

Plant-mediated effects in insect–pathogen interactions

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Interactions between insect herbivores and their pathogens can be modulated by host plants. Inter- and intraspecific differences in plant chemistry and structure can alter the susceptibility of insects to infection and the production and environmental persistence of pathogens. Whether plants can manipulate insect pathogens to act as ‘bodyguards’ and increase their own fitness remains to be shown. Reduced insect performance owing to poor plant quality can enhance the susceptibility of an insect to disease while these same phytochemicals can also reduce the effectiveness of entomopathogens in killing the host. As we discuss here, plants have an important role in the evolution of insect–pathogen relationships and a tritrophic perspective should thus be incorporated into the study of insects and their pathogens.

Insect pathogens in a tritrophic context

Plants have several complex direct and indirect defence strategies (see Glossary) against herbivores and plant pathogens, which include defensive chemicals and the manipulation of the natural enemies of herbivores, above and belowground [1–4]. Direct defence through constitutive or induced plant phytochemicals, can benefit the plant by reducing herbivore preference or performance [5,6]. Indirect defence by the recruitment of predators and parasitoids as ‘bodyguards’ can be achieved by the release of plant volatiles [7–9]. Determining how insect pathogens fit into this tritrophic framework provides new perspectives on host–parasite interactions.

Insects harbour a variety of pathogens (Figure 1), many of which are unidentified and little studied. The study of insect pathogens (entomopathogens) and plants has focussed largely on the impact of the plant (usually a crop) on the efficacy of pathogens as biological control agents [10]; a broader exploration of the insect–plant–entomopathogen relationship from the perspective of evolutionary ecology is enticing [11,12]. Here, we demonstrate that plants can influence the interactions between insect herbivores and their pathogens in numerous ways, and discuss the mechanisms that could be involved. Although plants can manipulate insect pathogens for their benefit, for them to use pathogens as ‘bodyguards’

requires variation in selectable plant traits that influence pathogen efficacy and increase plant fitness. Although these have yet to be demonstrated, it is clear that insect pathogens are affected by tritrophic interactions and that complex multitrophic relationships do exist.

Glossary

Allelochemicals: chemicals and other biomolecules produced by an organism that are toxic or inhibitory to the growth of other organisms.

Direct defence: production of substances or structures in plants that result in direct negative impacts on the second trophic level (herbivores or plant pathogens).

Indirect defence: production of substances or structures in plants that attract or enhance the negative effect of natural enemies on the second trophic level (herbivores or plant pathogens).

Induced plant responses: plant structures, nutrients and phytochemicals (including volatiles) produced in response to attack by a natural enemy, such as a plant pathogen or herbivore.

Oxidative stress: a form of physiological stress that occurs when the pro-oxidants:antioxidants ratio in tissues favours the former, resulting in increased damage to DNA, RNA, proteins and lipids by reactive oxygen species and other free radicals; can result in insects from exposure to UV irradiation, heavy metals and some plant phytochemicals. Some plant phenolics participate in redox cycling, propagating further cellular damage to vulnerable tissues, such as the insect midgut.

Peroxidases: with respect to their activity on phenolics, a group of enzymes that use hydrogen peroxide as a co-substrate to produce semiquinone free-radical intermediates, reactive oxygen species and quinones.

Phenolic, polyphenol: phenolics, which are ubiquitous in plants, comprise one or more hydroxyl groups (-OH) bound to one or more aromatic rings with a variety of R-groups [i.e. substituents, which in the case of phenolics, usually include ethers (-OCH₃) and glycosides] attached. Polymers of phenolics are referred to as polyphenols.

Phytochemicals: plant-produced compounds of varying structures and biological activities, including nutrients, allelochemicals, volatiles, proteins and peptides.

Polyphenol oxidase: class of enzymes characterised by their ability to catalyse aerobic oxidation of mono and dihydroxy phenols, commonly referred to as monophenoloxidase (monophenolase) and polyphenoloxidase (polyphenolase) activities, respectively; best known in insects for their role in the melanisation of cuticle, wound healing, immune responses, and plant defences against insects. Oxidised phenolics can covalently bind to proteins, reducing the nutritive quality of plant protein for herbivores, thereby reducing the growth of the herbivore.

Pro-oxidant: an atom or molecule that promotes oxidation of another atom or molecule by accepting electrons. Cells in a pro-oxidant state exhibit increased concentrations of reactive oxygen species, organic peroxides and other radicals.

Redox cycling: transformation of an oxidisable compound, such as a phenolic, which is oxidised and then re-reduced to its original form by biological antioxidants such as glutathione or ascorbate. This cycling amplifies the production of reactive oxygen species and depletes cellular antioxidant reserves that normally protect against radical damage.

Transmission: transmission of pathogens is a combination of innate larval susceptibility plus the likelihood of the insect encountering and ingesting a lethal dose of the pathogen. The transmission parameter can be estimated in within-season field experiments. To tease apart the specific components of transmission, it is necessary to compare host susceptibility, feeding rates and larval behaviour on each plant species.

