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HIGHLIGHTED STUDENT RESEARCH

Intraguild predation decreases predator fitness with potentially varying effects on pathogen transmission in a herbivore host

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Abstract

Predators and pathogens often regulate the population dynamics of their prey or hosts. When species interact with both their predators and their pathogens, understanding each interaction in isolation may not capture the system's dynamics. For instance, predators can influence pathogen transmission via consumptive effects, such as feeding on infected prey, or non-consumptive effects, such as changing the prey's susceptibility to infection. A prey species' infection status can, in turn, influence predator's choice of prey and have negative fitness consequences for the predator. To test how intraguild predation (IGP), when predator and pathogen share the same prey/host, affects pathogen transmission, predator preference, and predator fitness, we conducted a series of experiments using a crop pest (*Pseudoplusia includens*), a generalist predator (*Podisus maculiventris*), and a generalist pathogen (*Autographa californica* multicapsid nuclear polyhedrovirus, AcMNPV). Using a field experiment, we quantified the effects of consumptive and non-consumptive predators on pathogen transmission. We found that a number of models provided similar fits to the data. These models included null models showing no effects of predation and models that included a predation effect. We also found that predators consumed infected prey more often when choosing between live infected or live healthy prey. Infected prey also reduced predator fitness. Developmental times of predators fed infected prey increased by 20% and longevity decreased by 45%, compared with those that consumed an equivalent number of non-infected prey. While this research shows an effect of the pathogen on intraguild predator fitness, we found no support that predators affected pathogen transmission.

Keywords Epizootics · Intraguild predation · Prey quality · Resource quality · Biocontrol

Introduction

In natural and agricultural communities, predators and pathogens can drive the short- and long-term dynamics of their prey (Krebs et al. 2001) or hosts (Smith et al. 2009), respectively. Yet, interactions between a species and its natural enemies do not occur in isolation. For example, intraguild predation (IGP), which involves multiple interactions between species, occurs when two predators share prey and at least one predator consumes the other (Holt and Polis 1997). In a unidirectional IGP community, two predators compete for a shared prey, and one of those predators (i.e., intraguild predator) also consumes the other predator (i.e., the intraguild prey) (Rosenheim et al. 1995; Holt and Polis 1997). Similarly, predators often inadvertently ingest parasites and pathogens of their prey during consumption (Cirtwill and Stouffer 2015). In some instances, consumption of infected prey will result in trophic transmission (i.e., infection of the predator) when the prey serves as a

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In support of being a highlighted student project, we provide a unique look at intraguild predation focusing on predators, prey, and prey pathogens. Predators preferred infected prey, infected prey reduced predator fitness. However, we found no support that predators changed pathogen transmission dynamics. Our combination of field and lab experiments to examine both sides of these interactions is unique in these communities and our results point to interesting scenarios playing out in natural and agricultural systems. AJF designed the experiments, conducted field and lab studies, and wrote the manuscript. TAC provided insects, advised in insect rearing and experimental use, and made substantial edits to the manuscript. BDE advised in designing the experiment, assisted with field work, and advised writing of the manuscript.

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secondary host (Lafferty 1992). However, many ingested pathogens do not infect predators when consumed. In this instance, the predator has consumed an intraguild prey (i.e., the pathogen) and the pathogen is removed from the community (Johnson et al. 2010). This removal potentially changes disease transmission dynamics (Finke 2012). From the predator's perspective, these interactions may also influence the predator's fitness (Flick et al. 2016). While questions concerning predator–prey/host-parasite interactions have been tackled from a theoretical standpoint (e.g., Borer et al. 2007; Hilker and Schmitz 2008; Bate and Hilker 2014), few studies have empirically examined how both predator health and parasite transmission dynamics change in the same community (but see Rohr et al. 2015).

From the perspective of the pathogen, consumptive effects of predators can change population densities of infected and healthy prey (Packer et al. 2003). Density-dependent disease transmission rates or epizootic frequency may thus decline due to reduced host availability (Packer et al. 2003; Strauss et al. 2016). Conversely, predators can also increase disease incidence via sloppy predation (Strauss et al. 2016). Sloppy predators help spread disease through messy eating or defecation (Cáceres et al. 2009). For instance, without a predator in the system, pathogens can be unevenly distributed in the environment, resulting in heterogeneous transmission (D'Amico et al. 2005). Specifically, patchy distributions of pathogens could cause heterogeneous rates of exposure among hosts. If a predator is a sloppy eater, it could homogenize exposure among hosts by spreading the pathogen more evenly across the environment. Regardless of whether the predator reduces or increases transmission when feeding on infected prey, the consumptive effects of predators on the intraguild/infected prey, or the prey itself, may play an important role in determining disease dynamics.

