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Modelling the within-host growth of viral infections in insects

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Abstract

Insects are infected by a variety of pathogens, including bacteria, fungi and viruses, which have been studied largely for their potential as biocontrol agents, but are also important in insect conservation (biodiversity) and as model systems for other diseases. Whilst the dynamics of host-pathogen interactions are well-studied at the population level, less attention has been paid to the critical within-host infection stage. Here, the reproductive rate of the pathogen is largely determined by how it exploits the host; the resources supplied by the host in terms of size and condition; competition with other pathogens; and the speed with which it kills the host (death being an inevitable outcome for obligate-killing pathogens). In this paper we aim to build upon recent developments in the literature by conducting single infection bioassays to obtain data on growth and fitness parameters

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for phenotypically different and similar strains of nucleopolyhedroviruses in the Lepidopteran host *Spodoptera exigua*. Using these data, a simple mechanistic mathematical model (a coupled system of differential equations) is derived, fitted and parameter sensitivity predictions are made which support empirical findings. We unexpectedly found that initial growth of virus within the host occurs at a double-exponential rate, which contrasts with empirical findings for vertebrate host-pathogen systems. Moreover, these infection rates differ between strains, which has significant implications for the evolution of virulence and strain coexistence in the field, which are still relative unknowns. Furthermore, our model predicts that, counter to intuition, increased viral doses may lead to a decrease in viral yield, which is supported by other studies. We explain the mechanism for this phenomenon and discuss its implications for insect host-pathogen ecology.

Keywords: Lepidoptera, nucleopolyhedrovirus, consumer-resource dynamics, biocontrol, baculovirus

1. Introduction

Pathogens play an important role for many host organisms, ranging from population regulation [1] to species invasion [2]. These in turn, have applications for our understanding of issues such as disease control [3], pest control [4] and biodiversity [5]. However, much of our empirical understanding of host-pathogen ecology and evolution is derived at the population level (see Dwyer et al. [6] for example), and the crucial pathogen stages operating within the host are either simply over-looked or assumed to be non-dynamic, whereas, in reality, key pathogen fitness and virulence traits are often de-

10 terminated throughout the course of infection (e.g. environmental conditions).
11 For example, in the case of monarch butterflies, *Danaus plexippus*, rearing
12 infected larvae on different plant species alters parasite infection, replica-
13 tion and virulence [7]. This illustrates that population dynamics of hosts
14 and pathogens will be subject to feedback mechanisms from the pathogen
15 dynamics within the host.

16 This point is now being addressed by a number of authors, especially via
17 theoretical means. For example, Antia and Lipsitch [8] proposed a mathe-
18 matical model for an acute microparasite infection in a vertebrate host. This
19 model suggested that the within-host dynamics of the microparasite will be
20 a ‘race’ between parasite multiplication and the clonally expanding response
21 by the host immune system, resulting either in immune-mediated clearance
22 or host death. In a mathematically similar, but biologically different system,
23 Ellner et al. [9] modelled the within-host interaction of a fungal pathogen
24 in a coral. Here the fungal-immune system dynamics are rather complex
25 and spatially explicit, highlighting the importance of ‘immune response free
26 space’ which allows local rapid growth of the fungal infection. One appli-
27 cation of these types of models has been to improve our understanding of
28 pathogen evolution, which has revealed that the dynamics of the immune
29 system may select for parasites with intermediate within-host growth rates,
30 as this is when the number of transmission stages from infected hosts reaches
31 a maximum [10].

32 A large proportion of previous theoretical models based the infection dy-
33 namics on Lotka-Volterra interactions (see Alizon and van Baalen [11] for an
34 example and references therein) or models with a fixed kill rate by immunity

35 [12] and are aimed at vertebrate hosts, largely due to the applicability to
36 human health. In contrast, little work has been carried out for invertebrate
37 systems, particularly with empirical data to test the model.

38 The dynamics of pathogens within invertebrate hosts differ significantly
39 from those of vertebrates. Firstly, many insect pathogens are obligate killers,
40 in the sense that effective horizontal transmission may only be attained by
41 the death of the host, whereas for most vertebrates the infectious stages are
42 emitted throughout the course of infection. Secondly, since the host itself is
43 simply a resource for the virus to reproduce, the size and growth rate of the
44 host is crucial in determining the speed of pathogen replication and the yield
45 of infectious stages. Lastly, common to both vertebrates and invertebrates,
46 are innate immune mechanisms that can be either constitutively expressed
47 or induced on exposure to infection [13]. However, invertebrates lack ac-
48 quired immune responses, but their mechanistically simple innate effectors
49 are functionally sophisticated and can be highly efficient [14]. Therefore, the
50 vertebrate models do not lend themselves readily to invertebrate systems.

51 Ebert and Weisser [15] proposed a model for the dynamics of the within-
52 host growth of obligate-killing parasites, such as baculoviruses, and many
53 species of bacteria, bacteriophages, nematodes, fungi and microsporidia. Their
54 model assumes that pathogen biomass grows logistically, where the carrying
55 capacity (invertebrate host mass) is time-dependent (also assumed to be
56 logistic in growth) and crucially does not depend on the extent of infection
57 within the host. It is possible, however, that as the pathogen spreads through
58 host tissues it would interfere with metabolic processes and ultimately inhibit
59 the growth of the host. In this case, the host size at the time of death, and

60 therefore the ‘pathogen carrying capacity’ of the host, should depend on the
61 extent of host tissue infection. Typically, this is not the case in vertebrate
62 infections, and so the interaction between virus replication and host growth
63 rates has not been explicitly considered. In this paper we aim to address this
64 by developing a more biologically detailed model for the within-host growth
65 of obligate killing viruses of invertebrates, which is parameterised and vali-
66 dated against experimental data.

67 We base our model on baculoviruses, a group of double stranded DNA
68 obligate killing viruses, which have been particularly well studied because of
69 their utility as expression vectors and biocontrol agents [16]. Baculoviruses
70 can be subdivided into two distinct genera, Granuloviruses (GVs) and Nu-
71 cleopolyhedroviruses (NPVs - the focus of this paper), and are indirectly
72 transmitted pathogens, persisting outside their arthropod hosts as occlu-
73 sion bodies (OBs), a proteinaceous matrix in which the virus particles are
74 embedded. The OBs may contain many virus genomes. Hosts (primarily
75 Lepidoptera) become infected by consuming OBs when eating foliage. The
76 protein dissolves in the alkaline gut of the caterpillar allowing viruses to cross
77 the gut wall and then to start replicating. Overt infections result in the death
78 of the host a few days later. Body tissues are then dissolved with millions
79 of virus particles being produced as a result. These OBs persist in the en-
80 vironment until consumed by a new host or are degraded by environmental
81 factors.

