

Evolutionary Ecology of Multitrophic Interactions between Plants, Insect Herbivores and Entomopathogens

Ikkei Shikano¹

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Abstract Plants play an important role in the interactions between insect herbivores and their pathogens. Since the seminal review by Cory and Hoover (2006) on plant-mediated effects on insect–pathogen interactions, considerable progress has been made in understanding the complexity of these tritrophic interactions. Increasing interest in the areas of nutritional and ecological immunology over the last decade have revealed that plant primary and secondary metabolites can influence the outcomes of insect–pathogen interactions by altering insect immune functioning and physical barriers to pathogen entry. Some insects use plant secondary chemicals and nutrients to prevent infections (prophylactic medication) and medicate to limit the severity of infections (therapeutic medication). Recent findings suggest that there may be selectable plant traits that enhance entomopathogen efficacy, suggesting that entomopathogens could potentially impose selection pressure on plant traits that improve both pathogen and plant fitness. Moreover, plants in nature are inhabited by diverse communities of microbes, in addition to entomopathogens, some of which can trigger immune responses in insect herbivores. Plants are also shared by numerous other herbivorous arthropods with different modes of feeding that can trigger different defensive responses in plants. Some insect symbionts and gut microbes can degrade ingested defensive phytochemicals and be orally secreted onto wounded plant tissue during herbivory to alter plant defenses. Since non-entomopathogenic microbes and other arthropods are likely to influence the outcomes of plant–

insect–entomopathogen interactions, I discuss a need to consider these multitrophic interactions within the greater web of species interactions.

Keywords Biological control · Host–parasite interactions · Immune response · Microbiota · Nutrition · Tritrophic interactions

Introduction

Plants and insect herbivores harbor a variety of beneficial and pathogenic microbes. Within this multitrophic context, numerous complex interactions have been identified, including the alteration of plant defenses by plant and insect associated mutualistic symbionts and phytopathogens that can influence the performance of insect herbivores (Biere and Bennett 2013; Biere and Tack 2013; Shikano et al. 2017b). Much of the research on insect pathogens (entomopathogens) has focused on their development as biological control agents (Lacey et al. 2015). In the last decade, the use of microbial pesticides in the global market has grown considerably. This is mostly attributable to the European Union, where a decline in the use of conventional broad-spectrum chemical insecticides has coincided with an increase in the organic sector and a more favorable regulatory environment for companies to commercialize microbial pesticides (Lacey et al. 2015). Thus, it has become increasingly important to determine the extent to which the effectiveness of microbial pesticides will vary on different crop species, cultivars and varieties. Stimulated by the seminal review paper by Cory and Hoover (2006), there has concurrently been a growing number of studies that have focused on plant–insect–entomopathogen interactions from ecological and evolutionary perspectives.

✉ Ikkei Shikano
ius15@psu.edu

¹ Department of Entomology and Center for Chemical Ecology, Pennsylvania State University, University Park, PA 16802, USA

Plants are the main drivers of the interactions with their insect herbivores and the insect's pathogens. At the time of the Cory and Hoover (2006) review, research on these tritrophic interactions were mainly focused on the direct effects of toxic phytochemicals on entomopathogen persistence and infectivity (Fig. 1a) and on the growth rate of insect herbivores, which can influence their developmental (or age-related) resistance to entomopathogens (Fig. 1b). Since their review, there has been substantial strengthening in the areas of nutritional and ecological immunology. A surge in these research areas have revealed that host plants and dietary nutrients can influence insect immune functioning (Fig. 1b) (Lampert 2012; Ponton et al. 2011). Interestingly, some insects have demonstrated the ability to acquire certain phytochemicals and nutrients as a prophylactic or therapeutic response to entomopathogen risk or infection (Abbott 2014; De Roode et al. 2013). Additionally, an important factor that received little attention in plant–insect–entomopathogen interactions a decade ago was that the surface of plants and the guts of insects are teeming with diverse communities of non-entomopathogenic microbes (Engel and Moran 2013; Vorholt 2012; Whipps et al. 2008). Recent studies indicate that some of these microbes may modulate the tritrophic interactions by altering plant and insect immune responses (Biere and Bennett 2013; Biere and Tack 2013; Shikano et al. 2017b), suggesting that our understanding of plant–insect–entomopathogen interactions determined under sterile laboratory conditions may not necessarily extrapolate

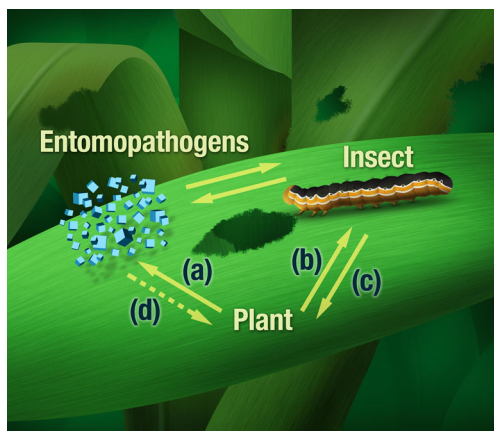


Fig. 1 The two-way interactions between insect herbivores and entomopathogens are strongly influenced by bottom-up plant effects. **a** Plant defensive chemicals can affect the persistence and infectivity of entomopathogens on plants. **b** Plant primary and secondary metabolites can influence the effectiveness of the insect's immune system and physical barriers against entomopathogens. Some insects can acquire certain plant metabolites that prevent or limit the severity of infection. **c** Feeding by insect herbivores induces the production and release of plant defensive chemicals that can influence entomopathogen infectivity and insect susceptibility to entomopathogens. **d** Entomopathogens might potentially mitigate negative plant effects by influencing the evolution of plant defenses. Illustration by Nick Sloff, Pennsylvania State University, University Park, PA, USA

to the field. Lastly, a key proposal made by Cory and Hoover (2006) was that plants may manipulate entomopathogens for their own benefit. However, empirical evidence for this is still lacking. Instead, I suggest that entomopathogens may impose selection pressure on plant traits that benefit both plant and entomopathogen fitness (Fig. 1d). Here, I review what has been learned in the last decade, with most of the focus on plant and insect interactions with three pathogen groups: bacteria, fungi and viruses. There are numerous mechanisms by which plants influence insect–entomopathogen interactions, but the generalities and context dependence of these mechanisms are still largely unknown.