While the main focus of much IGP research is on the consumptive effects of the intraguild predator, non-consumptive effects that alter the behavior of the intraguild prey can also influence disease transmission. In general, non-consumptive effects of predators change prey development and behavior (Preisser et al. 2005; Orrock et al. 2008). For example, prey may decrease movement to avoid detection by predators (Thiemann and Wassersug 2000; Reed and Levine 2005), which increases exposure (Thiemann and Wassersug 2000). Decreased movement could also decrease the contact between healthy hosts and pathogens in the environment, which also decreases the exposure (Finke 2012). Examples of developmental changes include instances where individual *Daphnia dentifera* increased in size due to chemical cues from *Chaoborus* predators. Larger individuals also increased their feeding rates leading to greater exposure to a yeast parasite resulting in increased parasite loads (Duffy et al. 2011). However, the empirical results are equivocal as predators can increase (Ramirez and Snyder 2009; Duffy et al. 2011; McCauley et al. 2011) or decrease (Coors and De Meester 2011) the likelihood of pathogen infection in their prey. Regardless of the direction, non-consumptive effects potentially play an important role in changing transmission dynamics.

From the perspective of the predator, many pathogens and parasites decrease the energetic value of the prey (Thieltges et al. 2013), which reduces predator survival and reproduction (Flick et al. 2016). For example, the predator *Podisus nigrispinus* was unable to survive more than three generations, when consuming only virus-infected *Anticarsia gemmatalis* (de Nardo et al. 2001). However, predators may prefer infected prey if they are easier to capture (Lafferty1992; Thomas et al.2005). Thus, in IGP systems, predators may affect disease transmission dynamics and prey infection status may affect predator fitness.

Using a tractable IGP system, consisting of an intraguild predator, an intraguild prey/pathogen, and a prey/host, we fit a series of disease transmission models to test if the presence of an intraguild predator, via consumptive and non-consumptive means, altered disease transmission dynamics. We also examined how changes in prey quality, via pathogen infection, affected predator preference and fitness. By assessing the influence of pathogen-infected prey on predators and predator influence on disease spread, the net effects of IGP community dynamics may emerge.

Materials and methods

Study system

We conducted a series of laboratory experiments to test how virus-infected larvae influenced predator fitness proxies and preference. We also conducted field experiments that tested consumptive and non-consumptive effects of predators on virus transmission. The system consisted of a single prey/host species, the soybean looper (*Pseudoplusia includens* Walker, Lepidoptera: Noctuidae), that can be consumed by a generalist predator, the spined soldier bug (*Podisus maculiventris* Say, Heteroptera: Pentatomidae), and infected by a lethal baculovirus, *Autographa californica* multicapsid nuclear polyhedrovirus (AcMNPV). The soybean looper is a widespread polyphagous multivoltine pest in soybean fields throughout North and South America (Herzog 1980; Smith et al. 1994; Bernardi et al. 2012). The spined soldier bug is a common predatory stink bug, with a distribution from Mexico to Canada, that feeds on crop pests including the soybean looper (O'Neil 1995; Yang 2000). AcMNPV, which consists of multiple copies of a double-stranded DNA virus within a protein coat or occlusion body, can infect a relatively large number of lepidopteran species during the larval stage (Goodman et al. 2001). Using this simplified

food web, we quantified how IGP interactions affect both the intraguild predator and the pathogen.

In this system, pathogen transmission occurs when the host, the soybean looper, consumes a lethal dose of virus. At the beginning of a disease outbreak, first-instar larvae hatch and a subset become infected by consuming contaminated leaf tissue; the leaf tissue can become inoculated via virus particles residing in the soil (Young and Yearian 1986). Once infected, the virus halts the host's growth and begins within-host replication as uninfected larvae continue to grow. In the final stages of the infection, the host liquefies and occlusion bodies spill out of the cadaver onto nearby leaves. Uninfected larvae, which have reached the third or fourth instar, become infected by consuming the newly contaminated leaf tissue (Elderd 2013).

We obtained the prey species—or the host in the system—soybean loopers, as eggs from Benzon Research Inc (Carlisle, PA, USA). We reared them on artificial diet (Southland Products Inc., Lake Village, AR, USA) at 27 °C and 16L:8D in individual one-ounce cups until they reached the appropriate instar. AcMNPV virus was amplified in larval hosts using previously collected virus and extracted in the lab. The spined soldier bug eggs came from a lab colony, maintained on *Trichoplusia ni* Hübner, (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* Smith, (Lepidoptera: Noctuidae) as described in Wittmeyer et al. 2001. For field experiments, we grew soybeans, variety Gasoy 17 (USDA-GRIN), a common food source for the soybean looper (e.g., Beach and Todd 1988).

Laboratory studies

The laboratory experiments examined predator preference and the fitness consequences of consuming infected prey. To infect larvae, recently molted fourth-instar soybean loopers were starved for 24 h, then fed a small cube of artificial diet with a 3 µl droplet of water containing 10^5 AcMNPV occlusion bodies. This dosage represents roughly 2500 times the lethal dose at which 50% (LD50) of fourth-instar larvae succumb to the virus (Kunimi et al. 1997). To ensure infection, larvae were used in experiments only if they consumed the entire diet cube and, thus, the full dose of virus.