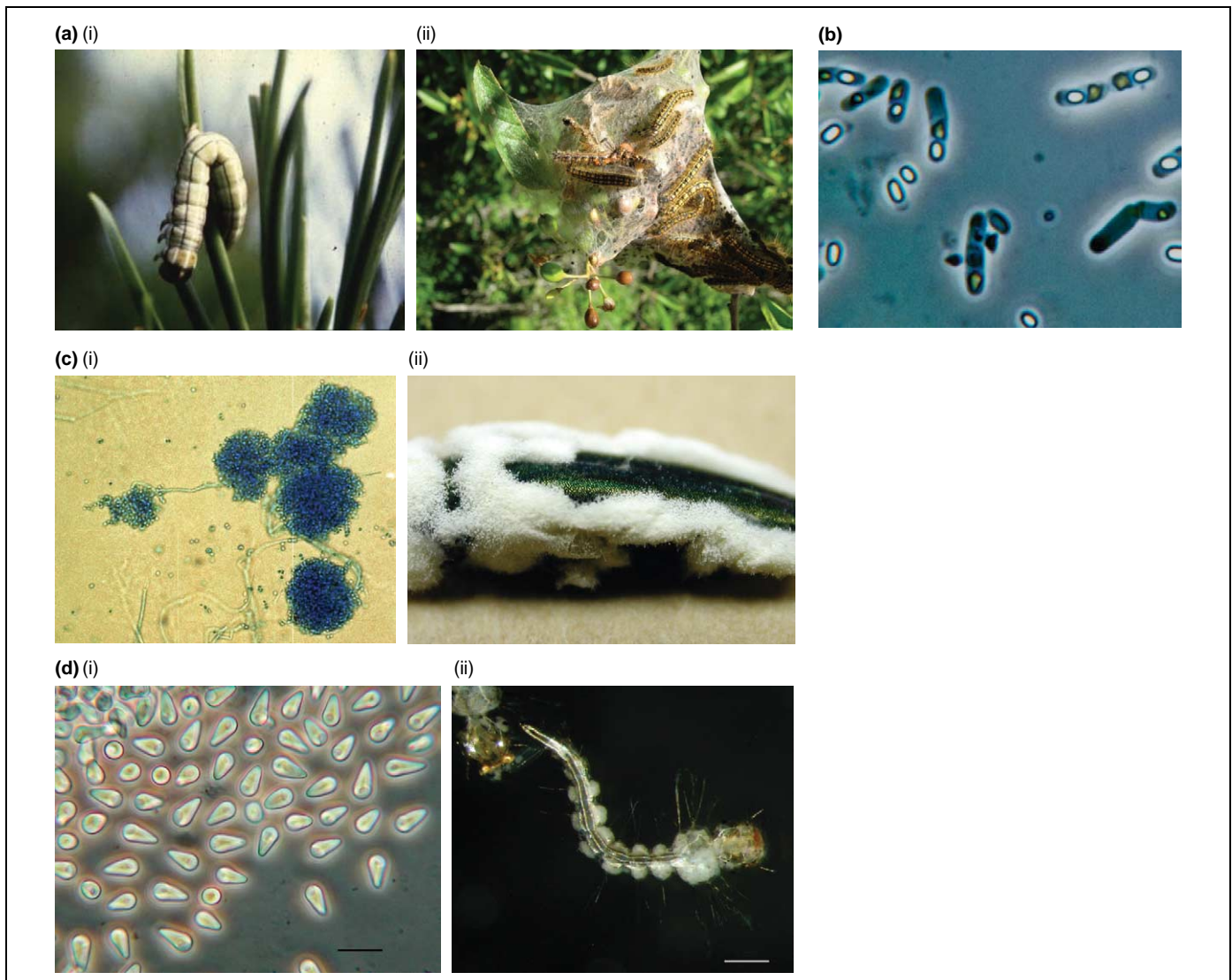


Figure 1. Examples of pathogens or pathogen-killed insects from the three major groups of pathogens. (a) (i) Pine beauty moth *Panolis flammea* larva killed by an NPV; (ii) Western tent caterpillar *Malacosoma californicum pluviale* family with NPV-infected larva. (b) Spores from the bacterium *Bacillus thuringiensis* var. *kurstaki*. (c) (i) Spores from the fungus *Beauveria bassiana* (stained with lactophenol blue); (ii) Emerald ash borer *Agrilus planipennis* killed by *B. bassiana*. (d) (i) Spores from the microsporidian *Edhazardia aedis*; (ii) *Edhazardia aedis* infection in a mosquito *Aedes aegypti* larva. Scale bars = 10 μ m (di), 1 mm (dii). Reproduced with permission from Joel Siegel (b); Leah Bauer (c) and Susan E. White and James J. Beceal (d).

