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The Parasitoid Factor in the Virulence and Spread of Lepidopteran Baculoviruses

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Abstract: Insect parasitoids and baculoviruses play important roles in the natural and strategic biological control of insects. The two parasites are frequent competitors within common hosts and much research has focused on the negative impact that baculoviral host infections have on parasitoids. This review summarizes the impacts that parasitoids may have on the virulence and spread of lepidopteran baculoviruses. By changing host behavior and development, parasitoids have been shown to decrease baculovirus virulence and productivity within parasitized baculovirus-susceptible hosts; however, studies of the tools used by hymenopteran parasitoids to overcome their hosts' immune systems, suggest that parasitoids may, in some cases, facilitate baculoviral infections in less susceptible hosts. Laboratory and field research have demonstrated that parasitoids can mechanically transmit baculoviruses between insects, and in this way, increase the efficacy of the viruses. Instances of new, more virulent isolates of baculoviruses have been recorded from specifically parasitoid-targeted hosts suggesting other possible benefits from the transmission or activation of baculoviruses by parasitoids.

Key words: Insect parasitoid; Baculovirus; Interactions

Baculoviruses and insect parasitoids are both common, highly evolved parasites of insects; each with survival strategies that allow them to target, damage and kill specific insect species while using the host tissues to multiply. Baculoviruses have been found in hundreds of primarily lepidopteran insect species in which they cause persistent infections and epizootics; and tens of thousands of parasitoid species have been identified, many of which also play key roles in suppressing host population densities. The use of baculoviral pesticides and indigenous or introduced parasitoids to suppress agricultural pests are recognized options in integrated pest management programs. Interaction between baculoviruses and parasitoids within common hosts is inevitable and early research primarily assessed the negative effects that the baculovirus may have on the parasitoid in this competition, mostly by killing the host before the parasitoid is able to develop (6, 21). Less frequently addressed is how the parasitoid is influencing the efficacy of an indigenous or applied baculovirus. Although the baculovirus-parasitoid relationship is not yet fully understood, research is unfolding a tapestry of interesting and complex interactions. Laboratory

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and field assessments have verified that parasitoids can mechanically transmit lepidopteran baculoviruses between hosts (33, 45). More recent studies have begun to recognize the ways in which hymenopteran parasitoids can manipulate the host's immune systems, suggesting the facilitation of viral pathogenesis in previously less susceptible hosts (54, 57). This review will attempt to summarize the recorded and potential impact that primarily hymenopteran parasitoids may have on specifically lepidopteran baculoviruses.

THE BIOLOGY OF THE TWO PLAYERS

Classified in the family Baculoviridae, baculoviruses are characterized as enveloped rod-shaped virions containing a circular double-stranded DNA genome that replicate in lepidopteran, dipteran and hymenopteran hosts (19). Although there are other types of viruses that infect insects, baculoviruses are the most frequently studied and implemented as pest management strategies due to their host specificity, pathogenicity and relative environmental persistence. The baculovirus family includes alphabaculoviruses (lepidopteran-specific nucleopolyhedroviruses, NPV) and betabaculoviruses(lepidopteran-specific granuloviruses, GV) which possess multiple or single virions within proteinaceous polyhedrin and granulin occlusion bodies, respectively (31). These baculoviruses are phylogenetically separated, based on genome sequence analyses, from the genera Gammabaculovirus (hymenopteranspecific NPV) and Deltabaculovirus (dipteran-specific NPV) (25, 31).

Lepidopteran baculoviruses cause infections in the larval host stages and generally result from the ingestion of viral occlusion bodies. Within susceptible insect hosts, the viral occlusion bodies dissolve in the gut and the occlusion-derived virus initiate infection in the midgut epithelial cells. Budded virus then moves into and replicates in other susceptible tissues. When a fatal infection occurs, characteristically the integrity of the infected larval lepidopteran host integument is lessened, resulting in the dissolution of the host tissues, releasing fresh new occlusion bodies on the surface of the host plant. The susceptibility of the exposed host insect and the ultimate phenology of the infection is a function of the virulence of the virus, the developmental status of the insect, stress imposed by the environment, and the dose of the viral inoculum. Consequently, not all infected hosts simply dissolve in a pool of virus. Covert baculoviral infections are present, and possibly frequent, in wild lepidopteran populations in which the virus may exist as latent (non-replicating) or persistent, sublethal infections (7, 32).