Predator preference

We tested predator preference for infected versus healthy prey using two different experiments, one with living and one with dead soybean larvae. For the live prey experiment, we reared infected fourth-instar soybean loopers for 72 h post-infection. After running preliminary tests to ensure that spined soldier bugs do not exhibit a preference for or against dusted larvae of any color (Flick, unpublished data), we dusted larvae with alternating colors of ultraviolet fluorescent powder to differentiate between larvae. We then placed one healthy and one infected live larva in a Petri dish (4.5 cm radius) with moistened filter paper, to maintain humidity levels. After allowing the larvae to acclimatize for one hour, we placed one soldier bug in the dish. We waited until the soldier bug continuously fed for ten minutes on one larva and recorded that larva as the soldier bug's preference. Preliminary experiments showed that a soldier bug will often probe a prey item for consumption. After feeding continuously for 10 min, the soldier bug rarely stopped a feeding bout, which often lasted an hour (Flick, personal observation). Soldier bugs that did not consume a larva within 24 h were omitted from the analyses. Of the 60 replicates, 39 spined soldier bugs consumed a larva within 24 h.

Differences in predator preference for live larvae may be driven by changes in the behavior of infected larvae, as compared to healthy larvae (e.g., prey aggression toward the predator). To differentiate between changes in prey behavior versus other predator preferences, we conducted a second experiment using dead infected and dead non-infected larvae. We infected fourth-instar larvae, then waited 24, 48, 72, 96, 120, 144, or 168 h before freezing the infected larvae, to create cadavers of increasing viral loads. We found no differences in preference among groups with different viral loads ($F_{6,97}=0.64$, $P=0.70$), thus we pooled those data. We placed one non-infected defrosted larva and one infected defrosted larva in a Petri dish and added an adult spined soldier bug (Online Appendix, Fig. A1). When a soldier bug continuously fed for ten minutes on one larva, we recorded that larva as the soldier bug's preference. Soldier bugs that did not consume a larva within 24 h were omitted from the analysis. Of the 140 replicates, 104 soldier bugs consumed a larva within 24 h.

Predator fitness

To quantify if predator fitness is affected by prey quality, soldier bugs were exclusively fed frozen non-infected or infected prey from hatching until death. We used two fitness metrics in our analyses—longevity and developmental time. Longevity was calculated as the time from adulthood to death and development was calculated as the time from first instar to adulthood. For females, we also recorded the number of eggs laid as a measure of fecundity. Spined soldier bugs were given one cadaver every other day, which is an adequate resource level to prevent starvation (Flick, unpublished data).

Disease transmission in the field

To quantify the effects of IGP on pathogen transmission, we manipulated virus density and the presence of a consumptive or non-consumptive adult spined soldier bug. We used

a fully factorial, randomized block study design. Each of the five blocks consisted of 12 soybean plants (60 plants total) one meter apart and individually bagged with insect-resistant mesh. The mesh prevents larvae from escaping the treatment and degradation of virus by UV light (Elderd et al. 2013). Each soybean plant was similar in size (approximately five trifoliate leaves) and had one of four virus (i.e., cadaver) densities (0, 15, 60, or 75 infected, first-instar larvae).

We infected newly-hatched, first-instar soybean loopers with a lethal dose of virus (10^5) in the lab. After three days, we placed them into the mesh bag and allowed them to freely move about the plant before they died of the viral infection. This ensured that the virus would be distributed more naturally across the plant's leaf tissue, as compared to spraying virus directly on plants (Elderd and Reilly 2014).

Each plant also received one of three-spined soldier bug treatments: consumptive predator, non-consumptive predator, and no predator. To test the non-consumptive effects of IGP on disease transmission, we snipped off the mouth parts before releasing the soldier bug into non-consumptive predator treatments. Surgically altering soldier bugs, so that they will hunt but not eat, has been shown to be an effective means for inducing prey behavioral responses, without significantly altering predator behavior (Thaler et al. 2012; Hermann and Thaler 2014). For the consumptive and non-consumptive predator treatments, one soldier bug was added to the plant.

After 4 days, during which the infected first-instar larvae died, we released 30 healthy, fourth-instar larvae on each plant along with the appropriate predator given the treatment. Soybean loopers fed for 4 days, after which we collected the soldier bugs and surviving soybean loopers. The soybean loopers were placed into individual one-ounce cups with artificial diet and monitored until death or pupation. Since infected individuals liquefy upon death, baculovirus infections were easily diagnosed. If any doubt as to the cause of death, the presence of occlusion bodies in the hemolymph, when viewed under a light microscope, confirmed baculovirus infection (Elderd et al. 2013). To ensure an adequate sample size to measure transmission, plots were included only if more than five of the original 30 soybean loopers survived the duration of the experiment. This resulted in two replicates of the consumptive predator treatment being excluded.