Food-mediated variations in pathogen virulence and host susceptibility can strongly affect the timing, duration and severity of epidemics in wildlife populations (Elder et al. 2013; Hall et al. 2009). Thus, determining how food-quality drives this variation is necessary, not only for improving the efficacy of microbial pesticides and understanding disease-mediated insect population dynamics, but also for predicting and controlling wildlife diseases, which could have implications for ecosystem health and biodiversity.

Nutrition-Mediated Effects on Insect Resistance against Entomopathogens

Nutritional studies have revealed the importance of dietary protein in the resistance of insects to entomopathogens. However, whether increased dietary protein improves or reduces host resistance varies among insect–entomopathogen systems, depending on how protein affects insect immune functioning and entomopathogen growth. Increased consumption of dietary protein relative to carbohydrate, either before or after pathogen challenge, enhanced the survival of larval lepidopterans against baculoviruses (Lee et al. 2006; Povey et al. 2013; Shikano and Cory 2015), a food-borne bacterium (*Bacillus thuringiensis*) (Shikano and Cory 2014), and a cuticle-entering opportunistic bacterium (*B. subtilis*) (Povey et al. 2009), but reduced the survival of Australian plague locusts (*Chortoicetes terminifera*) infected by a fungal entomopathogen (*Metarhizium acridum*) (Graham et al. 2014). The heightened resistance of lepidopterans to viral and bacterial infections were associated with higher immune activity on protein-rich diets (e.g. enhanced hemolymph antibacterial and phenoloxidase activities, hemocyte numbers and encapsulation response (Lee et al. 2006; Povey et al. 2009, 2013)) (see also a review on macronutrient effects on insect immune functioning (Ponton et al. 2011)). In locusts, increased consumption of carbohydrate relative to protein increased resistance to fungal infection, even though this reduced their immune functioning, because the fungus thrives on high nitrogen resources (Graham et al. 2014).

The effectiveness of insect immune responses against entomopathogens can also depend on the intake of dietary micronutrients. Ascorbic acid deficiency decreased the number of circulating hemocytes in codling moth, *Cydia pomonella*, larvae and increased their susceptibility to a fungus (*Beauveria bassiana*) and bacterium (*B. thuringiensis*) (Pristavko and Dovzhenok 1974). Deficiency in ascorbic acid also increased the susceptibility of tobacco budworm, *Heliothis virescens*, to baculovirus infection (AcMNPV) (Popham and Shelby 2009). Dietary trace metals may also play an important role in insect immune functioning and resistance to entomopathogens. The concentrations of multiple trace metals in the hemolymph of fourth instar tobacco budworms were altered in response to baculovirus (HzSNPV) infection (Popham et al. 2012). Iron in the hemolymph of greater wax moth, *Galleria mellonella*, larvae has been shown to be essential in clearing dead bacteria (*Xenorhabdus nematophila* and *Bacillus subtilis*) (Dunphy et al. 2002). Zinc added to Grace's medium increased the length of tobacco hornworm, *Manduca sexta*, plasmatocytes and enhanced plasmatocyte network formation (Willott and Tran 2002). Lastly, dietary selenium supplementation increased the resistance of the cabbage looper, *Trichoplusia ni*, to a baculovirus (AcMNPV), though the mechanism is unknown (Popham et al. 2005).

There can be considerable variation in macro and micronutrient content among plants. These can include variation between plant species, genotypes within a species, parts within a plant, ontogenetic stages, seasons and fertilization treatments (Genc et al. 2005; Grusak and DellaPenna 1999; Mattson 1980). Thus, nutritional studies suggest that variation in nutrients among plants play an important role in insect disease resistance. However, these studies isolate the effects of nutrients and ignore the complexity of interactions among plant nutrients and defensive allelochemicals that may influence insect–entomopathogen interactions. For example, plant allelochemicals can strongly alter insect nutrient intake and utilization (Simpson and Raubenheimer 2001), suggesting that the strength of insect immune functioning cannot be predicted from plant nutritional content in the presence of plant allelochemicals. Thus, plant-mediated effects on insect susceptibility to entomopathogens are far less clear-cut than nutritional effects.

Plant-Mediated Effects on Insect Resistance against Food-Borne Entomopathogens

Phytochemicals can impose conflicting effects on insect growth and immunity, while simultaneously interacting with nutritional factors. The majority of studies on plant-mediated changes in insect immune functioning (reviewed in Lampert (2012)) do not test if these immunological changes have any meaningful effects on entomopathogens. Thus, I focus on the