Parasitoids, primarily found in the orders of Hymenoptera and Diptera, are insects that parasitize invertebrate hosts, feeding on hosts tissues for most of their immature life cycle, but generally exist separately to reproduce as adults. Parasitoids are usually quite host specific, seeking and choosing hosts based on kairomonal cues found associated with the host or host plant, and inserting eggs inside, or on the surface, of host eggs, larvae or pupae, depending on the biology of individual parasitoid species. The resulting immature parasitoids may develop inside (endoparasitoid) or outside (ectoparasitoid) of the hosts feeding as solitary or gregarious species on host tissue or hemolymph, either immediately arresting host development or allowing the host to continue to feed and grow. In most cases the host is killed when, or shortly after, the parasitoid emerges. Parasitoids may

also feed on the host after piercing the body with the ovipositor (52).

BACULOVIRUS AND PARASITOID IMPACTS ON THE HOST

Invading parasites can initiate apoptosis in insect hosts in which superfluous, altered or malignant insect cells are eliminated by autodigestion of the cell by endogenous proteases (37) and lepidopteran larvae can slough baculovirus-infected midgut cells in order to defend themselves from a midgut initiated viral infection (53, 55). Baculoviruses have been found to contain two different anti-apoptotic genes which can block virus-induced host apoptosis and allow the virus to cause disease in the host (37, 20).

Many of the hymenopteran parasitoids in the families Ichneumonidae and Braconidae, produce venoms, polydnaviruses and/or ovarian proteins that are injected into the host together with the parasitoid egg(s). Polydnaviruses, which replicate in the parasitoid ovaries, infect but do not replicate in the host=s cells (56). Polydnaviruses have been shown to suppress host cellular immune responses, inhibit feeding, cause changes in hemocyte count or behavior, cause the appearance of new hemolymph polypeptides, inhibit phenoloxidase activity, prolong or arrest development, and influence hormone levels in hosts (1, 49*)*. Expression of the polydnaviral impact on parasitoid hosts varies and ranges from transient to continuous and may be delayed, or initiated within hours, post parasitization (11, 13, 50, 56).

In healthy insect hosts, defensive encapsulation begins with host granulocytes attaching to the surface of the invading foreign material where they lyse, releasing their contents over the surface. This allows the attachment of plasmatocytes, multiple layers of which form a capsule (56). Polydnaviruses in susceptible and semi-susceptible insect hosts have been recorded to induce host granulocytes to undergo apoptosis, host plasmatocytes to lose their capacity to adhere to foreign surfaces and to reduce the levels of host humoral defense by lowering lysozyme expression and inhibiting the synthesis of enzymes in the melanization process (48, 56). Hymenopteran parasitoid venoms have also been found to contain peptides, proteins and enzymes that can act to disrupt host cells and inhibit host defenses (57). A venom protein extracted from, *Pteromalus puparum*, an endoparasitoid of *Pieris rapae* which does not have a polydnavirus, was found to inhibit the spreading behaviour and encapsulation ability of host hemocytes (57).

IMPACT OF BACULOVIRUSES ON PARASITOIDS

Research has investigated whether the tactics of baculoviruses and parasitoids which enable them to overcome host defenses, also have the potential to impact one another. Parasitoids are not susceptible to infection by baculoviruses but are frequently reduced in size in virus-infected hosts (39) or killed due to the premature virus-induced mortality of the host (3). Parasitoid larvae have been found encapsulated in baculovirus-infected hosts (29). In 2006, Luo and Pang (37) demonstrated that a baculovirus expressing anti-apoptotic gene (s) inhibited parasitoid polydnavirus-induced host granulocyte apoptosis, which resulted in the parasitoid larvae becoming encapsulated by host blood cells. The degree of encapsulation of the parasitoids in this study was related to the time after parasitization, as the percentages of polydnavirus-induced

apoptosis in the total hemocyte population declined over time (37). A wild-type and recombinant *Autographa californica* multiple NPV was also reported to express a cathepsin-like protease that decreased the survival of the braconid parasitoid *Cotesia marginiventris* emerging from tobacco budworm larvae *Heliothis virescens* if the host was infected with the virus less than 72 h post parasitization (41). Consequently, an earlier general conclusion among researchers that postponing the exposure of parasitized larvae to a host baculovirus increased the percentage of successful parasitoid development, may be a function of more than just enabling the parasitoid sufficient time to develop and emerge from infected tissue before imminent virus-induced host mortality (3, 5, 14, 30).