Data analysis

For the predator preference data, we used a Chi-square goodness of fit test for binomial distributions. We predicted that soldier bug fitness would decrease following previous findings (Flick et al. 2016). Thus, we analyzed fecundity, longevity, and developmental time data using Welch two sample, one-tailed *t* tests.

To understand the transmission process in communities with pathogens, ecologists have long relied on the Susceptible-Infected-Recovered or SIR model, which quantifies infection dynamics during a disease outbreak (e.g., Anderson and May 1980; Borer et al. 2007; Roy and Holt 2008). For our analysis, we used two transmission equations (Dwyer et al. 1997) that assumed either individuals do not vary in their susceptibility to the virus (i.e., all individuals are the same) or individuals vary (i.e., individuals are heterogenous). If we assume that all larvae are equally susceptible to the pathogen, the change in susceptible individuals over time, dS/dt, is governed by the equation, $dS/dt = -\beta SV$. Here, V, is the amount of virus or, in this experiment, the number of first-instar cadavers. β is the transmission rate. The above equation can be integrated from the start of the experiment at time 0 to the end of the experiment at time W, which is four days for our study.

The integrated equation from 0 to W takes the form:

$$-\ln[1-\psi] = \beta V(0)W, \tag{1}$$

where ψ is the infection prevalence, $V(0)$ is the cadaver density at the start of the experiment (i.e., time 0), and W, is the duration of the experiment. Thus, $1-\psi$ is the proportion of susceptible individuals that did not become infected. In this linear equation, the transmission is dictated by the associated slope of the line, which is the transmission rate, β (Elderd 2018).

Disease dynamics can be decidedly non-linear (Hochberg 1991; Koelle and Pascual 2004). If individuals vary in their susceptibility to the virus, such that some individual larvae are more or less susceptible to infection, disease transmission becomes non-linear (Dwyer et al. 1997). The equation associated with the non-linear dynamics takes the following form: $dS/dt = -\overline{\beta}\left[S(t)/S(0)\right]^{C^2}SV$, where $\overline{\beta}$ is the mean transmission rate and C is the coefficient of variation of the mean transmission rate. $\left[S(t)/S(0)\right]^{C^2}$ scales the mean transmission rate such that transmission declines as the number of susceptible individuals decline. When integrated from time 0 to time W, the equation becomes:

$$-\ln[1-\psi] = \frac{1}{C^2}\ln\left(1+\overline{\beta}C^2V(0)W\right). \tag{2}$$

For Eq. 2, the transmission rate is no longer a single value like in Eq. 1 but now has an associated distribution around the mean transmission rate. As the coefficient of variation goes to zero, the non-linear equation's dynamics behave in a similar manner to the linear equation (Dwyer et al. 1997). When transmission dynamics are non-linear, adding an intraguild predator to a host–pathogen system potentially affects transmission dynamics in two ways—by either changing the mean transmission rate or its coefficient of variation, C.

Each of the treatments—consumptive predator, non-consumptive predator, and no predator—were fit to the linear (Eq. 1) and the non-linear (Eq. 2) equations. To examine whether consumptive effects alone affected transmission, we compared the consumptive predator treatment to the pooled data from the non-consumptive and no predator treatment. Non-consumptive effects may also be important in determining transmission. To quantify these effects, we tested the pooled data from the consumptive and non-consumptive predator treatments against the no predator treatment. Additionally, the null models, consisting of all the data pooled, were also fit to either the linear or non-linear equations, which assume no effect of predation on disease transmission. Given binary data (infected or healthy individuals), we used a binomial error distribution (McCullagh and Nelder 1989) to calculate the log-likelihood of the data. Akaike Information Criteria, which was corrected for small sample sizes (AICc), along with ΔAICc, AICc weights, and evidence ratios (Table 1) between models were used to compare across models (Burnham and Anderson 2003). We also calculated the variance inflation factor for the full model (Model 17 in Table 1) to check for overdispersion since binomial count data may be prone to overdispersion (Richards 2008). Given that the factor was close to 1, we did not correct the AICc scores for overdispersion (Burnham and Anderson 2003). To estimate the confidence intervals for the parameters associated with the linear and the non-linear equations (Eqs. 1 and 2, respectively) given the experimental treatments, we used *mle2* in the *bbmle* package (Bolker & R Development Core Team 2017). The confidence intervals are

Table 1 The eighteen models considered to assess whether intraguild predation via consumptive and non-consumptive interactions affects pathogen transmission