Introducing insect pathogens

Most research on entomopathogens is centred on three groups: viruses [primarily baculoviruses (nucleopolyhedroviruses, NPVs, and granuloviruses); bacteria (predominantly one species, *Bacillus thuringiensis*); and fungi (including Microsporidia) (Figure 1). Entomopathogenic nematodes are not included in the scope of our review because, although they are usually included within the microbial pest control agents, they are not strictly insect pathogens and, more importantly, they are distinct from the microparasites considered here in that they can host search. Several recent studies; however, have shown that insect nematodes can be manipulated by plants and that they are potentially important in driving above- and belowground interactions [3,4].

The extent to which plants can influence insect-pathogen relationships depends on pathogen (and host) life history and whether plants can directly or indirectly alter key processes, such as infection or environmental

persistence. The main difference among these pathogen groups is how they infect their hosts. Baculoviruses, bacteria and Microsporidia must be ingested to initiate infection, with body penetration usually beginning in the midgut of the insect; thus, plants are intimately involved at the point of infection. Most fungi penetrate the cuticle of the host and fungal spores germinate on the cuticle and invade the insect body, usually by a combination of physical force and enzymatic action; this makes fungi particularly sensitive to changes in microclimate. In addition, viruses and Microsporidia are obligate parasites (i.e. they need living host cells to reproduce) whereas some fungi and bacteria are facultative parasites (i.e. can replicate on substrates outside the host). Although entomopathogens are rapidly inactivated by UV irradiation, baculoviruses and many other insect pathogens produce structures or spores that enable them to survive outside of their hosts for prolonged periods, months or sometimes years in the case of baculoviruses, when they are in protected habitats. Thus,

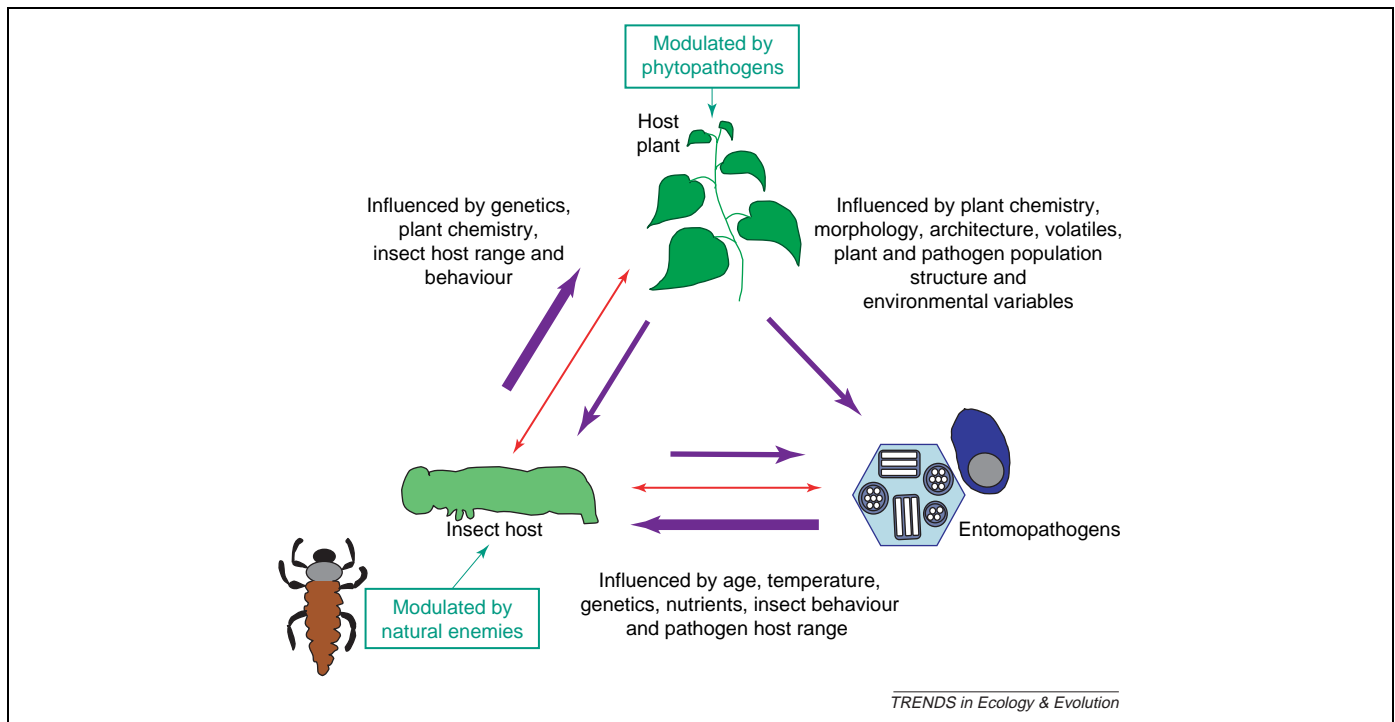


Figure 2. The theoretical framework in which insects, plants and entomopathogens can interact and the factors that influence the outcome. Arrow size represents the probable strength of the response and the double-headed arrows indicate where coevolution is expected. Whereas the plant can directly influence pathogen infectivity and viability, entomopathogen influence on the plant usually occurs indirectly via its effect on the insect. Plant phytochemicals can affect entomopathogens and their hosts negatively or positively: the outcome of the interaction, in terms of insect fitness, is dictated by which player is most strongly affected by the prevailing plant chemistry. The tritrophic interaction can be further influenced by the action of other natural enemies and infection of the plant host by phytopathogens, which, in turn, are likely to affect insect behaviour and plant quality.

many entomopathogens have a significant period of contact with host plant substrates, which, in turn, influences their environmental persistence.