few studies that have established a link among all three factors. A component of the cellular immune response (hemocyte numbers) and resistance to a baculovirus (TnSNPV) in cabbage loopers were significantly higher if the insects were fed broccoli foliage rather than cucumber foliage, though a component of the humoral immune response (phenoloxidase activity) was lower on broccoli (Shikano et al. 2010). Cabbage looper growth rate and hemolymph protein concentration (as a proxy for insect condition/health) were also higher in broccoli-fed insects, suggesting that a higher quality food plant for development was better for resistance to TnSNPV (Shikano et al. 2010). In a follow-up study, cabbage loopers grew faster and had higher humoral immune activity (i.e. higher hemolymph antibacterial and phenoloxidase activities) on cucumber foliage than cabbage foliage, but cucumber-fed larvae were more susceptible to *B. thuringiensis* and were equally resistant to TnSNPV as cabbage-fed larvae (Shikano et al. 2015b). Hemolymph protein concentration and the numbers of hemocytes did not significantly differ between larvae fed the two food plant treatments (Shikano et al. 2015b). These two studies suggest that plant-mediated effects on cabbage looper hemocyte numbers and hemolymph protein concentration could be predictive of resistance to TnSNPV. However, they did not confirm whether higher hemocyte numbers results in more encapsulation of virus infection foci or whether higher hemolymph protein means more antiviral proteins are available. Curiously, resistance to *B. thuringiensis* was not associated with plant-mediated effects on insect growth, condition or immune functioning (Shikano et al. 2015b).

A key component of resistance influenced by plant quality that has received little attention is the physical barriers of the insect. For instance, the peritrophic matrix (protective barrier lining the midgut) is known to provide some resistance against entomopathogens from reaching the midgut epithelium (Granados et al. 2001; Plymale et al. 2008; Vijendravarma et al. 2015) and short-term feeding (8 h) on different food plant species can alter the thickness and structure (number of layers) of the peritrophic matrix (Plymale et al. 2008). Variation in plant (or food) quality can also influence the identity and amount of salivary and digestive enzymes (Afshar et al. 2010, 2013; Brioschi et al. 2007; Broadway and Duffey 1986; Gruner and Taylor 2006; Peiffer and Felton 2005), which may enhance or hinder pathogen entry depending on the enzymes and entomopathogens involved. For example, the salivary enzyme glucose oxidase of the corn earworm, *Helicoverpa zea*, was antibacterial against *Serratia marcescens* and *Pseudomonas aeruginosa* (Musser et al. 2005). Multiple digestive enzymes in the silkworm, *Bombyx mori*, have been shown to have antiviral activity against its baculovirus (BmNPV) (e.g. Nakazawa et al. 2004; Ponnuel et al. 2003, 2012), but digestive enzymes are also involved in releasing infectious virus particles (occlusion derived viruses) from polyhedral occlusion bodies (Pritchett et al. 1984).

Moreover, short-term feeding on low nutrient food can induce compensatory feeding whereby insects ingest more food and allocate more biomass to the gut to improve digestive efficiency (Couture et al. 2016; Hawlena et al. 2011; Raubenheimer and Bassil 2007; Yang and Joern 1994). Gypsy moth, *Lymantria dispar*, larvae that fed for 4 days on a low nutrient diet still maintained the same growth rate as larvae on high nutrient diet by consuming almost fourfold more diet. These larvae also exhibited a fourfold increase in gut biomass relative to total body weight (Couture et al. 2016). The addition of plant secondary metabolites (salicinoids) reduced consumption rate and gut biomass allocation (Couture et al. 2016). While this study did not measure insect susceptibility to entomopathogens, nutrition and phytochemical-mediated changes in consumption rate will undoubtedly influence the ingested pathogen dose. The resulting plasticity in gut biomass might also influence the susceptibility of insects to entomopathogens by changing the surface area of the gut for entomopathogens to establish primary infections.

Deterioration in food quality (e.g. herbivore-induced plant defenses, reduced nutrient quality and starvation) is often associated with high herbivore population density, which is predictive of a higher likelihood of contacting diseased individuals (Fig. 2). This is particularly the case for density dependent, horizontally transmitted entomopathogens such as baculoviruses. For insects with short-generation times or those that are non-migratory, such as some forest Lepidoptera, poor food quality experienced by one generation may predict an even greater risk of disease for their offspring. Correspondingly, the immune functioning and resistance of some insects to entomopathogens can vary with the food quality experienced by their parents. Western tent caterpillars,



Fig. 2 High herbivore population density can often lead to deterioration in food quality and an increased likelihood of contacting diseased individuals. The photo shows crowded cabbage looper, *Trichoplusia ni*, larvae feeding on a cabbage plant. Notice the close proximity of larvae to each other and the small amount of remaining foliage. These are causing several larvae to feed on stems, which are less nutritious for *T. ni* than leaves. Photo courtesy of Michael Hrabar, Simon Fraser University, Burnaby, BC, Canada

Malacosoma californicum pluviale, that consumed herbivore-induced red alder (*Alnus rubra*) leaves, compared to non-induced leaves, produced offspring with significantly elevated encapsulation responses and marginally higher phenoloxidase activities, though hemocyte numbers and hemolymph protein concentration did not differ (Olson 2014). These offspring were also more likely to survive baculovirus challenge, and those that succumbed to infection took longer to die than offspring from parents reared on non-induced alder leaves (Olson 2014). The mechanism behind this plant-mediated transgenerational induction of offspring immunity is unknown. Antibacterial activities and protein and lipid concentration in the eggs did not differ between the parental food treatments, though the eggs produced by parents reared on induced leaves were smaller (Olson et al. 2017). Nutritional studies have found that larval cabbage loopers and Indian meal moths, *Plodia interpunctella*, under nutritional stress can also produce offspring with elevated immune functioning (hemolymph antibacterial and phenoloxidase activities) and resistance against baculoviruses and *B. thuringiensis* (Boots and Roberts 2012; Shikano et al. 2015a). In contrast to the smaller eggs produced by induced foliage-fed Western tent caterpillars, the heightened immunity in the offspring of nutritionally stressed cabbage loopers was associated with larger eggs (Shikano et al. 2015a). Importantly, the two nutritional studies reduced caloric value by diluting nutrients (Boots and Roberts 2012; Shikano et al. 2015a). However, parental diet quality did not influence offspring immunity in cabbage loopers when nutrition was manipulated by altering protein to carbohydrate ratios to maintain caloric value (Shikano et al. 2016). This suggests that the impact of changes in plant quality on cross-generational insect disease dynamics may depend on numerous factors, including the nutritional composition and content of the plants and the influence of plant defenses on the ability of insects to ingest and extract nutrients.

