliation the previous 2 years compared to those from Ontario with high defoliation in only the previous 1 year (pers. comm.), this could have reduced fitness for FTC from Alberta. Genomic differentiation in FTC is also related to geographic isolation and environmental conditions (MacDonald et al., 2022), which may have contributed to these results. Future research should also examine geographic variation in *N. disstriae* populations, which could alter the tri-trophic interaction we observed.

CONCLUSIONS

Our findings highlight both interactive and direct impacts of diet and microsporidia infection on FTC. They align with earlier reports of the direct impact of high population densities and reduced food quantity and quality on FTC survival and life history traits (Evenden et al., 2015; Hodson, 1941; Parry et al., 2003; Robison & Raffa, 1997) and support the conclusion that food quantity and quality are critical in outbreak and dispersal dynamics in forest Lepidoptera (Abbott & Dwyer, 2007; Fitzgerald, 1995; Smith & Goyer, 1986). Our results also reveal how diet quality can interact with a widespread entomopathogen in FTC populations, whose role in this species' outbreak dynamics remains poorly understood.

AUTHOR CONTRIBUTIONS

Leah Flaherty: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing – original draft; writing – review and editing. **Flavio Preti:** Data curation; formal analysis; investigation; methodology; writing – original draft. **Guncha Ishangulyyeva:** Investigation; methodology; resources; writing – review and editing. **Nadir Erbilgin:** Funding acquisition; investigation; methodology; resources; supervision; writing – review and editing. **Taylar Whidden:** Investigation; methodology; writing – review and editing. **Maya Evenden:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGEMENTS

This project was funded by MacEwan University and by an NSERC Discovery Grant held by ME. In-kind contributions (egg mass acquisition, diet ingredients and/or methodological support) were provided by Alberta Agriculture and Forestry (Caroline Whitehouse), Ontario Ministry of Natural Resources (Lia Fricano and Mike Francis) and the Great Lakes Forestry Centre (Amanada Roe, Jeff Fidgen and Chris Macquarrie). We are grateful to Alexandre Caouette, Dana Dunbar, Sophie Lang, Cameron Nordstrom, Rebecca Pacholuk, Ashley Pillay and Maria Ovcharenko for technical assistance.

CONFLICT OF INTEREST STATEMENT

The authors declare that they 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 request.

ORCID

Leah Flaherty https://orcid.org/0009-0007-2245-1880

REFERENCES

Abbott, K.C. & Dwyer, G. (2007) Food limitation and insect outbreaks: complex dynamics in plant-herbivore models. *Journal of Animal Ecology*, 76, 1004–1014.

Addy, N.D. (1969) Rearing the forest tent caterpillar on an artificial diet. *Journal of Economic Entomology*, 62, 270–271.

Appel, H.M. & Schultz, J.C. (1994) Oak tannins reduce effectiveness of thuricide (*Bacillus thuringiensis*) in the gypsy moth (Lepidoptera: Lymantriidae). *Journal of Economic Entomology*, 87, 1736–1742.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, 47, 817–844.

Barbehenn, R.V. & Martin, M.M. (1994) Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): roles of the peritrophic envelope and midgut oxidation. *Journal of Chemical Ecology*, 20, 1985–2001.

Bates, D., Mächler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67. Available from: https://doi.org/10.18637/jss.v067.i01

Becnel, J.J. & Andreadis, T.G. (2014) Microsporidia in insects. In: Weiss, L.-M. & Becnel, J.J. (Eds.) *Microsporidia: pathogens of opportunity*. Hoboken, NJ: John Wiley and Sons Inc., pp. 521–570.

Bracken, G.K. (1982) The bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae). Effects of dietary linolenic acid on pupal syndrome, wing syndrome, survival, and pupal fat composition. *The Canadian Entomologist*, 114, 567–573.

Bryant, J.P., Clausen, T.P., Reichardt, P.B., McCarthy, M.C. & Werner, R.A. (1987) Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortrix (*Choristoneura conflictana* (Walker)). *Oecologia*, 73, 513–517.

Carloye, L., Maddox, J.V. & Berenbaum, M.R. (1998) Influence of natural and synthetic compounds on the infectivity of a *Vairimorpha* (microspora) sp. in *trichoplusia ni* (Lepidoptera: Noctuidae) larvae. *Environmental Entomology*, 27, 976–983.