82 In this paper, we begin by describing bioassays carried out with lep-
83 idopteran hosts, in which we determine key life-history traits of the bac-
84 ulovirus and the within-host growth rate of the different strains of virus. We

85 empirically explore the possibility that the virus infection may impede host
86 growth rate, and whether this inhibition increases as the infection progresses
87 using statistical models. We then develop a novel mathematical model for
88 the within-host growth of the different strains of virus. This model incorpo-
89 rates the interactions observed and is parameterised by experimental data.
90 The ecological implications are then discussed.

91 **2. Infection Bioassays and Results**

92 *2.1. Materials & Methods*

93 *2.1.1. Insect and virus stocks*

94 *Spodoptera exigua* larvae were reared in continuous culture on artificial
95 diet [17]. Four different baculoviruses were used in this study; the Oxford
96 strain of *Mamestra brassicae* nucleopolyhedrovirus (*MbNPV*) [18], *Panolis*
97 *flammea* nucleopolyhedrovirus (*PaflNPV*) variant 4 [19], *Autographa californ-*
98 *nica* nucleopolyhedrovirus (*AcNPV*) strain C6 [20] and *Spodoptera exigua*
99 nucleopolyhedrovirus (*SeNPV*) [21]. Additional details can be found in the
100 electronic supplementary material (ESM).

101 *2.1.2. Determination of median lethal dose and mean time to death*

102 Three blocked bioassays were carried out to determine the median lethal
103 concentration (LC_{50}) and mean time to death of the four viruses in *S. ex-*
104 *igua*. Newly moulted third instar larvae of *S. exigua* were selected on the
105 basis of head capsule diameter and starved overnight at 28°C. Thirty insects
106 per treatment were then dosed by droplet feeding [22] with 1 μ l of the virus
107 concentrations specified. The time taken to administer each treatment was

108 recorded and the start time (T_0) taken as the mid-point of this. The ex-
109 posed larvae were transferred to individual pots of artificial diet and reared
110 at 28°C and checked after 24 hours, at which point any handling deaths were
111 removed. The larvae were subsequently checked every 12 hours until death
112 or pupation (if the host survived infection) and any levels of mortality and
113 time to death recorded. Details of our statistical methods can be found in
114 the ESM.

115 *2.1.3. Measurement of the within host-growth of baculoviruses*

116 Based on the data generated in the previous bioassays a virus concentra-
117 tion of 1×10^7 OBs/ml was selected for all four viruses as at this dose all
118 insects should be infected. Newly moulted third instar *S. exigua* larvae were
119 starved overnight and 200 larvae dosed with 1 μ l of either *AcNPV*, *MbNPV*,
120 *PafNPV* or *SeNPV* virus at a concentration of 1×10^7 OBs/ml. The larvae
121 were transferred to individual pots of artificial diet and reared at 28°C. Af-
122 ter 2 hours ten larvae were collected from each treatment. These were then
123 weighed and frozen at -20°C until DNA extraction. The process of weighing
124 and freezing 10 individual larvae was repeated at 12 hour intervals until all
125 remaining larvae had died from virus infection.

126 Details of the DNA extraction and quantification can be found in the
127 ESM.

128 *2.2. Bioassay Results*

129 *2.2.1. Infectivity and speed of kill of AcNPV, MbNPV, PaflNPV and SeNPV*
130 *in third instar larvae of S. exigua*

131 The mortality of third instar larvae of *S. exigua* was significantly differ-
132 ent between the viruses ($\chi^2 = 98.1$, $df = 4$, $p \leq 0.001$) although there was no
133 significant difference between the mortality induced by *AcNPV* and *MbNPV*
134 ($\chi^2 = 1.42$, $df=1$, $p=0.233$) (see Figure S1 (a) in the ESM). The mortal-
135 ity was significantly affected by dose of virus ($\chi^2 = 185.4$, $df=1$, $p < 0.001$)
136 but there was no significant interaction between dose and virus ($\chi^2 = 6.59$,
137 $df=4$, $p=0.159$). Overall *SeNPV* showed the highest mortality and *PaflNPV*
138 showed the lowest mortality, and in all cases mortality increased with dose.

139 Time to death was significantly different between the viruses (see Fig-
140 ure S1 (b) in the ESM), with a significant interaction between virus dose and
141 virus strain ($F_{4,799}=16.59$, $p \leq 0.001$). The time to death of *AcNPV*, *MbNPV*
142 and *SeNPV* decreased with increasing virus dose, however the slope of the line
143 for *PaflNPV* was not significantly different from zero ($F_{2,797}=1.355$, $p=0.259$)
144 showing that the speed of kill of this virus was unaffected by dose.

145 *2.2.2. Host growth and the within-host growth of baculoviruses in third instar*
146 *larvae of S. exigua*

147 Host weight showed distinct differences between infected and uninfected
148 insects (see Figure 1). Growth rates are curvilinear with time (minimally
149 adequate statistical models), and the degree of this curvilinearity varies
150 with virus strain (virus*time³, $F_{4,594}=3.41$, $p=0.009$) indicating that different
151 strains impede host growth to varying degrees. The uninfected larvae grow
152 to their peak in mass before a decrease in weight due to larvae preparing for

153 pupation (Figure 1 (e)). All infected larvae are smaller in mass in compari-
154 son with uninfected larvae, particularly at the later stages of infection prior
155 to virus induced death. Both *AcNPV* and *MbNPV* infected larvae showed
156 a similar decrease in host mass to controls at the latter stages of infection
157 (Figure 1 (a)-(b)), but no such effect was shown for *PaflNPV* and *SeNPV*
158 (Figure 1 (c)-(d)).

159 Taking the first 7 census host growth data points from each treatment,
160 when the \log_{10} weight grows linearly with time (Figure 1 (f) - minimally
161 adequate statistical model), the infected hosts (as one category) show a
162 significantly slower initial growth rate than their uninfected counterparts
163 ($F_{1,345} = 6.813$, $p=0.009$). Furthermore, the growth rates of control and in-
164 fected larvae were individually compared (virus*time, $F_{4,339}=4.617$, $p=0.001$)
165 indicating that NPV viral infections alter the growth of the host differentially
166 during the early stages of infection. *PaflNPV* and *SeNPV* infected larvae
167 showed significantly reduced initial growth rates compared to the uninfected
168 larvae (virus*time, $t_{1,339} = 3.645$, $p=0.0003$ and $t_{1,339} = 3.039$, $p=0.003$, re-
169 spectively). Interestingly, *AcNPV* and *MbNPV* infected individuals showed
170 no significant difference in initial growth rates when compared to their unin-
171 fected conspecifics.

172 The growth of the viruses within *S. exigua*, as measured by the proportion
173 of total DNA represented by viral DNA, also varied significantly between the
174 four viruses (see Figure 2). This relationship is highly non-linear and the
175 degree of non-linearity varies (virus*time⁵, $F_{3,594}=3.19$, $p=0.025$). All viral
176 treatments showed a log sigmoidal relationship with time, with all treatments
177 approaching an asymptotic proportion of DNA. Moreover, all treatments also

178 showed a decrease in the proportion of viral DNA shortly after inoculation,
179 with *SeNPV* and *AcNPV* showing the greatest reduction (approximately a
180 10-fold reduction). The four viruses also showed differences in the maximum
181 proportion of the host they converted to virus biomass. *AcNPV* had the
182 highest ratio of virus to host DNA with a peak of 45%. *SeNPV* was unable
183 to replicate as much viral DNA, peaking at 12.5%. *MbNPV* and *PaflNPV*
184 had the slowest speeds of kill and lowest proportion of viral DNA (8% and
185 10% respectively).