Evolutionarily, food quality can impose conflicting selection pressures on the dual functions of the insect's gut to serve as a barrier against entomopathogens and extract nutrients from food (Vijendravarma et al. 2015). Adaptation of *Drosophila melanogaster* populations during 160 generations of experimental selection to chronic larval malnutrition on a nutritionally dilute artificial diet increased their susceptibility to infection by the opportunistic food-borne bacterial pathogen *Pseudomonas entomophilya* (Vijendravarma et al. 2015). The increased susceptibility resulted from a higher predisposition of the peritrophic matrix to disintegrate upon infection and not from a weakened immune response or higher bacterial loads (Vijendravarma et al. 2015). Since different food plant species altered the thickness and structure of the peritrophic matrix of tobacco budworm, *Heliothis virescens* (Plymale et al. 2008), it is likely that the nutrition-mediated trade-off between the two functions of the fruit fly's gut will be influenced by phytochemicals in its natural foods.

Plant-Mediated Effects on Insect Resistance against Cuticle-Entering Entomopathogens

Plant-mediated effects on cuticle-entering pathogens are also complex. When challenged by a fungal entomopathogen (*Metarhizium anisopliae*), larvae of the rusty tussock moth, *Orygia antiqua*, which is a polyphagous insect that feeds on a wide range of deciduous trees and shrubs, had improved survival when reared on foliage from a willow species (*Salix myrsinifolia*) that contained higher concentrations of phenolic glycosides compared to a willow with lower concentrations (*S. viminalis*) (Sandre et al. 2011). In the absence of the entomopathogen, larvae survived better on the willow species that contained less phenolic glycosides. The study controlled for direct plant effects on conidia germination by exposing insects from both willow species to fungal conidia on foliage from the same plant species (birch). The plant-mediated differences in resistance was not associated with encapsulation rate (a component of immune functioning) or body condition (Sandre et al. 2011), suggesting that phenolic glycosides or their metabolites may have accumulated in the hemocoel and directly harmed the fungus; though dietary phenolic glycosides have been shown to increase immune functioning in another system (Del Campo et al. 2013).

The cuticle is a key component of resistance against many entomopathogens as it provides both physical and biochemical protection (Moret and Moreau 2012). For example, fungal germ tubes can be melanized as they pass through the cuticle before they enter the hemocoel (Golkar et al. 1993). Yet surprisingly, no studies have considered plant-mediated changes to the cuticle's defensive properties. A nutritional study demonstrated that the source of dietary protein (plant or animal based) can influence melanization of the insect cuticle (i.e. darkness of the cuticle) (Lee et al. 2008). Since the quality of plant protein is highly variable between plant species and between tissues within a plant (Felton 1996), plant-mediated effects on the cuticle deserve more attention.

Consumption of Phytochemicals and Nutrients to Increase Resistance against Entomopathogens

Some insects can take advantage of toxic phytochemicals by sequestering them to protect against predation and parasitism (Nishida 2002). Few systems have examined whether phytochemicals can protect herbivorous insects against entomopathogens. Monarch butterflies and their cardenolide-containing milkweed plants are probably the most thoroughly studied in this regard. Increasing cardenolide content of milkweed species consumed by monarch larvae infected by a protozoan parasite (*Ophryocystis elektroscirrha*) elevated their resistance and tolerance (De Roode et al. 2008; Gowler et al. 2015; Sternberg et al. 2012). Non-sequestering insects can

accumulate some phytochemicals in their tissues, depending on their ability, or lack thereof, to detoxify these compounds. For example, the steroidal glycoalkaloid α -tomatine in the diet of tomato fruitworm, *Helicoverpa zea*, infected by a fungal entomopathogen (*Nomuraea rileyi*) lowered larval mortality by reducing conidia production in vivo (Gallardo et al. 1990).

Importantly, while phytochemicals can afford some level of protection to insects against entomopathogens, it is possible that entomopathogens may adapt to the plants fed on by their hosts. De Roode et al. (2011a) showed that a protozoan parasite (*O. elektroscirrha*) compensated for the phytochemical-mediated reduction in effective infectious dose against monarch larvae by evolving higher virulence. Adaptation of entomopathogens to their host's food plants may be likely in stable plant–insect–entomopathogen systems, such as insect populations in geographically isolated areas dominated by a single plant species. For example, among three geographically distinct island populations of western tent caterpillars, *Malacosoma californicum phiviale*, on different dominant host plants, baculovirus isolates from two of the permanent host populations had the fastest speed of kill on the plant from which they were isolated (Cory and Myers 2004).