	Model	*k*	AICc	ΔAICc	AICc wt	Evidence ratio
1	No treatment effect (nonlinear)	2	105.77	0.00	0.24	1.00
2	Consumptive predator (linear), Non-consumptive/no (nonlinear)	3	106.32	0.55	0.18	1.32
3	No treatment effect (linear)	1	107.79	2.02	0.09	2.75
4	Consumptive predator (linear) Non-consumptive (nonlinear), no (linear)	4	108.04	2.27	0.08	3.11
5	Consumptive predator/non-consumptive (linear), no (linear)	2	108.65	2.88	0.06	4.22
6	Consumptive predator/non-consumptive (nonlinear), no (linear)	3	108.75	2.98	0.05	4.44
7	Consumptive predator (nonlinear), non-consumptive/no (nonlinear)	4	108.94	3.17	0.05	4.88
8	Consumptive predator (linear) Non-consumptive (nonlinear), no (nonlinear)	5	109.25	3.48	0.04	5.70
9	Consumptive predator (linear), non-consumptive (linear), No (linear)	3	109.34	3.57	0.04	5.96
10	Consumptive predator/non-consumptive (linear), no (nonlinear)	3	109.48	3.71	0.04	6.39
11	Consumptive predator (linear), non-consumptive/No (linear)	2	109.52	3.75	0.04	6.52
12	Consumptive predator/non-consumptive (nonlinear), no (nonlinear)	4	109.75	3.98	0.03	7.32
13	Consumptive predator (linear) Non-consumptive (linear), no (nonlinear)	4	110.34	4.57	0.02	9.83
14	Consumptive predator (nonlinear) Non-consumptive (nonlinear), no (linear)	5	110.87	5.10	0.02	12.81
15	Consumptive predator (nonlinear) Non-consumptive (linear), no (linear)	4	111.96	6.19	0.01	22.09
16	Consumptive predator (nonlinear), Non-consumptive/no (linear)	3	111.97	6.20	0.01	22.20
17	Consumptive predator (nonlinear) Non-consumptive (nonlinear), no (nonlinear)	6	112.30	6.53	0.01	26.18
18	Consumptive predator (nonlinear) Non-consumptive (linear), no (nonlinear)	5	113.17	7.40	0.01	40.45

The data collected were tested by fitting the linear (Eq. 1) or the non-linear heterogeneous (Eq. 2) equation to individual treatments or groups of treatments. The first column (Model) indicates the model tested, the second column (*k*) indicates the number of parameters, the third column (AICc) is the Akaike Information Criterion corrected for small sample sizes, the fourth column (ΔAICc) indicates the difference from the model and the highest-ranked model, and the penultimate column (AICc wt) is the weight of evidence for that model. The final column is the evidence ratio or relative likelihood of each model as compared to the model with the lowest AICc score or the top-ranked model (Burnham and Anderson 2003). For example, the top-ranked model is 1.32 times more likely than the model with the second-lowest AICc score. The model with the lowest AICc score is in bold. "No" refers to the no predator treatment. Treatments pooled for the analysis are denoted by a slash ("/")

based on the variance associated with the parameter fit for the linear equation or the variance–covariance matrix for the non-linear equation (Bolker 2008). All data were analyzed using R software version 3.4.3 (R Core Team 2013).

Results

Laboratory studies

Predator preference

Predator feeding preference differed depending upon whether or not the prey were alive. Soldier bugs chose live, infected soybean loopers twice as often as live, healthy soybean loopers (26 chose infected, 13 chose healthy, $\chi^2 = 4.33$, $P = 0.037$, $n = 39$), whereas they had no preference for infected or non-infected dead prey (55 chose infected, 49 chose non-infected, $\chi^2 = 0.35$, $P = 0.56$, $n = 104$).

Predator fitness

Metrics associated with predator fitness decreased when the predator fed on infected prey as compared to healthy prey (Fig. 1). Spined soldier bug longevity decreased by 45% when fed infected soybean loopers, as compared to healthy soybean loopers ($t_{10.5} = -3.06$, $P = 0.006$, Fig. 1a). Developmental times were 20% longer for predators reared on infected prey, when compared to healthy prey ($t_{79.9} = -1.78$, $P = 0.039$, Fig. 1b). Fecundity trended lower when females were fed infected prey, but the difference was not significant ($t_{5.4} = -1.43$, $P = 0.10$, Fig. 1c).

Disease transmission in the field

For the transmission experiments, we found essentially equal support among the top 12 ranked models (Table 1, ΔAICc < 4) and, therefore, cannot confidently draw biological conclusions with regards to the effect of predators on transmission. The null model with no differences between the consumptive predator, non-consumptive predator, and no predator treatments, which assumed host heterogeneity (Eq. 2), ranked the highest (24% of the support, Table 1, Fig. 2a). For the model ranked second with ΔAICc = 0.55 and 18% of the support (Table 1, Model 2), the addition of a non-consumptive predator had no effect on transmission since the model grouped the non-consumptive predator treatment with the no predator treatment (Fig. 2b). However, the addition of a consumptive predator changes transmission dynamics from non-linear to linear (Eqs. 2 to 1, Fig. 2c). The third-ranked model included no predator effect and assumed individuals do not vary in their susceptibility (Fig. 2d, 9% of the support). While infected prey affects predator fitness, predators appear to have little effect on disease transmission. If predators do, it comes from changes in the CV of the transmission rate due to consumptive predators (Table 2, Fig. 2b vs. c).