186 **3. Within-Host Virus Growth Mathematical Model**

187 Using the statistical model fitting above we have been able to demon-
188 strate differences in the growth dynamics of the 4 strains of virus within the
189 host. However, this analysis does not inform us of the importance of vari-
190 ous mechanisms and factors of viral infection. To address this we derive the
191 mathematical model below.

192 *3.1. The Model*

193 Let $H(t)$ and $V(t)$ be the mass of healthy host tissue and mass of virus
194 within the host at time t , respectively. We assume that the host is an inverte-
195 brate and thus has no acquired immunity [23]. Here, we only consider overt
196 infections where the initial dose of virus is sufficiently large such that the
197 innate immune response is negligible and cannot clear the infection, leading
198 to host death. The host grows with growth rate $r(t)$. Note that since we are
199 only interested in overt infections, it is not necessary to consider host growth
200 in the absence of infection, where the dynamics are considerably different
201 (e.g. overt infections will prevent the onset of pupation). Healthy host mass

202 is infected and converted into virus mass according to the mass action law
 203 with transmission coefficient $\beta(t)$. Here, the assumption is that all infected
 204 host tissue is converted into virus (to the best of our knowledge, it is not
 205 known if at the cellular level infected host cells produce ‘waste’, thus war-
 206 ranting a conversion efficiency parameter). These simplifying assumptions
 207 lead to the following model:

$$\frac{dH}{dt} = r(t)H - \beta(t)HV \quad (1a)$$

$$\frac{dV}{dt} = \beta(t)HV, \quad (1b)$$

208 where $H(t), V(t) > 0$ for all $t \geq 0$.

During the course of infection, the host becomes increasingly moribund and in the latter stages of infection the host almost completely stops eating and therefore stops growing. This is demonstrated in Section 2.2.2 and supported by additional and closely related findings [24]. Furthermore, we assume that increased viral loads will have greater effect on the host growth rate [24]. We model this by the following integral equation

$$r(t) = r_0 \exp\left(-a \int_0^t V(s)ds\right) \quad (1c)$$

209 where r_0 is the maximum host growth rate and a is the host growth reduction
 210 rate. Here, the growth rate decreases with the ‘experience’ of the infection.
 211 Note that for mathematical and numerical analyses it is useful to differentiate
 212 (1c) with respect to time.

In addition, we assume that as the virus converts an increasing proportion of host mass the infection rate decreases, and tends to zero as the virus proportion approaches a maximal limit, p . We model this by the functional

form

$$\beta(t) = \beta_0 \left(1 - \frac{V(t)}{p(H(t) + V(t))} \right) \quad (1d)$$

213 where β_0 is the maximum infection rate. This assumption essentially acts to
214 impose a ‘carrying capacity’ for the pathogen growth within the host since
215 the virus growth is limited by the size of the growing host. Hence, the
216 ‘carrying capacity’ is not a fixed parameter, but is dynamic with respect to
217 the interactions between the host and the virus. Note that the parameter p
218 acts as an upper limit for the proportion of virus mass within the infected
219 host, as we demonstrated empirically in Section 2.2.2. This is included as
220 not all of the available host mass may be infected (the host head capsule for
221 example), and therefore it is necessary to prescribe this limit via a reduction
222 in the potential carrying capacity. In addition, it should also be noted that
223 since $H(0) \gg V(0)$ it follows that $\beta(t) \geq 0$ for all $t \geq 0$.

Linear stability analysis (see Appendix A) of Model (1) reveals that there are an infinite number of locally stable equilibria which lie on the curve

$$\frac{V}{p(H + V)} = 1.$$

224 Thus, for given growth parameters, r_0 , β_0 and a , the equilibria obtained will
225 crucially depend on the mass of the host and the virus dose at the time of
226 infection.

227 3.2. Parameter Fitting

To fit the within-host infection Model (1) to the within-host virus growth data one must convert the proportion of virus data into virus mass data. To do this we assume that the fraction of sampled DNA that is virus DNA is

equal to the fraction of total host mass that is virus mass. Thus mathematically we have

$$\frac{\text{mass of virus DNA at time } t}{\text{total mass of DNA at time } t} = \frac{\text{mass of virus at time } t}{\text{total mass of infected host at time } t}. \quad (2)$$

228 Using the host and virus mass data we are able to fit the within-host
 229 infection Model (1) to the data and find the associated parameter values
 230 (see Appendix B for details). Here, we use some asymptotic properties of
 231 the model to find initial estimates of the model parameters and then all
 232 parameters are found simultaneously using the initial estimates. Note that
 233 we do not prescribe r_0 from the control data, but instead we find it from the
 234 simultaneous fitting on the infected data, which therefore takes into account
 235 the stochastic differences between treatments. A discussion on prescribing
 236 r_0 can be found in the ESM and Table S1. The fitted parameter values are
 237 listed in Table 1 and we compare the results of this parameter fitting with
 238 the data graphically in Figure 3.

239 From Figure 3 we see that Model (1) is an excellent fit to both the host
 240 and virus data, and we are able to capture all of the growth behaviour. All
 241 fits produce the characteristic log-sigmoidal virus growth and the sigmoidal
 242 growth of the host. However, due to the exceptionally fast speed of kill of
 243 *SeNPV* the deceleration of host growth is almost negligible, which is reflected
 244 in the low value of a . In contrast, *MbNPV* has the largest larvae at the time
 245 of death and thus the largest value for a . *PafNPV* and *SeNPV* have the
 246 largest infection rates, β_0 , whilst *MbNPV* has the smallest infection rate. The
 247 extent to which viruses can convert healthy host tissue into virus particles
 248 greatly differs between strains, ranging from approximately 2.8% for *MbNPV*
 249 to as much as 22% for *AcNPV*. The maximum host growth rate, r_0 , does not

250 change considerably between virus treatments. Moreover, the differences we
251 observe in the estimation of the initial masses, H_0 and V_0 , are largely due to
252 experimental variation.

253 *3.3. Model Predictions*

254 Using the fitted parameter values we can use Model (1) to predict the
255 effects of varying the initial dose of virus and the size of the host at the time
256 of infection.