In response to high pathogen risk, insects can engage in disease preventing behavior termed prophylactic medication (Abbott 2014; De Roode et al. 2013). Uninfected wood ants, *Formica paralugubris*, collected antimicrobial, antifungal terpene-containing resin from conifer trees and incorporated them into their nests to prevent disease in the colony (Chapuisat et al. 2007). These ants also applied significant amounts of endogenous formic and succinic acid on the resin, which greatly increased its antifungal activity (Brütsch et al. 2017). Diseased insects have also been demonstrated to medicate themselves in response to entomopathogen infection, termed therapeutic- or self-medication (Abbott 2014; De Roode et al. 2013). Honey bee colonies infected by a fungal entomopathogen (*Ascophaera apis*) decreased infection intensities in the colonies by increasing their foraging for resin, a form of group- or colony-medication (Simone-Finstrom and Spivak 2012). Self-medicating behavior by individual bees to cure themselves using single substances such as nicotine has also been suggested, but the strength and effectiveness of this behavior are weak (Baracchi et al. 2015). It is possible that insects rely on combinations of phytochemicals or “defensive cocktails” to therapeutically limit infections. For example, combinations of two floral phytochemicals, eugenol and thymol, had synergistic effects against the bumblebee gut trypanosome *Crithidia bombi* (Palmer-Young et al. 2017). The clearest demonstration of self-medication in insects, through intentional ingestion of phytochemicals, occurred in response to parasitization by tachinid flies (endoparasites). Parasitized woolly bear caterpillars, *Grammia incorrupta*, increased their ingestion of pyrrolizidine alkaloids, which increased their chances of survival (Singer et al. 2009). Even though monarch butterfly larvae

that are parasitized by the protozoan parasite *O. elektroscirra* are more resistant on higher cardenolide containing plants, they do not engage in self-medication by consuming more cardenolides (Lefèvre et al. 2012). Instead, infected monarchs engaged in transgenerational- or maternal-medication by preferentially laying their eggs on plants containing higher concentrations of cardenolides as there is a risk of vertically transmitting the parasite to their offspring (Lefèvre et al. 2010).

The infection-induced change in feeding behavior is not exclusive to phytochemicals. Nutritional studies have demonstrated that insects can alter their nutrient intake in response to entomopathogen infection. Larval lepidopterans increased their proportional intake of protein relative to carbohydrate in response to infection by bacterial (*B. subtilis*) and viral (baculoviruses) entomopathogens, either as a means to self-medicate or compensate for protein resources used in the immune response (Lee et al. 2006; Povey et al. 2009, 2013; Shikano and Cory 2016). Insects might also change their nutrient intake to limit resources for the entomopathogen. As mentioned previously, the fungal entomopathogen *Metarhizium acridum* flourishes on high nitrogen resources. In response to infection by *M. acridum*, locusts improved their chances of survival by increasing their intake of carbohydrate and decreasing their intake of protein (Graham et al. 2014).

The extent to which prophylactic and therapeutic medicating behavior occurs in nature and its effects on entomopathogen transmission dynamics are still largely unknown. For instance, we know little about the specificity of therapeutic compounds to different pathogens. Tomatine (1000 ppm) added to Noble agar completely inhibited the germination of *Paecilomyces fumosoroseus* but only inhibited the germination of *B. bassiana* by 45% relative to germination in the absence of tomatine (Poprawski et al. 2000). Consequently, the insect's self-medication behaviors are likely to be system and/or condition specific. Cabbage loopers only increased their protein intake in response to challenge by a generalist baculovirus (AcMNPV) at 24 °C but not at 32 °C, and not in response to its specialist baculovirus (TnSNPV) (Shikano and Cory 2016). In fact the increased protein intake by AcMNPV-challenged insects at 24 °C, though statistically significant, would only slightly increase the probability of survival and is unlikely to have any meaningful effects on transmission dynamics. Field studies are needed to examine whether food choice can actually influence disease dynamics in natural populations. Lastly, it is also possible that infection-induced behavior that has been interpreted as medicating behavior could somehow be benefiting pathogen fitness even if the behavior reduces host mortality (Karban and English-Loeb 1997). Some entomopathogens can manipulate the movement and feeding behavior of their hosts, which enhance entomopathogen distribution and persistence on plants (e.g. Andersen et al. 2009; Hoover et al. 2011; Mehlhorn 2015; Raymond et al. 2005; van Houte et al. 2012). However, pathogen fitness is rarely measured in studies of insect medication.

Potential for Entomopathogens to Influence the Evolution of Plant Defenses

Baculovirus occlusion bodies (transmission stages) can be inactivated by leaf exudates, as they wait to be ingested by susceptible hosts (Cory and Hoover 2006; Stevenson et al. 2010), and by herbivore-induced phytochemicals, which are released during the maceration of plant tissues by chewing insects (Cory and Hoover 2006). It seems counterintuitive for plants to harm the natural enemies of herbivores. Instead we might expect that entomopathogens should impose selection pressures on plant traits to minimize their harm. Wan et al. (2016) found that among 14 crop plant species, the lethality of a baculovirus against beet armyworms was only significantly reduced by a few plant species. Thus, plant-mediated inhibition of baculovirus infectivity may be an exception and not the rule.

We recently demonstrated that the levels of baculovirus-induced mortality in fall armyworms can differ with the intra-specific genetic variation in plants ingested with the virus (Shikano et al. 2017c). Induction of anti-herbivore defenses through exogenous application of the phytohormone jasmonic acid inhibited baculovirus (SfMNPV) lethality to varying degrees among the eight soybean genotypes tested (Shikano et al. 2017c). Induced plant traits that inhibited digestion in the fall armyworm were associated with reduced baculovirus lethality, but not traits that deterred feeding (Shikano et al. 2017c). Induction of higher levels of peroxidase activity, which is an enzyme involved in plant defense against herbivores, was associated with reduced baculovirus lethality on cotton and tomato plants (AcMNPV and HzSNPV, respectively) (Hoover et al. 1998), although this was not the mechanism on soybean plants (Shikano et al. 2017c). Interestingly, while the induction of another important enzyme in plant defense, polyphenol oxidase, did not inhibit baculovirus lethality (Hoover et al. 1998), incubation of *B. thuringiensis* with polyphenol oxidase significantly increased the lethality of *B. thuringiensis* (Ludlum et al. 1991). *B. thuringiensis* lethality increased even more if the bacteria was incubated with a combination of polyphenol oxidase and chlorogenic acid, both of which are key defensive phytochemicals in tomato plants (Ludlum et al. 1991). Since genotypes within a plant species can vary in their levels and compositions of constitutive and induced defensive compounds, these findings suggest that there may be selectable plant defensive traits that can enhance entomopathogen efficacy. Such traits could be targeted in plant breeding programs to improve the compatibility of plant defenses with entomopathogens to maximize plant protection.