Chippendale, G.M., Beck, S.D. & Strong, F.M. (1965) Nutrition of the cabbage looper, *Trichoplusia ni* (Hübn.) – I. Some requirements for larval growth and wing development. *Journal of Insect Physiology*, 11, 211–223.

Colasurdo, N., Gélinas, Y. & Despland, E. (2009) Larval nutrition affects life history traits in a capital breeding moth. *Journal of Experimental Biology*, 212, 1794–1800.

Cole, R.J. (1970) The application of the "triangulation" method to the purification of *Nosema* spores from insect tissues. *Journal of Invertebrate Pathology*, 15, 193–195.

Cook, S.P., Webb, R.E., Podgwaite, J.D. & Reardon, R.C. (2003) Increase mortality of gypsy moth *Lymantria dispar* (L.) (Lepidotera: Lymantriidae) exposed to gypsy moth nuclear polyhedrosis virus in combination with the phenolic gycoside salicin. *Journal of Economic Entomology*, 96, 1662–1667.

Cory, J.S. & Hoover, K. (2006) Plant-mediated effects in insect-pathogen interactions. *Trends in Ecology & Evolution*, 21, 278–286.

Donaldson, J.R. & Lindroth, R.L. (2007) Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. *Ecology*, 88, 729–739.

Ebling, P.M. & Dedes, J. (2015) *Preparation of artificial diets*. Great Lakes Forestry Centre Insect Production Services. IPS.010.003. Sault Ste. Marie: Great Lakes Forestry Centre, Natural Resources Canada.

Ethier, J., Gasse, M., Lake, K., Jones, B.C., Evenden, M.L. & Despland, E. (2015) The costs of colour: plasticity of melanin pigmentation in an outbreaking polymorphic forest moth. *Entomologia Experimentalis et Applicata*, 154, 242–250.

Evenden, M.L., Lopez, M.S. & Keddie, B.A. (2006) Body size, age, and disease influence female reproductive performance in *Choristoneura conflictana* (Lepidoptera: Torticidae). *Annals of the Entomological Society of America*, 99, 837–844.

Evenden, M.L., Mori, B.A., Sjostrom, K.D. & Roland, J. (2015) Forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), mate-finding behavior is greatest at intermediate population densities: implications for interpretation of moth capture in pheromone-baited traps. *Frontiers in Ecology and Evolution*, 3, 1–11. Available from: https://doi.org/10.3389/fevo.2015.00078

Falk, M.A., Lindroth, R.L., Keefover-Ring, K. & Raffa, K.F. (2018) Genetic variation in aspen phytochemical patterns structures windows of opportunity for gypsy moth larvae. *Oecologia*, 187, 471–482.

Fitzgerald, T.D. (1995) *The tent caterpillars*. Ithaca, NY: Cornell University Press.

Fitzgerald, T.D. & Costa, J.T., III. (1986) Trail based communication and foraging behaviors of young colonies of forest tent caterpillars (Lepidoptera: Lasiocampidae). *Annals of the Entomological Society of America*, 79, 999–1007.

Gassmann, A.J., Stock, S.P., Tabashnik, B.E. & Singer, M.S. (2010) Tri-trophic effects of host plants on an herbivore-pathogen interaction. *Annals of the Entomological Society of America*, 103, 371–378.

Goulson, D. & Cory, J.S. (1995) Responses of *Mamestra brassicae* (Lepidoptera: Noctuidae) to crowding: interactions with disease resistance, colour phase and growth. *Oecologia*, 104, 416–423.

Hagerman, A.E. & Butler, L.G. (1980) Condensed tannin purification and characterization of tannin-associated proteins. *Journal of Agricultural and Food Chemistry*, 28, 947–952.

Hemming, J.D.C. & Lindroth, R.L. (1995) Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia*, 103, 79–88.

Hodson, A.C. (1941) *An ecological study of the forest tent caterpillar, Malacosoma disstria Hbn., in northern Minnesota*, Vol. 148. University of Minnesota Agricultural Experiment Station Technical Bulletin.

Hunter, M.D. & Schultz, J.C. (1993) Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia*, 94, 195–203.

Hwang, S.Y. & Lindroth, R. (1997) Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia*, 111, 99–108.

Hwang, S.Y., Lindroth, R.L., Montgomery, M.E. & Shields, K.S. (1995) Aspen leaf quality affects gypsy moth (Lepidoptera: Lymantriidae) susceptibility to *Bacillus thuringiensis*. *Journal of Economic Entomology*, 88, 278–282.