257 In Figure 4 (a) we see that an increase in virus dose leads to a reduction
258 in the yield of virus and host size at the time of host death. At first, this
259 may seem counterintuitive, as one might expect that an increased dose may
260 lead to an increased yield. However, the mechanism behind this phenomenon
261 is a combination of two processes. Firstly, an increased dose has a greater
262 initial negative effect on the host growth rate, resulting in smaller hosts,
263 and therefore the dynamic virus ‘carrying capacity’ is reduced. Secondly, a
264 greater viral dose increases the initial infection rate and therefore the virus
265 infects a larger proportion of host more quickly, thus causing a decreased
266 yield at death. Furthermore, extensive parameter variation, such as initial
267 host size and host growth rate (not presented here), suggests that this is
268 ubiquitous under our model assumptions.

269 In Figure 4 (b) we see the effect of varying the size of the host at the time
270 of initial infection. As one might expect, in most cases, as host size increases,
271 the virus yield and the size of host at the time of death both increase. This is
272 because the size increase simply acts as an increased virus carrying capacity
273 and there is a longer period for the virus to replicate before it has a large
274 negative effect on the host growth rate. A similar scenario occurs when the

275 maximum host growth rate is increased, corresponding to an increase in the
276 host diet or environmental quality (Figure 4 (c)).

277 The effects of varying virus parameters can be seen in Figure 4 (d)-(f).
278 In Figure 4 (d) we see that, as one might expect, increasing the rate at
279 which the host growth is reduced by the virus infection causes a decrease
280 in host mass which in turn reduces the virus yield. In contrast, one might
281 expect that increasing the infection rate of the virus would increase the virus
282 yield, however, in Figure 4 (e) we see that the opposite is true. This is due
283 to the increase in maximum infection rate (β_0) causing the virus mass to
284 utilise more of the host mass more quickly, resulting in earlier saturation.
285 Therefore, the host is increasingly moribund and suffers from a reduction in
286 growth rate, final host mass and hence a reduction in virus yield. Finally, in
287 Figure 4 (f) we show the effect of varying the zero infection virus proportion,
288 p . Intuitively, we see an increase in proportion of host that the virus can
289 infect causes an increase in viral yield, which in turn reduces the host mass
290 since the host growth rate is reduced by the additional virus mass.

291 4. Conclusions & Discussion

292 It is well known that genetically similar virus strains show differences in
293 pathogenicity, speed of kill and yield [19], but here we have shown that they
294 also differ in how they may impact host growth and replicate within it. We
295 have demonstrated empirically that virus infection impedes the growth rate
296 of the host, with some viruses doing so from the early stages of infection,
297 and that this inhibition increases as the infection progresses and has conse-
298 quences for the outcome of infection. Four genotypically similar strains of

299 pathogen were found to have differences in traits associated with pathogen
300 fitness (speed of kill, pathogenicity), and to impede host growth to differing
301 degrees (parameter a varied by more than an order of magnitude). This led
302 to a lack of correlation between the standard phenotypic traits (speed of kill,
303 pathogenicity) and the efficiency with which the different viruses converted
304 host tissue to virus (for which *AcNPV* had the highest ratio of virus DNA
305 to host DNA and *MbNPV* had the lowest).

306 Using the fitted parameter values from the within-host infection model,
307 we have seen that *AcNPV* is relatively slow at infecting healthy host tissue.
308 On the other hand, it is clear from the fitted parameter values and the
309 simulations that *SeNPV* is the fastest growing virus, but it does not convert
310 a high ratio of host mass into virus mass. From these parameter-fitting
311 results we can conclude that there is a lack of correlation between initial
312 virus growth rate (β_0) and both the speed of kill and mortality. For example,
313 *SeNPV* does have the highest initial growth rate and it also has the fastest
314 speed of kill and mortality. In contrast, *PaflNPV* has the slowest speed of kill
315 and lowest mortality, but does not have the lowest initial virus growth rate
316 (this belongs to *MbNPV*). This would suggest that one cannot predict speed
317 of kill, mortality and virus yield from initial virus growth rate alone and that
318 these pathogen fitness parameters are a result of a number of interacting
319 processes.

320 Surprisingly, empirical investigation into parasite growth rates has been
321 largely neglected in invertebrate hosts [13]. Our experimental data highlight
322 the initial fast speed at which virus replication occurs. Using an approxima-
323 tion of our mathematical model (B.3), we have shown that the initial virus

324 growth rate is double exponential ($V(t) \approx e^{e^t}$). This is in stark contrast
325 to previous theory of obligate killing parasites, where more simplistic logis-
326 tic growth curves have been assumed [15], thus underestimating initial virus
327 growth. Indeed, for the prodigious theory of human diseases the within-host
328 growth of viruses is often shown to be significantly slower ($V(t) \approx e^t$) [25],
329 even before innate or adaptive immune responses slow the within-host spread
330 of disease. This further highlights the differences between the complexity of
331 vertebrate and simplicity of invertebrate hosts and their diseases. The main
332 reason for this difference is the speed at which the host grows. In vertebrate
333 systems host growth is assumed to be constant, since the speed of replication
334 of the pathogen is much faster than the growth rate of the host (Steinmeyer
335 et al. [26] for example).

336 Interestingly, the fitted statistical model (Figure 2) shows a decline in
337 virus abundance for each of the virus genotypes at approximately 10-20 hours
338 post infection. Why this occurs remains unclear. A possible explanation is a
339 sloughing defence mechanism [27] or simply loss of virus particles on the outer
340 body of the larva from droplet feeding, which illustrates the sensitivity of the
341 molecular method used. It may also be attributed to more complex cellular
342 and humoral mechanisms of immunity and both have been implicated in
343 insect resistance to baculoviruses [28]. In terms of our results, the decline of
344 the virus abundance is likely to have some small effect on the fitted parameter
345 values, in particular underestimating the β_0 values. Further study is clearly
346 required to ascertain the precise cause of the reduction in virus abundance
347 at the early stages of an overt infection and to understand the implications
348 for the host and virus growth dynamics.

From our observations we hypothesise that the speed of kill is strongly dependent on the rate at which the virus grows within the host, how quickly the virus replication rate reaches a plateau (if at all) and how much the virus impedes host growth, but the exact relationship is not immediately obvious. For example, *PafI*NPV has the slowest speed of kill, whilst in contrast *Mb*NPV has the smallest maximum infection rate, β_0 . Therefore, using a simple single parameter to predict the speed of kill is not possible. Furthermore, the biological mechanism behind host death (i.e. timing of host death relative to infection levels) is still relatively undetermined. Previous models have assumed that host death occurs when pathogen fitness is maximised [15]. Ebert and Weisser [15] assumed that the fitness of the obligately killing pathogen, F , is given by

$$F(t_{\text{kill}}) = V(t_{\text{kill}})e^{-mt_{\text{kill}}} \quad (3)$$

349 where $V(t_{\text{kill}})$ is the number of transmission stages at the time of host death,
 350 t_{kill} , and m is the background host mortality. Maximising (3) with respect to
 351 the time of host death gives the optimal speed of kill. Ebert and Weisser [15]
 352 found that under their model assumptions for the within-host virus growth
 353 the optimal killing time approximately corresponded to the period of time
 354 during which viral replication rate significantly decreases. However, applying
 355 this optimisation to our within-host viral growth model, parameterised for
 356 our 4 strains of NPV, results in nonsensical optimal speeds of kill, even for
 357 a wide range of background mortalities. This suggests that the speed of
 358 kill of baculovirus infections may be more complex than simple pathogen
 359 fitness optimisation or that pathogen fitness is not suitably described by (3).
 360 Moreover, our empirical data do not support the finding that the killing

361 time occurs at a point of rapid deceleration in the viral replication rate. In
362 contrast, we find that rapid viral growth, which has been shown in many
363 host-pathogen systems [29], is followed by a prolonged period of deceleration
364 towards a stationary final viral mass (see also Evans et al. [30]). However, this
365 deceleration is less pronounced for some viral strains, in particular *SeNPV*.