Intraspecific genetic variation is fundamental for species to respond rapidly to environmental conditions. Insect herbivores can exert selective pressures on plant defense traits and different herbivore species can favor specific defense traits (Zust et al. 2012). Moreover, the use of chemical

insecticides to suppress insect herbivores can change the favored plant traits to select for plant genotypes that grow more competitively against other genotypes and species (Agrawal et al. 2012). In addition to the differential effects of soybean genotypes on baculovirus lethality, we have shown that the ingestion of baculovirus occlusion bodies with different soybean genotypes can influence the yield of occlusion bodies produced in the host (Shikano et al. 2017a). For obligate-killing pathogens like baculoviruses, the number of hosts killed and the number of occlusion bodies released from each killed host into the environment to infect new hosts are important factors for transmission dynamics. Thus, it is conceivable that there may be selection for plant traits that minimizes their detrimental effects on entomopathogen efficacy, particularly in natural systems with high insect pressure and ample entomopathogen reservoirs or in agricultural systems that consistently use microbial insecticides. Because of these intraspecific plant effects and the patchily distributed nature of entomopathogens, the plant traits favored by entomopathogen protection may differ from those selected by the broad suppression of insects by chemical insecticides. On the other hand, since the most important roles of plant defense chemicals is probably for protection against herbivores and plant pathogens, it may be too speculative to think that plant genotype-mediated variation in entomopathogen efficacy could be beneficial enough for plants to warrant selective changes in their defense traits. Adaptation of entomopathogens to the phytochemicals in their host's food plants, as discussed earlier, may be more likely.

Another possibility for entomopathogen-driven evolution of plant traits is through the endophytic colonization of plants by entomopathogenic fungi. Endophytic colonization by entomopathogenic fungi can protect plants from insects (Ownley et al. 2010). For example, fungal isolates from five genera (*Beauveria*, *Hypocrea*, *Gibberella*, *Fusarium* and *Trichoderma*) colonized the roots, stems and leaves of fava beans (*Vicia faba*) and common beans (*Phaseolus vulgaris*) and killed 100% of pea leafminers (*Liriomyza huidobrensis*) on the plants. Interestingly, two *Beauveria* isolates (G1 LU3 and S4SU1) colonized roots, stems, and leaves of *P. vulgaris* but only the root and stem of *V. faba*, indicating that the ability of endophytic entomopathogenic fungi to colonize can vary by plant species and parts within a plant (Akutse et al. 2013). Moreover, opium poppy (*Papaver somniferum*) plants were shown to vertically transmit *B. bassiana* via seeds (Quesada-Moraga et al. 2014). It is still not known if intraspecific genetic variation in plants can influence the ability of endophytic entomopathogenic fungi to colonize and vertically transmit. If it does, protection from these fungi under high insect pressure may select for plant genotypes that favor endophytic colonization. It would also be interesting to see if plants can use these fungi as bodyguards by modifying their physiology during herbivory to enhance or invite endophytic colonization.

Complex Species Interactions: Other Herbivores, Microbes and Sublethal Entomopathogen Doses

Insects share their food plants with many other herbivores, including those that feed on the same or different parts of plants and may have different modes of feeding that differentially influence plant defenses (Fig. 3a). For example, aphids indirectly increased the virulence and transmission potential of a monarch butterfly parasite through aphid-induced changes in the chemical composition of the shared milkweed plant (De Roode et al. 2011b).

Insects will also encounter a wide array of microbes that inhabit the surface of their food plants (Fig. 3b–d), including sublethal doses of their pathogens. Surviving a previous entomopathogen-challenge has been associated with increased resistance to the same entomopathogen upon subsequent exposure (termed immune priming) (e.g. Pham et al. 2007; Tidbury et al. 2011) and this immunity can be transferred maternally to protect offspring (Sadd and Schmid-Hempel 2006; Shikano et al. 2015a; Tidbury et al. 2011). The mechanism of maternal or transgenerational immune priming against bacterial pathogens can occur through the binding of the egg-yolk protein vitellogenin to pathogen-associated molecular patterns, which then transports the bacterial cell-wall pieces into the developing oocytes (Salmela et al. 2015). The transferred bacterial fragments in the eggs appears to act as the immune elicitor for the offspring (Freitak et al. 2014; Salmela et al. 2015).