Jones, B.C. & Despland, E. (2006) Effects of synchronization with host plant phenology occur early in the larval development of a spring folivore. *Canadian Journal of Zoology*, 84, 628–633.

Jones, B.C. & Evenden, M.L. (2008) Ecological applications of pheromone trapping of *Malacosoma disstria* and *Choristoneura conflictana*. *Canadian Entomology*, 140, 573–581.

Kononchuk, A.G., Martemyanov, V.V., Ignatieva, A.N., Belousova, I.A., Inoue, M.N. & Tokarev, Y.S. (2021) Susceptibility of the gypsy moth *Lymantria dispar* (Lepidoptera: Erebidae) to *Nosema pyrausta* (Microsporidia: Nosematidae). *Insects*, 12, 1–10.

Kyei-Poku, G. & Sokolova, Y.Y. (2017) The microsporidium *Nosema disstriae* (Thomson 1959): fine structure and phylogenetic position within the *N. bombycis* clade. *Journal of Invertebrate Pathology*, 143, 90–103.

Lee, K.P., Simpson, S.J. & Wilson, K. (2008) Dietary protein-quality influences melanization and immune function in an insect. *Functional Ecology*, 22, 1052–1061.

MacDonald, Z.G., Snape, K.L., Roe, A.D. & Sperling, F.A.H. (2022) Host association, environment, and geography underlie genomic differentiation in a major forest pest. *Evolutionary Applications*, 15, 1749–1765.

Majerus, M.E. (1998) *Melanism: evolution in action*. Oxford: Oxford University Press.

Mason, C.J., Peiffer, M., Hoover, K. & Felton, G. (2023) Tomato chemical defenses intensify corn earworm (*Helicoverpa zea*) mortality from opportunistic bacterial pathogens. *Journal of Chemical Ecology*, 49, 313–324. Available from: https://doi.org/10.1007/s10886-023-01420-7

Matsuki, M. & MacLean, S.F. (1994) Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia*, 100, 141–152.

Mikkola, K. & Rantala, M.J. (2010) Immune defence, a possible nonvisual selective factor behind the industrial melanism of moths (Lepidoptera). *Biological Journal of the Linnean Society*, 99, 831–838.

Myers, J.H. & Cory, J.S. (2013) Population cycles in forest lepidoptera revisited. *Annual Review of Ecology, Evolution, and Systematics*, 44, 565–592.

Najar, A., Landhäusser, S.M., Whitehill, J.G.A., Bonello, P. & Erbilgin, N. (2014) Reserves accumulated in non-photosynthetic organs during the previous growing season drive plant defenses and growth in aspen in the subsequent growing season. *Journal of Chemical Ecology*, 40, 21–30.

Palo, R.T. (1984) Distribution of birch (*Betula* spp.). willow (*Salix* spp.) and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *Journal of Chemical Ecology*, 10, 499–520.

Parry, D. & Goyer, R.A. (2004) Variation in the suitability of host tree species for geographically discrete populations of forest tent caterpillar. *Environmental Entomology*, 33, 1477–1487.

Parry, D., Herms, D.A. & Mattson, W.J. (2003) Responses of an insect folivore and its parasitoids to multiyear experimental defoliation of aspen. *Ecology*, 84, 1768–1783.

Parry, D., Spence, J.R. & Volney, W.J.A. (1998) Budbreak phenology and natural enemies mediate survival of first-instar forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, 27, 1368–1374.

Popham, H.J.R. & Shelby, K.S. (2009) Ascorbic acid influence the development and immunocompetence of larval *Heliothis virescens*. *Entomologia Experimentalis et Applicata*, 133, 57–64.

Preti, F., Flaherty, L. & Evenden, M.L. (2023) Effects of larval host and natural microsporidian infection on adult life history traits of the forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, 52, 648–658. Available from: https://doi.org/10.1093/ee/nvad042

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, 11, 41–65.

Pristavko, V.P. & Dovzhenok, N.V. (1974) Ascorbic acid influence on larval blood cell number and susceptibility to bacterial and fungal infection in the codling moth, *Laspeyresia pomonella* (Lepidoptera: Tortricidae). *Journal of Invertebrate Pathology*, 24, 165–168.