366 To this end, in the ESM we have covaried the speed of kill alongside the
367 other model parameters for two contrasting strains: *AcNPV* and *SeNPV*,
368 where the former exhibits a strong saturation effect. In each case, a faster
369 speed of kill leads to reduced viral yield and small hosts at the time of
370 death, as one would expect. However, for *AcNPV*, some parameters are more
371 sensitive to the speed of kill than others - the most sensitive parameters being
372 the initial host mass and viral dose parameters. In contrast, for *SeNPV*, the
373 speed of kill has a large effect on host mass at the time of death and the
374 viral yield for each parameter variation. Therefore, we must conclude that
375 for viruses that exhibit weak saturation, the speed of kill will have a large
376 effect on the viral yield. Moreover, if the speed of kill is greatly altered by
377 either (i) inoculating different insect instars or (ii) changing the viral dose
378 concentration, then viral yield will be greatly affected. Conversely, if the
379 host's environment can affect other model parameters, resulting in different
380 speeds of kill, then there may be no significant change in the viral yield.

381 Our results show that pathogen infection slows the growth rate of the
382 host, even at the early stages of infection. Surprisingly, there are relatively
383 few studies that empirically demonstrate a reduction in host growth rate (but
384 see Burand and Park [24]), but this is often suggested since parasites cause
385 harm to their hosts as an unavoidable consequence of parasite reproduction.

386 Our model predicts that a greater virus dose will increase this effect, which
387 agrees with evidence from a similar host-pathogen system [24]. To date, the
388 exact reason behind the reduction of host growth rate is unknown, but a
389 number of possible mechanisms have been put forward. These include host-
390 virus competition for nutrients at the cellular level [24] and the expression of
391 the viral ecdysteroid UDP-glucosyl transferase (EGT) gene which alters host
392 hormones related to host development [31]. Our model does not explicitly
393 state the origins of the growth rate reduction, but we simply incorporate
394 this effect as a composition parameter on the host-virus growth dynamics,
395 which produces a good fit. This enables us to detect differences between virus
396 strains and thus yields, which in terms of transmission in the field is critical,
397 as yield has a direct effect on the abundance of overwintering inoculum.

398 Our model predicts that larger hosts at the time of infection result in
399 larger viral yields, which supports experimental results of others [30]. Coun-
400 terintuitively, our model reveals that larger viral doses may decrease viral
401 yield. This result has been discovered experimentally in other closely related
402 systems [30, 32], but this is not always the case [33], perhaps due to the trade-
403 offs between dose, speed of kill and virus yield obscuring this phenomenon. In
404 terms of maximising transmission, the virus will increase its yield with lower
405 doses, but this will trade-off against the probability of infection. Therefore,
406 transmission is likely to be maximised for some intermediate dose. Our result
407 also contrasts with vertebrate within-host theory, where Steinmeyer et al. [26]
408 found that increasing viral dose increased the peak viral load, whilst empiri-
409 cal evidence suggests the contrary, as found in sheep inoculated intranasally
410 with a type O foot-and-mouth disease virus [34]. Here the authors suggested

411 the reason for this is that cell-mediated immune mechanisms responded more
412 quickly to high doses than lower doses, the result being increased inhibition
413 of viral replication.

414 It has been suggested that environmental stress increases host suscep-
415 tibility to infections and reduces host ability to resist parasite growth and
416 reproduction, thus benefiting parasites. This suggestion stems from expected
417 costs of immune defence; hosts in poor condition should have fewer resources
418 to be allocated to immune function. However, the alternative hypothesis
419 for the response to environmental stress is that hosts in poor condition pro-
420 vide fewer resources for parasites and/or suffer higher mortality, leading to
421 reduced parasite growth, reproduction and survival [35]. Under the assump-
422 tion that poorer quality diet results in a lower host growth rate (r_0), our
423 model predicts a reduction in virus yield, and so supports the latter hypoth-
424 esis.

425 Despite the focus of most host-pathogen work concentrating on single in-
426 fections, as we have studied here, molecular techniques have revealed that
427 many infections in insect hosts are caused by several pathogen genotypes
428 which differ phenotypically in their interaction with the host [19]. One ex-
429 ample is the pine beauty moth, *Panolis flammea*, in which a plethora of
430 genetically distinct strains of NPVs have been isolated from a single host.
431 These strains have been found to differ phenotypically in parameters corre-
432 lated with fitness, including the speed with which the pathogen kills the host
433 and the subsequent yields of OBs [19], which may act as non-lethal syner-
434 gists by interacting with secondary virus strains but are not themselves lethal
435 [36]. The simplest assumption would be that competition between genotypes

436 within a host is a race to gain the greatest share of resources (host tissues)
437 [37], as in the tragedy of the commons [38]. As a consequence, mixed infec-
438 tions may lead to reduced transmission between hosts. Hence, understanding
439 within-host dynamics of multiple infections is essential for understanding the
440 impact of multiple pathogens in the field.

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447 **Table**

Table 1: Fitted parameter values for the infection model using the method outlined in Section 3.2 for the total host mass and within-host mass growth of $AcNPV$, $MbNPV$, $PafNPV$ and $SeNPV$ in third instar larvae of *S. exigua*. The bracketed numbers correspond to the 95% bootstrapped confidence interval for each parameter.