Overall, given the relatively similar AICc scores for the higher-ranked models, our results were inconclusive with regard to a predator effect on disease transmission. While the null model with heterogenous transmission was ranked the highest, the model ranked second should also be considered given the relatively similar AICc scores (Table 1). In this model, the consumptive predator treatment is best described by the linear equation, which assumes all individuals are alike. This is further supported by examining the fit of these data to the non-linear equation, which assumes individuals

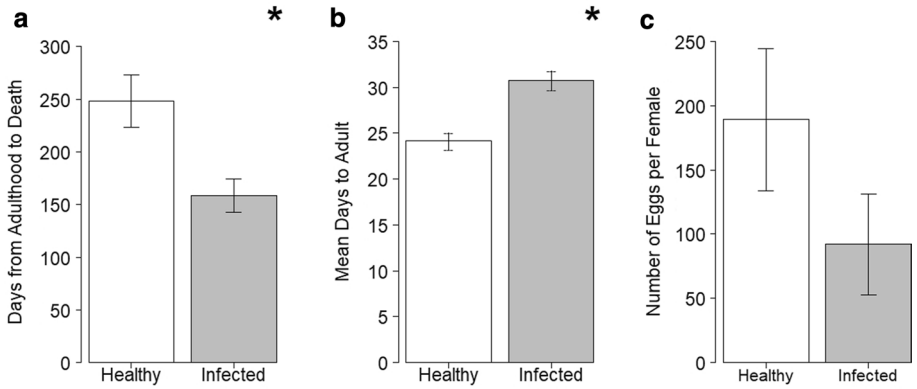

Fig. 1 The effects of consuming AcMNPV infected soybean loopers compared to healthy soybean loopers on longevity, development, and fecundity of spined soldier bugs. Soldier bugs reared on healthy prey lived longer ($P = 0.006$), **a**), and developed quicker ($P = 0.034$), **b**). The difference between soldier bugs reared on healthy prey and those reared on infected prey was not significant for fecundity ($P = 0.10$), **c**). Error bars represent standard errors. Note that statistically significant ($P < 0.05$) differences between treatments are marked by an asterisk (*) in the upper right corner of the graph

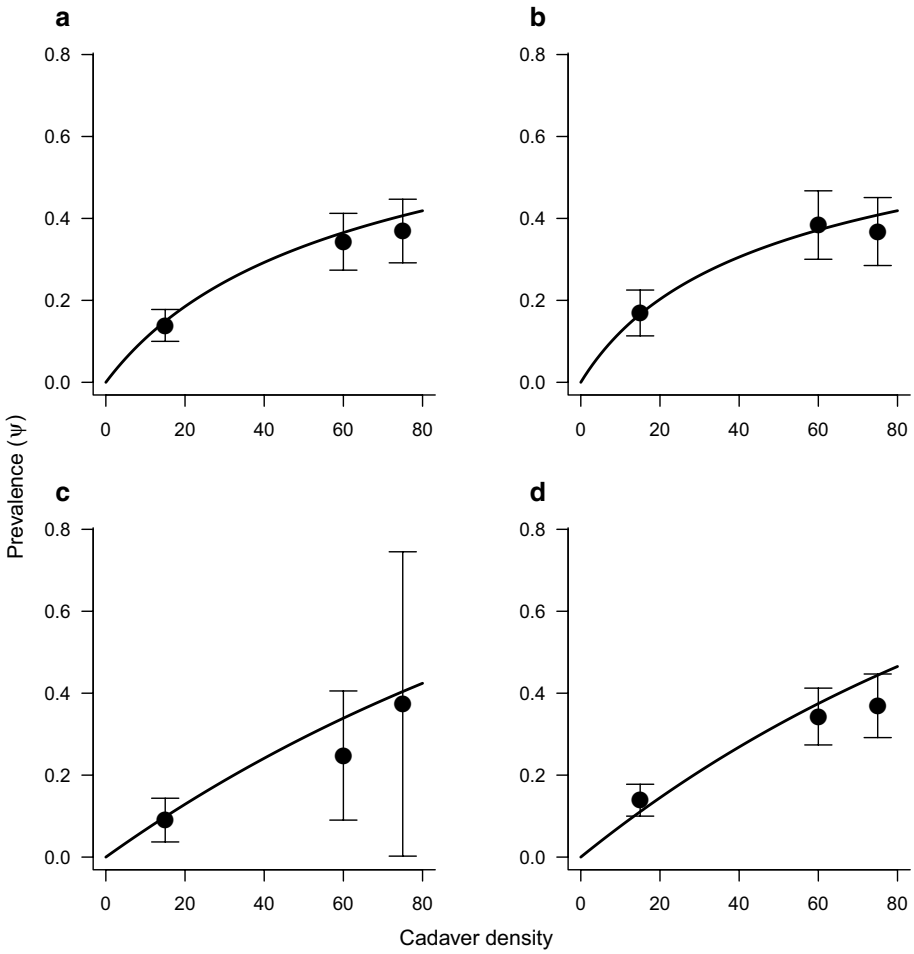

Fig. 2 The top three ranked models of disease transmission of soybean loopers in field experiments as a function of infection prevalence (ψ in Eqs. 1 and 2) and cadaver density. **a** Null model with no predator effects assumes host heterogeneity using the non-linear heterogenous equation (Eq. 2). **b** Non-consumptive predators and no predators were grouped and fit best by the heterogeneous non-linear transmission equation while **c** consumptive predators are best represented by the linear transmission equation (Eq. 1). At high virus densities, consumptive predators increase disease transmission as compared to **b**. **d** Null model with no predator effect that assumes no host heterogeneity. The lines represent model estimates, the closed circles indicate means, and error bars represent standard errors