As a baculoviral host infection can decrease the survival of a parasitoid, it is apparent that it would be in the parasitoid's best interest to avoid infected hosts in the field. Research to date has shown that many, although not all, hymenopteran parasitoids are able to discriminate to varying degrees, between infected and non-infected hosts (8, 16, 17, 35). For example, both *Venturia canescens* and *Meteorus gyrator* females were shown to reduce the number of eggs that they inserted into betabaculovirus-infected Indian meal moth, *Plodia interpunctella* and *Lacanobia oleracea* larvae, respectively, based on the level of the host=s infection (38, 45).

IMPACT OF PARASITOIDS ON BACULOVIRUSES

As hymenopteran parasitoids can suppress their hosts' immune responses (23, 49), at least temporarily, it is possible that parasitoids could allow a baculovirus to infect and possibly kill a host that would normally be more resistant to the infection (54, 56). To date, however, there is no evidence that the pathogenicity of baculoviruses increases in already highly susceptible (permissive) hosts that have been parasitized by a polydnavirus-equipped hymenopteran parasitoid (Table 1). For example, Murray *et al* (40) reported that the LC_{50} for cotton bollworm larvae, *Helicoverpa armigera* NPV at two days post parasitization with *Microplites demolitor* was higher in parasitized versus non-parasitized hosts. Fall armyworm, *Spodoptera frugiperda* larvae were not more susceptible to infection by the SfMNPV when parasitized by *Chelonus insularis* or *Campoletis sonorensis* as determined by lethal concentrations and time to mortality, although both parasitoids inject a polydnavirus at oviposition (16). Santiago-Alverez *et al* (46) noted that a difference between the decreased virulence of *Agrotis segetum* GV in the host larvae parasitized by *Apanteles telengai* versus the retained virulence of the same virus in *Campoletis annulata* parasitized larvae may be due to the fact that the host moulted twice before the emergence of the first parasitoid and only once when parasitized by the second, with the moult possibly clearing the viral infection in the midgut epithelium (15). It is important to remember that parasitoids also affect the host=s development by inhibiting feeding or weight gain or by prolonging or arresting development (3) and consequently they impact the nutrient levels of various host tissues (1). As most of the baculovirus polyhedra production takes place in tissues that are the most nutrient rich such as the fatbody (1, 49), the parasitoid may reduce the replication, genetic composition and virulence of co-habitating lepidopteran baculoviruses (18, 49).

Table 1. Parasitoid impact on lepidopteran baculoviral virulence

Parasitoid	Virus[a]	Host larvae	Original host susceptibility[b]	Order of exposure[c]	Impact of parasitoid on virulence of virus	Reference
Braconidae						
Apanteles glomeratus	PbGV	*Pieris brassicae*	P	Par=d first	None; decreased at one level	(26)
Apanteles telegai	AzGV	*Agrotis segetum*	P	Par=d first	Decreased virulence	(46)
Meteorus gyrator	LoGV	*Lacanobia oleracea*	P	Par=d first	Decreased virulence	(38)
Meteorus gyrator	LoGV	*Lacanobia oleracea*	P	GV first	Nothing significant	(38)
Chelonus insularis	SfMNPV	*Spodoptera frugiperda*	P	Par=d first	None; decreased productivity	(18)
Microplites croceipes	HzMNPV	*Heliothis zea*	P	NPV first	None; increased time to death	(14)
Microplites demolitor	HaNPV	*Helicoperpa armigera*	P	Par=d first	Decreased virulence	(40)
Cotesia congregata	R-AcMNPV	*Manduca sexta*	Semi-P	Par=d first	Increased virulence	(54)
Chelonus inanitus	AcMNPV	*Spodoptera littoralis*	NP	Par=d first	Increased virulence	(44)
Ichneumonidae						
Campoletis annulata	AsGV	*Agrotis segetum*	P	Par=d first	Nothing significant	(46)
Campoletus sonorensis	SfMNPV	*Spodoptera frugiperda*	P	Par=d first	Nothing significant	(16)
Hyposoter exiquae	TniNPV	*Trichoplusia ni*	P	Par=d first	Decreased virulence	(2)
Campoletis sonorensis	*R*-AcMNPV	*Heliocoverpa zea*	NP	Same time	Increased virulence	(53)