Ingestion of non-entomopathogenic bacteria (*Escherichia coli* and *Micrococcus luteus*) have also been shown to induce the activity of antibacterial enzymes and the expression of immune-related proteins in the hemolymph of cabbage loopers (Freitak et al. 2007) and greater wax moth larvae (Freitak et al. 2014). Non-entomopathogenic bacteria are always present on plants in the field (Fig. 3b). This suggests that insect immune systems are continuously maintained at elevated levels, which would be energetically costly for the insect. Cabbage loopers that ingested these non-entomopathogenic bacteria had reduced pupal weight and prolonged development (Freitak et al. 2007). An alternative is that the immune priming may have resulted from the novelty of the bacteria to the insect, which included an enteric anaerobic bacterium (*E. coli*) and an obligate saprotrophic aerobe (*M. luteus*) that colonizes the skin and mucous membranes of warm-blooded animals. In greater wax moth larvae, the immune responses to *E. coli* and *M. luteus* were significantly weaker than the immune responses against entomopathogenic bacteria (*Serratia entomophila* and *Pseudomonas entomophila*) (Freitak et al. 2014). Thus, while consumption of non-entomopathogenic bacteria may have induced a statistically significant immune response, the strength of this immune priming may have little influence on resisting a subsequent pathogen challenge. In a more ecologically relevant system, ingestion of non-

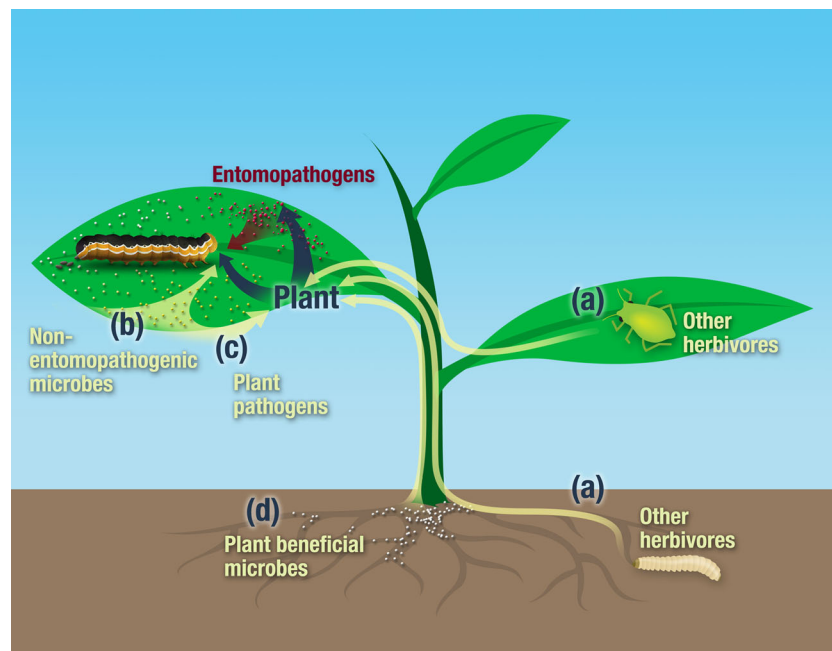


Fig. 3 The tritrophic interactions between plants, insect herbivores and entomopathogens are influenced by other species interactions. **a** Insect–entomopathogen interactions can be influenced by differential changes in plant quality induced by the dynamic community of herbivores feeding on the same plant, including those that feed on different plant tissues and use different modes of feeding. **b, c, d** Diverse communities of microbes inhabit plants, and many of these microbes can influence plant–insect–entomopathogen interactions. **b** Ingestion of non-entomopathogenic microbes (and sublethal doses of entomopathogens) with plant tissue

can alter the immune functioning of insect herbivores. Some of these microbes can colonize the insect gut and competitively exclude entomopathogens. Some insects can orally secrete gut microbes onto wounded plant tissue during feeding to suppress plant defenses, which is likely to alter plant–insect–entomopathogen interactions. **c** Plant pathogens and **d** plant beneficial microbes can influence insect resistance to entomopathogens by changing plant defenses and nutritional quality. Illustration by Nick Sloff, Pennsylvania State University, University Park, PA, USA

entomopathogenic phyllosphere bacteria (*Pseudomonas fluorescens* and *P. syringae*) that are commonly associated with the cabbage looper’s food plants had no significant impact on immune functioning (hemolymph antibacterial and PO activities and hemocyte numbers) or resistance to the entomopathogens *B. thuringiensis* and a baculovirus, though the presence of the bacteria reduced the cabbage looper’s growth rate and condition (Shikano et al. 2015b).

Microbes on the phyllosphere can colonize and persist in insect midguts (Mason and Raffa 2014; Priya et al. 2012) and plant species and genotype can influence the community composition of gut microbiota (Broderick et al. 2004; Mason et al. 2015; Tang et al. 2012). Lab-reared beet armyworms, *Spodoptera exigua*, challenged by its baculovirus (SeMNVP) suffered significantly higher mortality and produced more viral occlusion bodies when larvae were reared on artificial diet without antibiotics to preserve gut microbiota (*Enterococcus* and *Enterobacter* spp.) than on diet containing antibiotics (Jakubowska et al. 2013). In contrast, the gut bacterium *Streptococcus faecalis* of lab-reared greater wax moth larvae has been shown to competitively suppress infection by multiple ingested entomopathogens (*Pseudomonas aeruginosa*, *Proteus mirabilis*, and *B. thuringiensis*) (Jarosz 1979). Importantly, the gut microbiota of lab insects reared on artificial diet are typically dominated by only a few culturable

species (e.g. Broderick et al. 2004; Jakubowska et al. 2013; Johnston and Crickmore 2009). By comparison, the gut microbial communities of wild insects feeding on plants are dramatically more diverse, and the composition of these communities can vary widely by plant species (Broderick et al. 2004; Chung et al. 2017; Mason and Raffa 2014). How the gut microbiota of wild insects influences entomopathogen efficacy remains to be tested. Moreover, insect gut microbes can be involved in the degradation of defensive phytochemicals (Mason et al. 2014; Pilon et al. 2013; Welte et al. 2016) and can be orally secreted onto wounded plant tissue during herbivory to alter plant defenses (Acevedo et al. 2017; Barr et al. 2010; Casteel et al. 2012; Chung et al. 2013). Thus, it is highly likely that insect gut microbes may also influence insect susceptibility and entomopathogen virulence by modulating plant physiology and chemistry.