How can plants manipulate insect pathogens?

Many reviews of tritrophic interactions are plant focussed, with descriptions of direct plant defences and of indirect manipulations of natural enemies by plants to reduce damage from herbivores or plant pathogens [13,14]; however, the impact of these interactions on plant fitness has rarely been measured. Studies involving plants and entomopathogens tend to be centred on improving the control of insect pests with pathogens through reducing insect fitness. The concept of direct and indirect effects can also be applied to how plants affect entomopathogens with resulting consequences for insect–pathogen relationships (Figure 2).

Many entomopathogens persist in the environment and this pathogen reservoir has a major role in the infection of new hosts. The most direct way that a plant influences an entomopathogen is through the leaf surface (phylloplane) (Box 1). For example, some plants produce alkaline exudates containing basic ions (e.g. Zn^{2+} , Mg^{2+} and Ca^{2+}) that can inactivate baculoviruses, probably by the premature dissolution of occlusion bodies [15]. This is particularly the case with cotton *Gossypium hirsutum* and soybean *Glycine max* [16]. The phylloplane is also the main point of plant contact in the infection of insects by fungi [17,18]. For example, mortality of the pea aphid *Acyrtosiphon pisum* as a result of the fungus *Pandora neoaphidus* was greater on plants with reduced wax

bloom, which increased adhesion and germination of the conidia on the insect cuticle [19]. Leaf waxiness is a variable plant trait that can respond to selection in environments where fungi are major mortality factors for damaging insect herbivores. Additionally, the architecture of the plant (via the degree of shading) can influence how long entomopathogens persist before degradation by UV irradiation [15]. For example, the NPV of the winter moth *Operopthera brumata* persisted longer on Sitka spruce *Picea sitchensis* than on either common oak *Quercus robur* or heather *Calluna vulgaris* [20], thereby increasing the likelihood of virus infection for larvae feeding on spruce.

Whereas the phylloplane is likely to be the major site for direct effects of the plant on the entomopathogen, leaf phytochemicals can participate in direct antagonism (or synergism) with the pathogen. For example, biologically activated phytochemicals can bind to occlusion bodies in the larval midgut and reduce the subsequent infectivity of the virus to host insects [21]. This interaction can be modulated by the digestive processes of the insect host and, thus, could also be influenced by indirect, insect-mediated effects (Box 1).

Many examples demonstrate that insect mortality can vary up to 50-fold when entomopathogens, mainly viruses and bacteria, are ingested on different host plants [15,22,23]. Baculoviruses have been studied most extensively, primarily because they are highly pathogenic, diverse and have relatively narrow host ranges. For example, baculovirus-induced mortality can differ, depending on the plant species ingested with the viral

Box 1. Potential points of interaction of phytochemicals during infection

Figure 1 illustrates the stages at which phytochemicals could impact pathogen infection in larval Lepidoptera. Because they are the best studied group, this example shows the infection route of baculoviruses; however, several processes apply to other ingested pathogens. The gut lumen is in blue and the haemocoel (body cavity) is in pink. The peritrophic matrix lining the midgut is constantly secreted at the anterior midgut, surrounding the food bolus as it moves through the gut. The baculovirus occlusion body must be broken down and the virus particles released before infection can be initiated in the midgut. To initiate infection, virus particles pass through the peritrophic matrix to reach and bind to midgut receptors, enter the nuclei of gut cells, replicate and move into the tracheal (respiratory) system to establish a systemic infection. Phytochemicals are hypothesised to affect all of these processes via several mechanisms (a–i):

(a) Conditions on the leaf surface (e.g. pH and ionic strength) dissolve occlusion bodies (OBs), resulting in rapid inactivation by UV irradiation, reducing pathogen persistence on the phylloplane [15].

(b) Insect salivary enzymes, such as glucose oxidase, produce hydrogen peroxide, which inactivates the pathogen [57,58]. Insect saliva can also suppress the induction of allelochemicals [59].

(c) Virus OBs form aggregates with phenolics, and/or accumulate a shell of cross-linked phenolics and/or proteins, resulting in an inability to release occlusion-derived virus particles (ODVs) [21].

(d) ODVs are damaged or inactivated by phenolic binding and/or redox cycling, generation of free radicals, or other chemical processes, such that they are unable to bind to midgut receptors.

(e) The receptors are altered by similar processes as described in (d), and no longer support ODV binding.

(f) Peritrophic matrix (PM) permeability to virus particles is diminished by phytochemical impacts on its structure and/or physiology.

(g) Oxidative stress damages midgut cells to the point where they cannot support viral replication.