[a]baculovirus as described in reference, not necessarily as recognized by ICTV; [b]P = permissive, NP = non-permissive; [c]Par=d = parasitized.

In other studies, Washburn *et al* (53, 54) evaluated the impact of a recombinant virus based on the broad spectrum *Autographa californica* alphabaculovirus, AcMNPV, on a non-permissive host, the corn earworm, *Helicoverpa zea* and a semi-permissive host, the tobacco hornworm *Manduca sexta* when parasitized by *C. sonorensis* and *Cotesia congregata*, respectively, by injecting the parasitoids' polydnavirus into the hosts (Table 1). Both hosts became susceptible to infection by the baculovirus which the authors attributed to the impact of the polydnavirus on the host cellular immune response. Similarly, the parasitoid *Chelonus inanitus* polydnavirus increased the susceptibility of the Egyptian cottonworm, *Spodoptera littoralis* to AcMNPV although the species is normally highly resistant to infection by the virus (Table 1) (44).

It has also been suggested that the within-host competition between the parasitoid and the baculovirus could increase the virulence of the virus or activate a latent host virus, as the two parasites are competing for the host resources (18, 51). When *S. frugiperda* larvae were exposed to parasitism by both *C. insularis* and SfMNPV, one genetically distinct isolate of the virus was found in parasitized hosts that showed increased virulence and pathogenicity (18). Stolz and Makkay (51) found a betabaculovirus disease developed in cabbage looper *Trichoplusia ni* larvae that were parasitized by *Hyposoter exiguae* while viral disease was never associated with the unparasitized larvae and the authors hypothesized that a sublethal infection in the *T. ni* larvae developed into overt disease as a consequence of parasitism by the *H. exiguae*.

PARASITOID TRANSMISSION OF BACULOVIRUSES

Years of research assessing the ability of parasitoids to physically move host baculoviruses from one host to another clearly demonstrate that parasitoids are able to mechanically transmit host baculoviruses (3, 4, 8, 14, 27, 30, 33-35, 38, 39, 39, 42, 45, 58, 59). Earlier transmission studies did not use molecular tools to assess the transmission and relied on host virus-induced mortality to verify transmission by the parasitoid

which probably underestimated the incidence of sublethal parasitoid-mediated transmission. Low, sublethal baculoviral infections in lepidopteran larvae that allows emergence of adults are probably common (7, 32). The repeated evidence that host baculoviruses can be mechanically transmitted between infected hosts indicates that the parasitoids can increase the impact of a host baculovirus treatment by spreading it to other uninfected hosts over time and space. It has been recognized that in a similar manner, parasitoids can also function as mechanical vectors of other pathogens, such as microsporidia, bacteria and fungi (43) and other types of viruses (36).

The transmission of the baculoviruses occurs when the ovipositor or parasitoid body surface or gut becomes contaminated (30). In overtly infected hosts where a high proportion of the virus is occluded, simple horizontal transmission of the virus by surface contamination of feeding surfaces would be necessary to infect other susceptible insects, as the virus needs to be ingested and passed through the alkaline environment of the host=s midgut to dissolve the protein coat and release the infectious virions (45). The mechanical transmission of baculoviruses on a contaminated parasitoid ovipositor inserted into host hemocoel would need to transfer occlusion derived or budded virions to cause an infection.