Phytopathogens and plant-associated beneficial endophytes and soil microbes also influence plant nutritional quality and defense (Fig. 3c, d) (Choudhary and Varma 2016; Pieterse et al. 2014; Stout et al. 2006), which can influence insect–entomopathogen interactions. For instance, infection of Chinese cabbage leaves by a phytopathogenic fungus (*Alternaria brassicae*) slowed the growth rate of mustard leaf beetle, *Phaedon cochleariae*, larvae by reducing leaf quality (Rostás and Hilker 2003). Consequently, this increased larval

mortality to an entomopathogenic fungus (*Metarhizium anisopliae*) by increasing the period of vulnerability (Rostás and Hilker 2003). Arbuscular mycorrhizal fungi associated with milkweed plants influenced the interaction between monarch butterflies and their protozoan parasites (Tao et al. 2015). Parasite virulence, host resistance and host tolerance were differentially affected depending on the arbuscular mycorrhizal fungi's effects on the primary (phosphorus) and secondary (cardenolides) chemistry of different milkweed species (Tao et al. 2015).

It is increasingly clear that non-entomopathogenic microbes play important roles in plant–insect–entomopathogen interactions. Variation in the compositions of insect and plant-associated microbes among host species and genotypes suggest that the microbes' effects on entomopathogens are likely to be system specific. Tools to identify and quantify microbes and their functions are becoming increasingly available. These tools should facilitate more studies to determine the influences of individual and communities of microbes on complex multitrophic interactions.

Future Challenges

It is clear that plant–insect–entomopathogen interactions are intertwined within a complex web of species interactions. An important question going forward is whether our understanding of these multitrophic interactions based on controlled laboratory experiments will translate to field conditions. Few studies have examined plant–insect–entomopathogen interactions in the field. Raymond and Hails (2007) demonstrated that both host plant species and phenology in the field can influence the levels of infection and the numbers of infectious stages produced by infected larvae of the winter moth, *Operophtera brumata*, fed foliage of oak and Sitka spruce trees treated with a baculovirus (*O. brumata* NPV). Elder et al. (2013) examined the effects of induced anti-herbivore defenses (hydrolyzable tannins) in red oaks (*Quercus rubra*) on the susceptibility of gypsy moth larvae to a baculovirus in the field. They found that on induced foliage, average infection rates were lower at lower virus density but higher at higher virus density, compared to non-induced foliage. AIC analysis demonstrated that induction produced a linear relationship between virus transmission and virus density. This implied that induction strongly reduced variability in infection risk because high levels of variability in infection risk between individuals produces nonlinear virus transmission. Their model suggests that the inducibility of hydrolysable tannin defenses in oaks may play an important role in the alternating outbreaks of gypsy moth in forests with a high frequency of oaks, compared to the uniform outbreaks in forests with a low frequency of oaks. While these studies extend beyond the laboratory, they restricted groups of larvae to individual

branches or plants. This limits insect movement, thereby restricting their exposure to variations in plant quality and consequently may have influenced infection rates.

A major difference between laboratory and field conditions is the dose of entomopathogens insects are likely to encounter. In the laboratory, most studies use either a range of doses in a bioassay or a single dose that produces moderate levels of infection, such as an LD₅₀. One of the biggest gaps in understanding the dynamics of entomopathogens in the field is that almost nothing is known about the distribution and amount of transmission stages that are on wild plants, even under epizootic conditions. Plants may influence the numbers of infectious transmission stages produced in infected hosts. For example, the plant species fed on by infected hosts can differentially select baculovirus genotypes, which vary in their infectivity, speed of kill and yield (Hodgson et al. 2002, 2004). Even plant genotypes and induced defenses in the foliage consumed by infected hosts can influence the numbers of baculovirus transmission stages produced (Shikano et al. 2017a). Entomopathogen-killed cadavers contain highly concentrated numbers of infectious transmission stages, meaning that insects in the field may be most likely to ingest/contact doses at the extremes, sublethal doses and extremely high doses, rather than moderately lethal doses. Extremely high doses in the field may overwhelm any plant and nutrition-mediated effects on insect–entomopathogen interactions. Few studies have examined how the natural distributions and amounts of entomopathogen transmission stages on plants influence transmission dynamics, and the findings are unclear. Transmission of the recombinant and wild-type baculovirus AcMNPV in cabbage loopers in the field depended more on the numbers of virus-killed cadavers (i.e. inoculum patches) on the plants than the size of the cadavers (i.e. size of inoculum) (Hails et al. 2002). In contrast, larger patches of baculovirus (*Panolis flammea* NPV) inoculum (i.e. size of cadaver) induced higher mortality in the following generation of cabbage moth (*Mamestra brassicae*) larvae on the same plants, even when fewer patches were present (Hesketh and Hails 2015). The contradictory results could have arisen from any number of differences between the two studies including the viruses and hosts used, though both studies used cabbage plants. Knowing more about the occurrence and distribution of entomopathogens is crucial to any further understanding of tritrophic interactions.

While plants can clearly mediate the interactions between insects and their pathogens, little is still known about if or how these interactions extend to the occurrence and intensity of epizootics. Key future challenges include improving our knowledge of what entomopathogens insects encounter in the field, at what doses and how often insects encounter these doses (e.g. uniformly distributed low doses or patchy high doses). Without this knowledge, extrapolation of our current understanding of multitrophic interactions to natural processes

should be made with caution. Only through the use of ecologically relevant doses and distributions of entomopathogens could we comprehend the importance of plant-mediated effects on insect–entomopathogen interactions in the field, not to mention the myriad of ways that complex natural species interactions may influence these multitrophic interactions.

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