Parameter	$AcNPV$	$PafNPV$	$MbNPV$	$SeNPV$
Initial Host Mass (g), H_0	5.0642×10^{-3} (4.4941 $\times 10^{-3}$, 5.6508 $\times 10^{-3}$)	5.6441×10^{-3} (4.0462 $\times 10^{-3}$, 6.3307 $\times 10^{-3}$)	5.9695×10^{-3} (4.5804 $\times 10^{-3}$, 7.7104 $\times 10^{-3}$)	4.3949×10^{-3} (3.7634 $\times 10^{-3}$, 4.5878 $\times 10^{-3}$)
Virus Dose (g), V_0	1.3089×10^{-9} (7.2990 $\times 10^{-10}$, 3.3345 $\times 10^{-9}$)	3.5492×10^{-9} (1.9431 $\times 10^{-9}$, 6.9817 $\times 10^{-8}$)	9.1399×10^{-9} (5.6659 $\times 10^{-9}$, 2.1487 $\times 10^{-8}$)	2.4957×10^{-10} (1.7004 $\times 10^{-10}$, 5.1954 $\times 10^{-10}$)
Max. Host Growth Rate (h^{-1}), r_0	3.7446×10^{-2} (3.4601 $\times 10^{-2}$, 4.2140 $\times 10^{-2}$)	2.3584×10^{-2} (2.0334 $\times 10^{-2}$, 3.2734 $\times 10^{-2}$)	3.4451×10^{-2} (2.8663 $\times 10^{-2}$, 4.1988 $\times 10^{-2}$)	3.6758×10^{-2} (3.4622 $\times 10^{-2}$, 3.7876 $\times 10^{-2}$)
Zero Infection Virus Proportion, p	2.1588×10^{-1} (1.3742 $\times 10^{-1}$, 3.5856 $\times 10^{-1}$)	4.9161×10^{-2} (3.8357 $\times 10^{-2}$, 6.5778 $\times 10^{-2}$)	2.8096×10^{-2} (1.6406 $\times 10^{-2}$, 4.3981 $\times 10^{-2}$)	4.6904×10^{-2} (4.5363 $\times 10^{-2}$, 1.7088 $\times 10^{-1}$)
Max. Infection Rate ($g^{-1}h^{-1}$), β_0	16.750 (13.760, 17.022)	16.530 (14.142, 21.722)	6.9231 (5.4750, 7.7177)	20.151 (16.354, 21.331)
Host Growth Reduction Rate (h^{-1}), a	1.9390 (0.9933, 4.5449)	7.9301 (2.3150, 22.454)	31.813 (20.025, 128.84)	5.0346 (1.2807, 5.5024)

448 **Appendix A. Model Analysis**

Since $r(t) \rightarrow 0$ as $t \rightarrow \infty$ the nullclines are given by $\beta(t) = 0$ for both $H' = V' = 0$, and thus are given by

$$\frac{V}{p(H + V)} = 1.$$

449 Hence, the nullclines for both of the coupled differential equations completely
450 overlap and therefore the equilibria, which are given by the intersection of
451 the nullclines, are defined by a curve.

452 Proof of our claims on stability will be reported elsewhere; here we sim-
453 ply sketch the details. Straightforward linear stability analysis reveals the
454 existence of a centre manifold. The long-term behaviour critically depends
455 on the initial conditions. The system will blow up if the initial dose is suf-
456 ficiently large relative to the initial host size, that is, if $\beta(0) < 0$. However,
457 given that the virus dose is small compared to the initial host size it is bio-
458 logically reasonable to assume that $\beta(0) > 0$. Then, since the equilibrium is
459 given by $\beta(t) = 0$ it can be shown that the system tends to the equilibrium.
460 Moreover, the fast and slow dynamics are calculable for the manifold.

461 **Appendix B. Parameter Fitting**

462 Here we outline the method used for the parameter fitting of the math-
463 ematical model (1). The results of these methods are listed in Table 1 and
464 shown graphically in Figure 3. For this we use a two-stage process:

1. Since we have assumed that in the absence of virus the host initially grows exponentially, we fit the curve

$$H(t) = H(0)e^{r_0 t} \tag{B.1}$$

465 where $H(0) = H_{0_{\text{cont}}}$, to the initial growth phase of the control data,
 466 thus giving the initial estimate of the maximum growth rate r_0 and the
 467 initial host mass $H_{0_{\text{cont}}}$ for the control data. We then use r_0 and the
 468 total mass of the infected hosts data to find the initial size of the host,
 469 H_0 .

470 To find the initial estimates of the maximum infection rate, β_0 , and
 471 the initial dose, V_0 , we use the fact that initially the amount of virus
 472 within the host is small. Hence we may approximate model (1) by

$$\frac{dH}{dt} = r_0 H \quad (\text{B.2a})$$

$$\frac{dV}{dt} = \beta_0 H V. \quad (\text{B.2b})$$

Solving (B.2) we obtain

$$V(t) = V_0 \exp \left\{ \frac{\beta_0 H_0}{r_0} (e^{r_0 t} - 1) \right\} \quad (\text{B.3})$$

473 where $V(0) = V_0$, for the initial growth of the virus. Hence one can
 474 use (B.3) to fit to the initial part of the virus data to find the initial
 475 parameter estimates for β_0 and V_0 .

476 It is not possible to find initial estimates for the host growth reduction
 477 rate, a , and the zero infection virus proportion, p , using techniques
 478 similar to those above. Therefore, we fit the model to both the total
 479 host mass and virus mass data simultaneously by making use of the
 480 previously found initial parameter estimates.

481 2. The set of six initial parameter estimates are then fitted to the data si-
 482 multaneously where the previously found parameter estimates are used
 483 as ‘good’ initial guesses. Since the data exhibit growing variance over

484 the course of the infection, we perform a log-transformation to account
485 for this [39].

486 All fitting is achieved by the method of least squares using a modified
487 Levenberg-Marquardt algorithm [40] and implemented in MATLAB®, using
488 a Runge-Kutta method for solving the differential equations numerically.
489 This fitting method, often referred to as “trajectory matching” [41] or “model
490 calibration” [42], has been successful in fitting in other biological datasets (see
491 Harrison [43] for example). Our method works well here since the time-series
492 has little process noise and we assume that all the error is from observation,
493 but for noisy data more complex methods can be used, such as a gradient
494 matching method [44].

495 The bootstrapped confidence intervals are calculated from 10000 boot-
496 strapped data sets (with replacements), to which the model is fitted using
497 the parameter estimates as initial guesses.

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619 **Figure 1**

620 Mean weight of third instar larvae infected with *Ac*NPV, *Mb*NPV, *Pafl*NPV,
621 *Se*NPV and uninfected control. The lines show the fitted values for (a)
622 *Ac*NPV (log weight = $-2.2827 + 0.0104 \times \text{time} + 1.4605 \times 10^{-4} \times \text{time}^2 -$
623 $1.3546 \times 10^{-6} \times \text{time}^3$), (b) *Mb*NPV (log weight = $-2.3446 + 0.0180 \times \text{time} -$
624 $4.9347 \times 10^{-6} \times \text{time}^2 - 4.0265 \times 10^{-7} \times \text{time}^3$), (c) *Pafl*NPV (log weight =
625 $-2.3832 + 0.0183 \times \text{time} - 9.0041 \times 10^{-5} \times \text{time}^2 + 1.1677 \times 10^{-7} \times \text{time}^3$),
626 (d) *Se*NPV (log weight = $-2.2662 + 0.0146 \times \text{time} - 4.7435 \times 10^{-5} \times \text{time}^2 +$
627 $1.8663 \times 10^{-7} \times \text{time}^3$) and (e) uninfected controls (log weight = $-2.2718 +$
628 $0.0119 \times \text{time} + 1.3297 \times 10^{-4} \times \text{time}^2 - 9.8279 \times 10^{-7} \times \text{time}^3$). In (f)
629 the mean weights are plotted for the various treatments, along with fit-
630 ted lines for uninfected controls (log weight = $-2.2829 + 0.01644 \times \text{time}$),
631 *Ac*NPV (log weight = $-2.2973 + 0.01444 \times \text{time}$), *Mb*NPV (log weight =
632 $-2.2963 + 0.01527 \times \text{time}$), *Pafl*NPV (log weight = $-2.2947 + 0.01131 \times \text{time}$)
633 and *Se*NPV (log weight = $-2.2392 + 0.01219 \times \text{time}$).