(h) Oxidative stress can damage infected midgut cells [40], causing them to slough off before the virus establishes a systemic infection [32].

(i) Plant-derived chemicals cross the midgut or initiate signalling cascades, which alter host physiology sufficiently to impact normal immune function, or reduce cell permissiveness to infection. Diet also alters immunity in insects and can decrease pathogen-induced mortality [47].

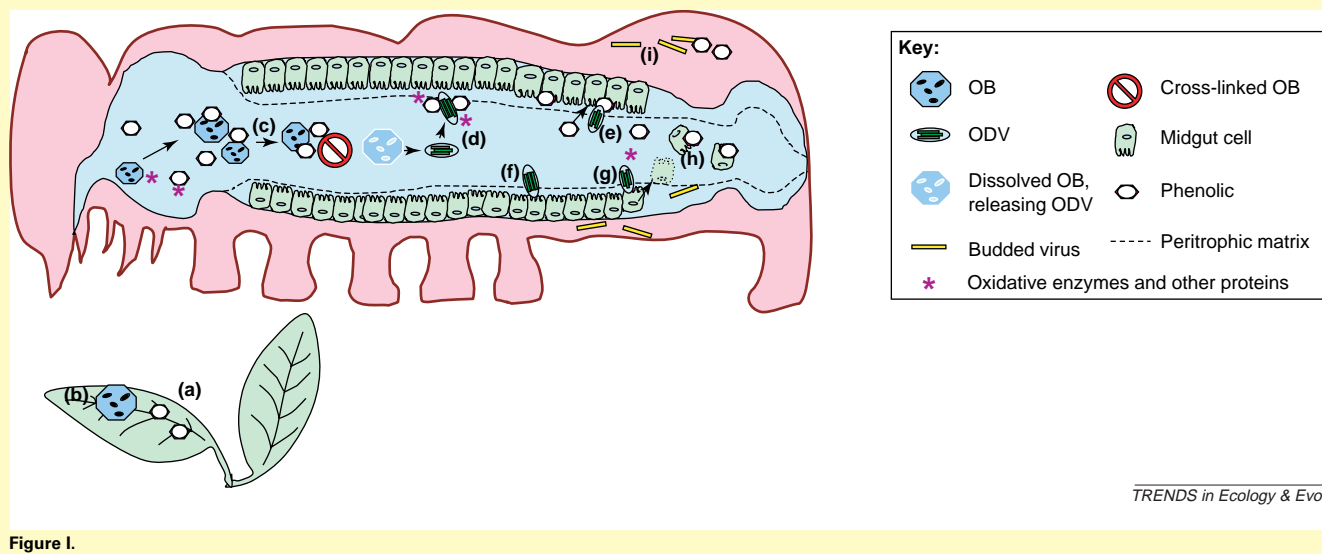


Figure 1.

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inoculum, for larvae of the gypsy moth *Lymantria dispar* [24,25], cotton bollworm *Helicoverpa zea* [26,27], beet armyworm *Spodoptera exigua* [27] and tobacco budworm *Heliothis virescens* [28,29]. In general, phytochemicals and virus must be delivered together to have an effect on mortality from virus [30–32], although preconditioning with foliage [33] or feeding on foliage throughout the instar in which the insect is challenged with virus [31] might be necessary for plant effects to occur in some systems.

The impact of plant species is not restricted to altering mortality: pathogen speed of kill can also vary, as well as the resultant yield of virus occlusion bodies [34]. For example, for *H. zea* and *H. virescens* larvae, virus yield was considerably lower (up to tenfold) on cotton, compared with soybean and crimson clover *Trifolium incarnatum* [35]. Within-plant variation can also have an impact: *Helicoverpa zea* was more susceptible to NPV on vegetative than on reproductive structures in a range of plants, including soybean and clover [26]. In addition,

intra-specific variation can be associated with altered speed of kill, virus yield and even the infectivity of progeny virus [35].

Plants can have an indirect effect on pathogen fitness through the host insect, although this has received less attention than the impact of phytochemistry at the point of infection. Indirect effects are likely to arise mainly through two routes: alteration of the susceptibility and/or the behaviour of the insect host. Many phytochemicals, especially allelochemicals and nutrients, can modify the physiology and growth of the insect host, affecting its susceptibility to infection [26]. Insect behaviour can determine whether the insect host will encounter entomopathogens; thus, increases in activity or behavioural changes that promote insect contact with pathogen reservoirs or increase feeding rate, will increase the likelihood of infection. We suspect that the insect–pathogen relationship will also vary as a function of differences in palatability among different species of host plant (or different cultivars) and that this will influence

acquisition of a lethal pathogen dose and, therefore infection rates.

Can entomopathogens be manipulated to defend plants against insect herbivores?