R Core Team. (2022) *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

Robison, D.J. & Raffa, K.F. (1997) Effects of constitutive and inducible traits of hybrid poplars on forest tent caterpillar feeding and population ecology. *Forest Science*, 43, 252–267.

Roland, J. (1982) Melanism and diel activity of Alpine *Colias* (Lepidoptera: Pieridae). *Oecologia*, 53, 214–221.

Rosa, E. & Saastamoinen, M. (2020) Beyond thermal melanism: association of wing melanization with fitness and flight behaviour in a butterfly. *Animal Behaviour*, 167, 275–288.

Scalbert, A. (1991) Antimicrobial properties of tannins. *Phytochemistry*, 30, 3875–3883.

Shi, J., Chen, F. & Keena, M.A. (2015) Differences in wing morphometrics of *Lymantria dispar* (Lepidoptera: Erebidae) between populations that vary in female flight capability. *Annals of the Entomological Society of America*, 108, 528–535.

Shikano, I. (2017) Evolutionary ecology of multitrophic interactions between plants, insect herbivores and entomopathogens. *Journal of Chemical Ecology*, 43, 586–598.

Sipell, W.L. (1962) Outbreaks of the forest tent caterpillar, *Malacosma disstria* Hbn., a periodic defoliator of broad-leaved trees in ontario. *The Canadian Entomologist*, 4, 408–416.

Smirnoff, W.A. (1967) Effects of some plant juices on the ugly-nest caterpillar, *Archips cerasivoranus*, infected with microsporidia. *Journal of Invertebrate Pathology*, 9, 26–29.

Smith, J.D. & Goyer, R.A. (1986) Population fluctuations and causes of mortality for the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), on three different sites in southern louisiana. *Environmental Entomology*, 15, 1184–1188.

Sun, R., Hong, B., Reichelt, M., Luck, K., Mai, D.T., Jiang, X. et al. (2023) Metabolism of plant-derived toxins from its insect host increases the success of the entomopathogenic fungus *Beauveria bassiana*. *ISME Journal*, 17, 1693–1704. Available from: https://doi.org/10.1038/s41396-023-01480-3

Talloen, W., van Dyck, H. & Lens, L. (2004) The cost of melanization: butterfly wing coloration under environmental stress. *Evolution*, 58, 360–366.

Thomson, H.M. (1959) A microsporidian parasite of the forest tent caterpillar, *Malacosoma Disstria* Hbm. *Canadian Journal of Zoology*, 37, 217–221.

Thomson, H.M. (1960) *The possible control of a budworm infestation by a microsporidian disease*, Vol. 16. Ottawa, ON, Canada, Canada Department of Agriculture Bi-monthly Progress Report, p. 1.

van Frankenhuyzen, K. & Liu, Y. (2016) Infection by *Nosema fumiferanae* (Microsporidia: Nosematidae) and feeding on previous–year's foliage do not interact in affecting late instars of the spruce budworm (Lepidoptera: Tortricidae). *Canadian Entomology*, 148, 163–170.

van Hezewijk, B., Wertman, D., Stewart, D., Béliveau, C. & Cusson, M. (2018) Environmental and genetic influences on the dispersal propensity of spruce budworm (*Choristoneura fumiferana*). *Agricultural and Forest Entomology*, 20, 433–441.

Wilson, G.G. (1977) Effects of the microsporidia *Nosema disstriae* and *Pleistophora schubergi* on the survival of the forest tent caterpillar. *The Canadian Entomologist*, 109, 1021–1022. Available from: https://doi.org/10.4039/Ent1091021-7

Wilson, G.G. (1984) Pathogenicity of *Nosema disstriae*, *Pleistophora schubergi* and *Vairimorpha necatrix* (Microsporidia) to larvae of the forest tent caterpillar, *Malacosoma disstria*. *Zeitschrift für Parasitenkunde*, 70, 763–767.

Wilson, K., Cotter, S.C., Reeson, A.F. & Pell, J.K. (2001) Melanism and disease resistance in insects. *Ecology Letters*, 4, 637–649.

How to cite this article: Flaherty, L., Preti, F., Ishangulyyeva, G., Erbilgin, N., Whidden, T. & Evenden, M. (2024) Independent and interactive effects of diet and entomopathogenic microsporidia on an outbreaking forest insect defoliator. *Agricultural and Forest Entomology*, 1–14. Available from: https://doi.org/10.1111/afe.12616