634 **Figure 2**

635 The within-host growth of (a) *Ac*NPV, (b) *Mb*NPV, (c) *Pafl*NPV and
636 (d) *Se*NPV in third instar larvae of *S. exigua* as measured by the proportion
637 of total DNA represented by viral DNA. The lines show the fitted values
638 for *Ac*NPV (log proportion = $-4.6000 - 0.3706 \times \text{time} + 0.0181 \times \text{time}^2 -$
639 $2.7824 \times 10^{-4} \times \text{time}^3 + 1.8302 \times 10^{-6} \times \text{time}^4 - 4.4369 \times 10^{-9} \times \text{time}^5$), *Mb*NPV
640 (log proportion = $-5.4454 - 0.0473 \times \text{time} + 0.0010 \times \text{time}^2 + 2.0390 \times$
641 $10^{-5} \times \text{time}^3 - 3.3223 \times 10^{-7} \times \text{time}^4 + 1.1557 \times 10^{-9} \times \text{time}^5$), *Pafl*NPV
642 (log proportion = $-5.6825 - 0.1018 \times \text{time} + 0.0062 \times \text{time}^2 - 8.3174 \times$

643 $10^{-5} \times \text{time}^3 + 4.3875 \times 10^{-7} \times \text{time}^4 - 8.0036 \times 10^{-10} \times \text{time}^5$) and *Se*NPV
644 (log proportion = $-6.2862 - 0.2996 \times \text{time} + 0.0172 \times \text{time}^2 - 2.8257 \times 10^{-4} \times$
645 $\text{time}^3 + 1.9346 \times 10^{-6} \times \text{time}^4 - 4.8002 \times 10^{-9} \times \text{time}^5$).

646 **Figure 3**

647 The total host mass and within-host mass growth of (a) *Ac*NPV, (b)
648 *Mb*NPV, (c) *Paf*NPV and (d) *Se*NPV in third instar larvae of *S. exigua*.
649 The asterisks denote total host mass, plus signs denote virus mass from the
650 experimental data and the solid and dashed lines show the results of the
651 fitted Model (1) for the parameter values in Table 1. Note that the virus
652 axes are in a \log_{10} scale.

653 **Figure 4**

654 The effects of the virus parameters in infections. Here we run simulations
655 of Model (1) using the parameters in Table 1 for *Ac*NPV. In each graph the
656 total host mass (solid line) and the virus yield (dashed line) at the time of
657 host death is plotted against (a) virus dose, V_0 , (b) initial host mass, H_0 ,
658 (c) maximum host growth rate, r_0 , (d) host growth reduction rate, a , (e)
659 maximum infection rate, β_0 , and (f) zero infection virus proportion, p . The
660 time to death is 160 hours. Note that qualitatively similar results hold for
661 *Mb*NPV, *Se*NPV and *Paf*NPV.