Parasitoid-mediated baculoviral transmission may also have an important impact on baculoviral virulence. Isolates of NPV rarely contain a single genotype and infections by more than one virus genotype might be beneficial to the virus as the host immune system may be more stressed and therefore overcome by having to fight multiple infections (10, 28). It has also been suggested that cross-infection of a host insect with a dose of a heterologous virus may activate a persistent host virus (9). In other cases, the host insect may be resistant to oral infection by the virus but susceptible to budded virus injected into the host (24). The introduction of the viral factor that results in these overt viral infections could theoretically be carried out by a surface contaminated parasitoid ovipositor and may explain mysteries such as the new, highly virulent multiple NPV variant of unknown origin, but not considered to be a latent host virus, found in *T. ni* larvae following parasitization with the parasitoid *Cotesia marginiventris* (22).

Passive mechanical transmission of baculoviruses involves a susceptible host insect chancing on and consuming the virus and would be expected to be more likely under high population densities (32). As the parasitoids are host specific and seek out hosts into and onto which they would transfer the virus, parasitoid transmission of baculoviruses is more targeted. Theoretically this type of transmission would be expected to be of higher importance to the natural spread of baculoviruses in low host population densities (9, 45).

FIELD EVIDENCE OF A PARASITOID FACTOR

Field studies that specifically address the impact of parasitoids on baculovirus efficacy and not just the impact of the virus on the parasitoid, are rare and some of the implications are still untested. In 1915 it was suggested that the gypsy moth, *Lymantria dispar* MNPV was unintentionally introduced into the USA with imported parasitoids and the incidence of the alphabaculovirus was found to be positively correlated with the incidence of parasitoids in populations of *L. dispar* in the eastern USA (6, 43).

When LdMNPV was released in experimentally established populations of disease free larvae, the spread of the virus could only be attributed to the dispersal of larval gypsy moth for the first few weeks (12) and it was hypothesized that the spread of the NPV may be due to mechanical vectoring by a parasitoid. A correlation was similarly made between outbreaks of the betabaculovirus associated with the western grape leaf skeletonizer, *Harrisina brillians* in California (47) and the presence of two common parasitoids. The ability of the parasitoids to transmit the virus was later supported in controlled laboratory trials (33, 42).

In the few field or semi-field trials in which of the impact of a parasitoid on a baculovirus has been recorded, the parasitoids appear to have positive effects on viral efficacy and overall host suppression. For example, in greenhouse trials with LoGV treated tomato plants, the braconid, *Meteorus gyrator* was shown to: discriminate between healthy and infected *L. oleracea* larvae; mechanically transmit the virus at a low level; significantly increase total host mortality in combination with the virus; and decrease tomato damage (38). *Apanteles glomeratus* released in field plantings of brussel sprouts appeared to discriminate between PbGV-infected and healthy *Pieris brassicae* larvae and were shown to accelerate the initial spread of the betabaculovirus, although no increase in the number of virus-induced mortalities was recorded (27). The braconid *Microplites croceipes* was shown to mechanically transmit *Heliothis* NPV to *Heliothis virescens* (4) and in both single plant and large cage soybean field tests, viral-induced mortality in *H. virescens* larvae occurred when HvNPV contaminated *M. croceipes* were released onto infested plants (59). The resulting transmission resulted in viral-induced host mortality that was shown to be a function of the density of the parasitoids released.

CONCLUSIONS

Although laboratory research indicates that hymenopteran parasitoids can reduce the pathogenicity of baculoviruses in susceptible hosts, an albeit small number of field research trials assessing the impact of the parasitoid on the baculovirus to date, indicate that this within host impact does not appear to reduce the overall impact of an applied baculovirus on host mortality. Parasitoids actively spread or accelerate the spread of the virus within susceptible hosts and this ability has been demonstrated to result in increased baculoviral efficacy under field conditions. Host baculoviral infections obviously have the capacity to lower parasitoid population densities, however many parasitoids appear to have the ability to avoid or reduce their use of virus-infected hosts and it is probable that a strategically timed baculoviral biopesticide may have low impact on host parasitoid populations. The emerging research on how parasitoids suppress host immune responses evokes many questions relating to the ways in which they may influence lepidopteran baculoviral infections, including how parasitoids may impact the susceptibility of non-target Lepidoptera to field treated baculoviruses. Parasitoids are expected to occur more frequently in ecosystems where baculoviruses are replacing more broad spectrum chemical insecticides as pest management strategies and it is important for researchers to continue to evaluate, integrate, and maximize the efficacy of both parasites.

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