Plant effects on pathogens could be a by-product of variability in plant characteristics that are associated with local adaptation or resistance to natural enemies and UV damage. Alternatively, plant–entomopathogen interactions could be evolved responses. Any process by which the plant directly alters the efficacy of the entomopathogen could be manipulated by the plant, through either numerical responses, in which the entomopathogen population size is increased, and/or functional responses, in which pathogen efficacy is enhanced [12]. Plants could increase the impact of pathogens by enhancing pathogen persistence on the plant surface, increasing the encounter rate between the insect and the pathogen and altering insect susceptibility to disease [12]. These possibilities require further study.

Interestingly, plant volatiles can affect entomopathogenic fungi. The fungus *Neozygites tanajoae* produced more conidia when exposed to volatiles from leaves damaged by the cassava green mite *Mononychellus tanajoae* than when exposed to clean air [36]. However, for the pea aphid *A. pisum* and the fungus *P. neoaphidus*, infection levels did not change even though the number of conidia germinating increased on damaged broad bean *Vicia faba* plants [37]. Plant volatiles could influence the behaviour of herbivores by altering their foraging activity such that the risk of pathogen infection for the herbivore is increased [38]. A particularly intriguing study on the interaction between the green peach aphid *Myzus persicae* and a densovirus showed that plants can passively transport these viruses through the phloem, enabling infection of healthy aphids on infested leaves [39]. In essence, the plant functions as the vector of an insect

pathogen and this could increase plant fitness and be selected for if the trait has a genetic basis.

Whereas plants could manipulate entomopathogens, pathogens are less likely to be reliable as protectors of plants, as compared with predators and parasitoids, because they lack mobility and cannot respond from afar to plant cues. This could be overcome if plants could differentially attract infected insects. However entomopathogens vary in their host specificity and many baculoviruses only infect species from a narrow host range. Thus, a specific response would be required by the plant to attract the ‘right’ host species, with the added risk of attracting more herbivores in the attempt to increase the number of diseased insects. Many fungi have wider host ranges and could be better generalist herbivore control agents for plants. Combined with their reliance on plant phylloplane conditions for survival and germination, fungi might be the most promising contenders of the entomopathogens as ‘bodyguards’. The concept of plants manipulating insect pathogens to increase plant fitness remains purely speculative, although the possibility of more-complex interactions involving entomopathogens and plants does exist.

Constitutive and induced phytochemicals: a tradeoff between effects on the insect and the pathogen

Although many studies indicate that interactions between plants and entomopathogens in the insect midgut alter (usually decrease) pathogen infectivity, the mechanisms that drive these changes are not clear, in part owing to the many phytochemicals that can influence the course of disease in insects (Table 1). The behaviour of many phytochemicals has little relevance outside the context of the specific chemical mixture and the midgut environment: correlations between mortality of host insects and plant phenolic levels do not necessarily confirm causal relationships [40–42]. Phytochemicals such as phenolics,

Table 1. Phytochemical impacts on insect–entomopathogen interactions

Phytochemical(s)	Pathogen–insect interaction ^{a,e}	Effects on insect host as phytochemical level or reaction products increase	Refs
Catecholic phenolics	AcMNPV–tobacco budworm <i>Heliothis virescens</i> ^b	Increased mortality from virus and slower time to death	[29] ^f
Polyphenols:	HzSNPV–cotton bollworm <i>Helicoverpa zea</i> ^b	Decreased mortality from virus	[66] ^f
	AcMNPV–tobacco budworm ^b ; LdMNPV–gypsy moth <i>Lymantria dispar</i> (weak effect) ^b	Increased mortality from virus	[29,67] ^f
Condensed tannins	HzSNPV–cotton bollworm ^c	Decreased mortality from virus	[31] ^g
Hydrolysable tannins	HzSNPV–cotton bollworm ^b ; LdMNPV–gypsy moth ^b	Decreased mortality from virus	[24,31 ^g ,64 ^f]
Polyphenol oxidase + phenolics	AcMNPV–tobacco budworm ^b	Minor decrease in mortality from virus and/or no effect	[28,29] ^f
Peroxidases + phenolics	HzSNPV–cotton bollworm ^d	Decreased mortality from virus	[21] ^d
	<i>Bacillus thuringiensis</i> (<i>Bt</i>)–cotton bollworm ^c	Enhanced mortality by <i>Bt</i>	[68] ^d
Reactive oxygen species, free radicals	AcMNPV–tobacco budworm ^b	Decreased mortality from virus	[28 ^f ,29 ^f ,41 ^{d,f}]
Alkaloids (tomatine)	AcMNPV–tobacco budworm ^c	Decreased mortality from virus	[41] ^f
Furanocoumarins (xanthotoxin)	<i>Nomuraea rileyi</i> –cotton bollworm ^c	Reduced mortality by fungus	[69] ^g
	<i>Variomorpha</i> (Microspora) sp.–cabbage looper	Longer survival times	[70] ^g
	<i>Trichoplusia ni</i> ^c		

^aMethods vary from virus delivered with foliage but insects reared on artificial diet before and after challenge with virus (i.e. simultaneous exposure^b), to exposure to foliage before, during and after exposure to virus (i.e. exposure throughout^c), with exposure referring to delivery of the virus with respect to ingestion of foliage, to the pathogen being treated *in vitro* before being used to challenge the host insect^d.