Table 2 Best-estimates (90% confidence intervals) of the transmission rates for the linear (Eq. 1) and non-linear heterogeneous equations (Eq. 2) and best-estimate of the Coefficient of Variation (90% confidence intervals) for the heterogeneous equation for the individual or combined treatments

	Linear model[a] Transmission rate, β	Heterogeneous model[a] Transmission rate, $\overline{\beta}$	Heterogeneous model Coefficient of variation, C
Null	1.96 (1.78, 2.14)	3.21 (2.37, 4.60)	1.47 (0.97, 1.91)
Consumptive predator	1.72 (1.37, 2.13)	1.73 (1.37, 2.15)	0.13 (NA, NA)[b]
Non-consumptive predator	2.31 (2.01, 2.63)	4.96 (3.00, 10.42)	1.67 (1.03, 2.34)
No predator	1.75 (1.51, 2.02)	2.93 (1.76, 5.78)	1.54 (0.43, 2.31)
Consumptive predator and non-consumptive predator	2.01 (1.82, 2.21)	3.93 (2.72, 6.33)	1.66 (1.15, 2.17)
Non-consumptive predator and no predator	2.11 (1.88, 2.37)	3.30 (2.29, 5.15)	1.36 (0.64, 1.93)

[a]Note that estimates of β and $\overline{\beta}$ are raised to the 10^{-3}

[b]Confidence Interval estimates of the coefficient of variation for the consumptive predator fit to the heterogenous transmission equation (Eq. 2) could not be calculated using *bbmle* (Bolker and R Development Core Team 2017) due to the relatively flat likelihood surface (Online Appendix, Fig. A3).

differ in their susceptibility. The estimate of the CV for the non-linear equation is extremely low for consumptive predators (Table 2), which results in transmission dynamics that are similar for the non-linear and linear equations (Online Appendix, Fig. A2). While there is enough curvature in the likelihood surface to find the local minima for the non-linear equation (Eq. 2) fit to the consumptive predator treatment, the likelihood surface is relatively flat (Online Appendix, Fig. A3a), which makes it difficult to calculate the confidence intervals. For the non-linear equation that combines the non-consumptive and no predator treatments (Online Appendix, Fig. A3b), confidence interval estimates were

obtained. In terms of disease dynamics, linear transmission with consumptive predators results in a larger number of infected hosts at higher cadaver density, when compared to non-linear transmission (Fig. 2b vs. c).

Given their ΔAICc scores, a number of other models had support as well (Burhnam and Anderson 2003). Subsequent models (Table 1, Models 3–12) vary in their support in terms of whether the predator treatments had an impact on transmission dynamics. In total, the null models had 33% support as compared to 67% support for models containing a predator effect. In general, these models group the treatments in a variety of different ways and part of the similarity in AICc scores between these models may stem from the similar values for the transmission rate and CV across treatments (Table 2).

Discussion

Our results show that in an IGP system a pathogen can influence a predator's fitness (Fig. 1) but the predator may or may not influence pathogen transmission in the host (Table 1, Fig. 2). Spined soldier bugs preferred infected live prey to healthy live prey (when both are alive), which can have negative fitness consequences (Fig. 1). For the pathogen, the addition of a predator did not clearly affect transmission dynamics given the wide variety of support for models with no predator effects and for models with predator effects (e.g., Fig. 2a vs. b, c). Since there were a number of models that also had some support with ΔAICc values less than four (Burnham and Anderson 2003), we cannot safely conclude that predators had an effect or no effect.

While other transmission models were supported by the data (Table 1, Models 3–12), insight can be gained by examining the differences between the two top-ranked models. The top-ranked model had 24% of the support but only 1.32 times more support than the model ranked second. In the top-ranked model, predator addition had no effect. In the model ranked second with 18% support, consumptive predators are fit with the linear equation (Eq. 1) and the grouped data from non-consumptive predators and no predators are fit with the non-linear equation (Eq. 2). This may result from sloppy predation of infected larvae. Since virus and occlusion body production begins within 16 h post-infection (Granados and Lawler 1981), fourth-instar larvae in high virus treatments are likely infected when consumed. If virus density is high, predators are more likely to consume infected prey and spread the virus when the predator moves the prey while feeding, which soldier bugs do (Flick, personal observation and Online Appendix, Fig. A1). Thus, the predator could be taking a heterogeneous virus-contaminated environment and transforming it into a homogenous environment where larvae have a high likelihood of consuming virus wherever they feed. This would result in the linear equation, which assumes no differences in susceptibility or infection risk, better fitting the consumptive predator data than the non-consumptive and no predator data. The above explanation should be taken with the caveat that the null model, which assumes host heterogeneity (Eq. 2), is the highest-ranked model.