^eBaculovirus nomenclature is based on the host from which the virus was first isolated. The host species is abbreviated to a two-letter code (accepted species) or a four-letter code (putative species), usually based on the first two letters of the latin binomial. NPVs can have virus particles containing either multiple (MNPV) or single (SNPV) nucleocapsids.

^fBioassays on foliage; phytochemical(s) implicated by correlation or functional relationship.

^gBioassays using diet incorporation of phytochemical(s).

Box 2. The gypsy moth–NPV story: complexity in interpreting tritrophic interactions

The gypsy moth *Lymantria dispar* (Figure 1) feeds on a variety of deciduous tree species in which feeding damage can induce chemical changes that, in turn, negatively affects insect growth and reproduction [60]. *Lymantria dispar* NPV is commonly associated with the outbreak and then collapse of gypsy moth populations. This system has been the focus of intense empirical and theoretical study on the role of pathogens in driving insect outbreaks [61,62].

Bioassays have shown that virus-induced mortality varies with the host plant ingested with the virus, and this effect is correlated with levels of hydrolysable tannin (polyphenol) [24]. Thus, a potential tradeoff of opposing effects exists, such that high polyphenol levels can negatively affect the insect, whereas the same chemicals can protect their larvae from disease. Interactions between plant quality, the insect and NPV were predicted to destabilise gypsy moth dynamics at intermediate tannin levels [63]. Higher levels of defoliation of red oak *Quercus rubra* were associated with lower levels of NPV-induced mortality in assays of field-collected foliage within the same season, suggesting that phytochemical induction provides a refuge from disease for the insect [64]. The mechanisms responsible, however, were not clear.

Correlations were also found between increased foliar gallotannin levels and increased insect resistance to NPV, but only in the following year. It is not clear whether this was a result of induced gallotannins, variation in constitutive levels of phytochemicals, or other causes. This relationship was re-examined using transmission experiments in the field. No differences in hydrolysable tannin levels in oaks were found as a result of defoliation by gypsy moth larvae within the same larval period

(\approx 1 month), and no differences in virus transmission were observed [65]. Further field studies found no difference in transmission levels later in the year (although allelochemical levels were not measured) [48].

Thus, whether induced plant responses have a role in the long-term interaction between gypsy moths and NPV in natural populations is still not clear. Part of the reason might be the difficulty of extrapolating from laboratory studies to the field, where the infection dynamics might be different, particularly in relation to the numbers of pathogen propagules ingested. Our understanding of the influence of induced and constitutive phytochemicals is only likely to be resolved by the detailed monitoring of all three players at a range of temporal and spatial scales in the field, combined with laboratory studies that more closely represent field conditions.



Figure 1. The gypsy moth *Lymantria dispar* larva. Reproduced with permission from Jim McNeil.

especially oxidised phenolics and other reactive products produced during redox cycling, are hypothesised to alter many steps of the infection pathway (Box 2). If a generalisation can be made, it is that the outcome will be a tradeoff between the insect and the pathogen as to which is most affected by the phytochemicals. Most allelochemicals appear to interfere with pathogen infection at the level of the fore- and midgut, in some cases via redox cycling. In the field, however, these same allelochemicals can reduce larval growth and development rates, and could make the insects more susceptible to disease, possibly via oxidative stress (Figure 2).

Induced plant responses are perhaps the most fascinating area for tritrophic interactions. However, their impacts on entomopathogens are poorly studied and the findings equivocal. The system that has received the most attention is the gypsy moth and its NPV (Box 2). Rapid and delayed induced plant responses can have negative and positive impacts on individual herbivore performance [43] but the net results of these effects have not been described for herbivores in relation to their pathogens. Reduced plant suitability is usually accompanied by a decline in herbivore growth rate (or relocation), although this can be compensated for by increased feeding. The potential consequences of induced plant changes for entomopathogen–host dynamics are complex and interpretations must be made with care. For example, the induction of plant peroxidases in cotton and tomato using Lepidoptera reduced baculovirus mortality in one study [28] but not in another [35]. The difference between these two studies lies in their methodology; the first focussed on the influence of specific induced allelochemicals at the time of pathogen challenge in the midgut, although insects were otherwise reared under identical

conditions on artificial diet. In the second experiment, larvae were reared on their respective plants until after pathogen challenge. Thus, changes in insect growth rate (larvae always weighed less on wounded plants) might have obscured the specific influence of induced chemicals on initial infection in the midgut. Smaller insects often require lower pathogen doses to initiate infection and if insects eat more to compensate for reduced plant quality, they would also increase their risk of exposure to a lethal pathogen dose.

Tradeoffs in plant effects also occur in tritrophic interactions involving parasitoids [44,45]. Induced changes in plant quality that could affect insect–entomopathogen interactions also occur from responses to herbivore attack belowground. Both above- and belowground herbivory can stimulate soil microbial activity, plant nitrogen acquisition and plant growth [46]. Diets with higher levels of nitrogen can influence pathogen-induced mortality and larval feeding behaviour [47]. Thus, multitrophic induced plant responses add another level of complexity to the insect–pathogen balance. Induced responses might not necessarily benefit the plant as they could reduce the ability of the natural enemy to act as a plant ‘bodyguard’ and the outcome of these interactions will be context dependent.