The similarity between the highly ranked models' AICc scores may be due to the high degree of variability in consumptive predator transmission for the high cadaver treatments (Fig. 2c). To determine if predators change transmission dynamics and, if they do, what is driving the change, a different experimental approach along with an increase in sample size may be needed. When fitting non-linear equations to data, a regression-based approach with few replicates but more density treatments performs better when fitting simulated data (Inouye 2001; Gotelli and Ellison 2004). Thus, a regression-based approach with an increase in the number of different virus levels may help elucidate the means by which predators affect transmission dynamics.

From a predator perspective, we found that spined soldier bugs had reduced longevity and increased developmental time when consuming infected soybean loopers compared to healthy soybean loopers. Since virus particles are not digested by the predators, infection could reduce the resource quality of the prey. However, our results differed from some past studies. Lee and Fuxa (2000) found that soldier bugs reared on infected caterpillars had similar survival to those reared on healthy caterpillars, while Abbas and Boucias (1984) found that consuming infected prey did not significantly reduce soldier bug developmental times. The above may arise from differences in the experimental design. When soldier bugs are fed ad libitum (e.g., Abbas and Boucias 1984; Lee and Fuxa 2000), differences in nutritional value may be overwhelmed by increased feeding on infected prey. For instance, soldier bugs fed for shorter periods of time on infected prey compared to healthy prey (Abbas and Boucias 1984) and consumed significantly more infected prey (Bell et al 2004). However, other studies that did not take an ad libitum approach like ours found similar results to ours (de Nardo et al. 2001; Down et al. 2004). This general pattern—ad libitum feeding resulting in no fitness differences, while more realistic scenarios show a decrease in fitness—agrees with the conclusions drawn from a meta-analysis examining predator fitness (Flick et al. 2016). However, during epizootics, when pest populations are relatively high, there may be enough prey to counteract the negative effects of consuming infected prey (something close to ad libitum feeding).

We also found that spined soldier bugs exhibited a preference for infected prey when the soybean loopers were alive. Since we found no differences in preference when soybean loopers, healthy or infected, were dead, this suggests that

live infected prey have some behavioral trait that make them more appealing than live healthy prey. Baculovirus infections can influence host behavior, such as increasing the propensity to climb (Katsuma et al. 2012; van Houte et al. 2012), which may cause prey to move to exposed areas that are more open to predation. However, generalist predators across numerous studies do not exhibit a consistent preference for infected or healthy prey. Instead, there was a great deal of variability in the mean effect size for predator choice with some studies showing a preference for infected prey and others showing avoidance (see Fig. 3 of Flick et al. 2016). Differences in aggressiveness toward the predator may result in different effects of virus infection on prey selection. For example, some prey are relatively aggressive (Marston 1978; Silva et al. 2012), and pathogen infections can increase sluggishness thus decreasing prey aggressiveness.

In IGP systems, the quality or productivity level of the resource can determine whether the system collapses or is maintained. For example, if the resource is of low productivity, the theory predicts that the intraguild predator will exclude the intraguild prey (Polis et al. 1989). In a predator, pathogen, and prey community, low host densities are likely to drive pathogens extinct as would potentially be the case in our low recovery plots with a consumptive predator. When the resource is highly productive, like a crop pest, the intraguild prey, the pathogen, may outcompete the intraguild predator (Polis et al. 1989). When infected prey negatively influence predator fitness, and the pathogen is ubiquitous, predators may be excluded from the community (e.g., de Nardo et al. 2001) unless there are sufficient resources. The above is based on the theoretical results of the long-term equilibrial dynamics of the system. While we only examined the short-term dynamics, our results help to support these long-term predictions.

Intraguild predation is common in nature (Polis et al. 1989; Arim and Marquet 2004) and increasing evidence points to the need to invoke IGP in systems in which pathogens play a major role (Thomas et al. 2006; Borer et al. 2007; Cáceres et al. 2009; Rohr et al. 2015). While we focused on the short-term dynamics, the long-term dynamics remain to be tested empirically, though theoretical perspectives can help to guide the way. In disease-driven systems, as well as systems in which IGP occurs between two predators, the importance of non-consumptive effects appear to be gaining attention and traction (Raffel et al. 2010; Rohr et al. 2015). However, in our system, we could not definitively conclude that predators, either due to consumptive or non-consumptive means, affected disease transmission.

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Author contribution statement AJF designed the experiments, conducted field and lab studies, and wrote the manuscript. TAC provided insects, advised in insect rearing and experimental use, and made substantial edits to the manuscript. BDE advised in designing the experiment, assisted with field work, and advised writing of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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