Can host plant variability influence insect–pathogen population dynamics?

Host plant species can differentially affect pathogen traits, such as the speed of kill, productivity and resulting host mortality, in addition to altering insect growth, condition and behaviour. The interaction between parameters will in turn determine insect–entomopathogen dynamics. Whether plant variation influences the development of

disease epizootics or the dynamics of insects and their pathogens in natural populations remains undetermined. Long-term data sets on insects and their pathogens are rare. Temperate forest Lepidoptera, many of which are polyphagous, are among the most-studied groups in terms of population dynamics and disease epizootics, however, there is no indication of host plant effects. A particularly good example, the western tent caterpillar *Malacosoma californicum pluviale*, undergoes regular population cycles every eight to ten years in south-western British Columbia and NPV epizootics are associated with population declines [48]. Although some spatially distinct populations feed on different host-plant species, no obvious difference in insect population fluctuations occurs (although there is no evidence that host plant can influence NPV-induced mortality [49]).

Only one study has examined the role of host plant species on entomopathogen transmission in the field. Bioassays indicated that gypsy moth larvae were more susceptible to NPV on white oak *Q. alba* than on red oak *Q. rubra*. However larvae also fed more on red oak and therefore would potentially ingest more virus on that host species [50]. These two effects cancelled each other out, and viral transmission on the two tree species was the same. A key factor here might be pathogen concentration: insects can release millions of propagules when they die from an entomopathogen infection and this could result in susceptible larvae consuming high doses that would overwhelm any inhibitory effects of the host plant. By contrast, most studies on insect–pathogen interactions are carried out in the laboratory with much lower doses, thus results do not necessarily extrapolate to natural populations.

Plant quality and insect resistance to pathogens

From an evolutionary perspective, a fascinating recent finding is that the costs of resistance to *B. thuringiensis* in the cabbage looper moth *Trichoplusia ni* vary with host plant species, and the size of these costs increase as the suitability of the plant as a food source declines [51]. If the costs and even development of resistance are context dependent, this has wide implications for the use of entomopathogens as bioinsecticides, and for the interplay between host resistance and pathogen virulence in natural populations. Preliminary evidence also suggests that entomopathogen populations become specialised on different host plants. Herbivorous insects can also become specialised on different host plant species [52]. Is it possible that there is a coevolutionary interaction between the entomopathogen and the plant?

At first, this might seem unlikely because most entomopathogens cannot infect plants. However, many pathogens are ingested with plant material and persistence on the plant surface is often a major component in the entomopathogen life cycle. Insect pathogens, particularly baculoviruses, also exhibit high levels of genotypic and phenotypic variation [53]. Infection of the pine beauty moth *Panolis flammea* with two NPV genotypes revealed that mortality varied depending on the host plant–genotype combination [54]. This implies that virus populations could become adapted to the locally abundant

host plant. Results from another field system, *M. c. pluviale* and its NPV, support this (Figure 1 [49]). Virus isolates performed better, as shown by a greater speed of kill, on the host plant from which they were isolated [49]. Although the mechanisms behind these findings are as yet unknown, they could relate to changes in the virus that reduce the binding of phytochemicals to either the occlusion body or the virus particles. It does suggest, however, that the relationship between the plant and the pathogen is far from static.

Complexity and future challenges

Thus far, only the tip of the pyramid of complex multi-trophic interactions has been exposed. The biggest challenge is to address if and how host plants mediate entomopathogen infection in field populations at the individual and population levels. Questions for future exploration include: what is the net result of the balance between plant-mediated suppression of infection and phytochemical stressors on the insect host? Does the diet choice of the insect affect the likelihood of surviving pathogen challenge? Can different host plants influence the development and frequency of disease epizootics and at what scale? From a plant-centred viewpoint, it is now apparent that there are several mechanisms by which plants could enhance the effectiveness of pathogen populations for their benefit, but this concept needs to be tested directly through the determination of whether plant-mediated changes in pathogen efficacy can increase plant fitness by protecting plants against insects.

It is not difficult to envisage more complex, naturally occurring interactions, such as plant pathogens indirectly influencing entomopathogen-induced mortality [55]. Interactions between more mobile natural enemies, possibly modulated by plant volatiles, are also likely to influence the distribution of pathogen propagules and pathogen encounter rates [56]. However, this also raises the issue of how enhancing entomopathogen efficacy might interact with the effectiveness of other natural enemies and the net effect on plant and herbivore populations. Although entomopathogens, with their difficulties of detection and identification, present new challenges to the tritrophic paradigm, they also cannot be overlooked as important players. Only through consideration of these interactions will it be possible to understand the impacts of entomopathogens in the complex web of plant–herbivore–natural enemy relationships.

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