Figure 1

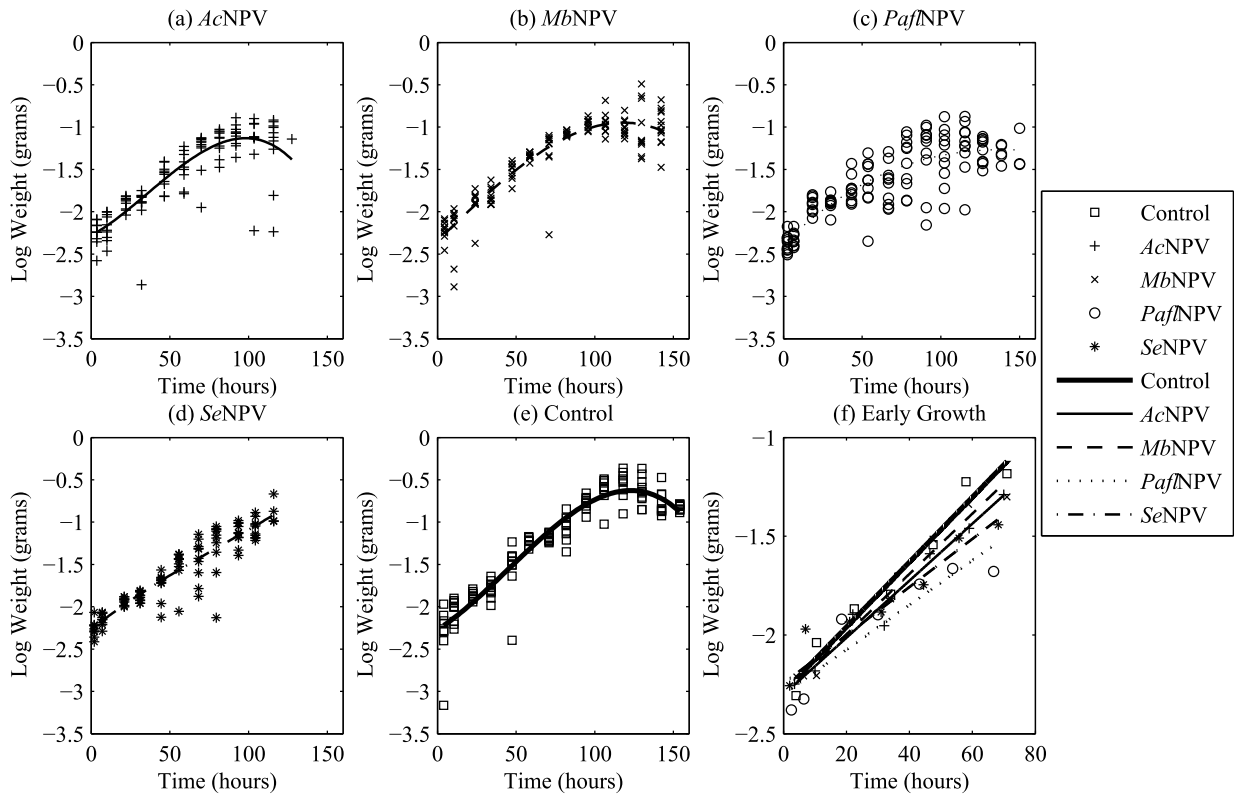


Figure 2

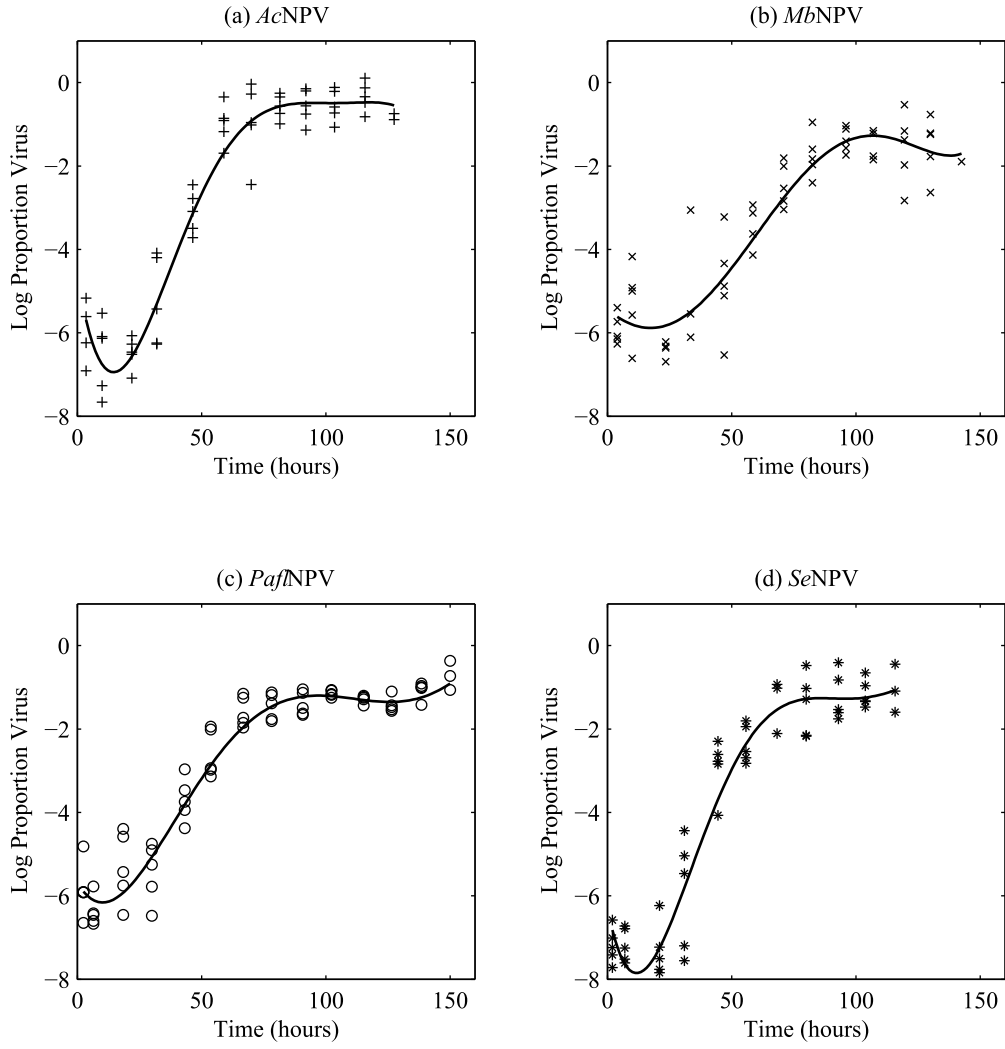


Figure 3

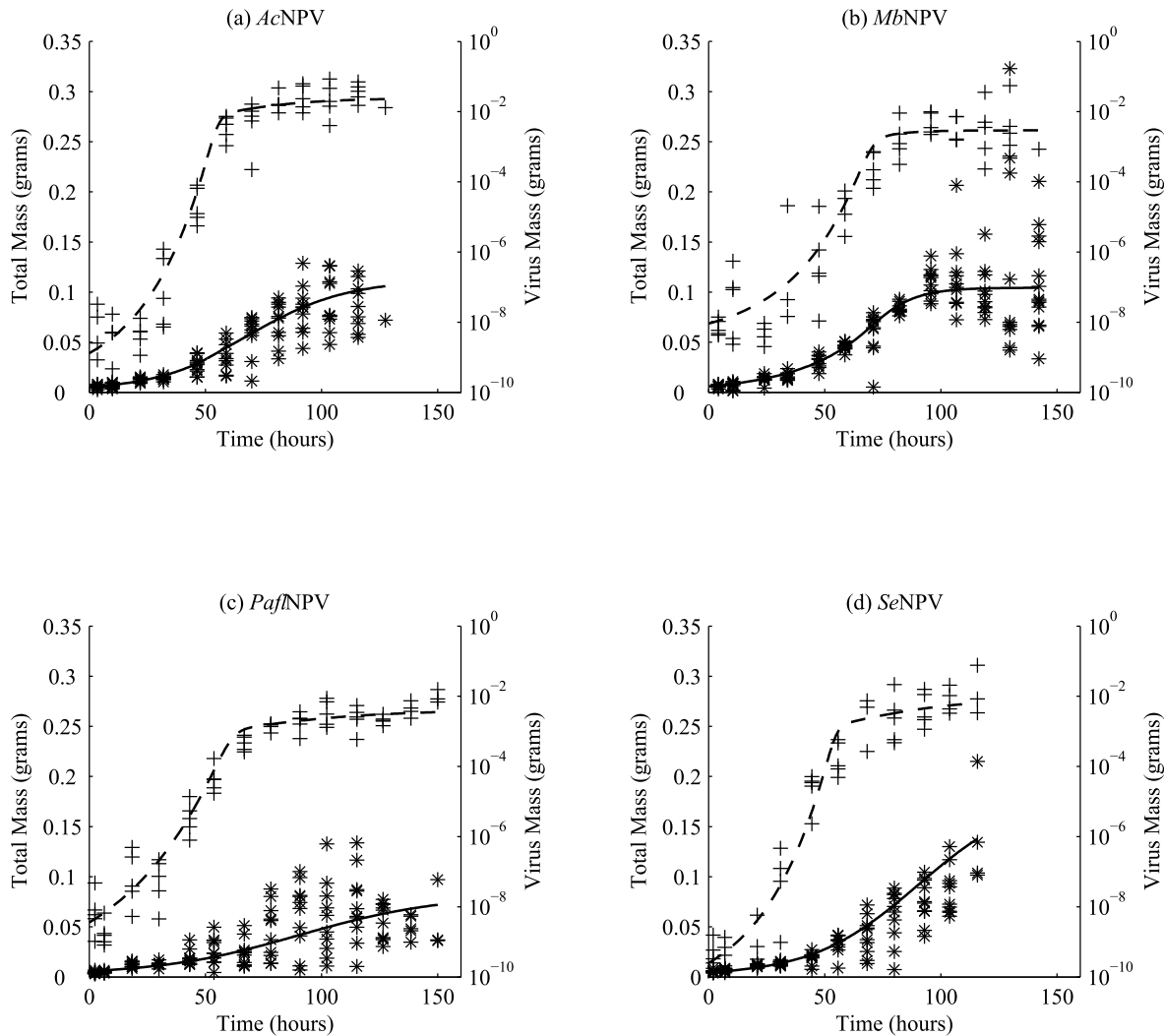
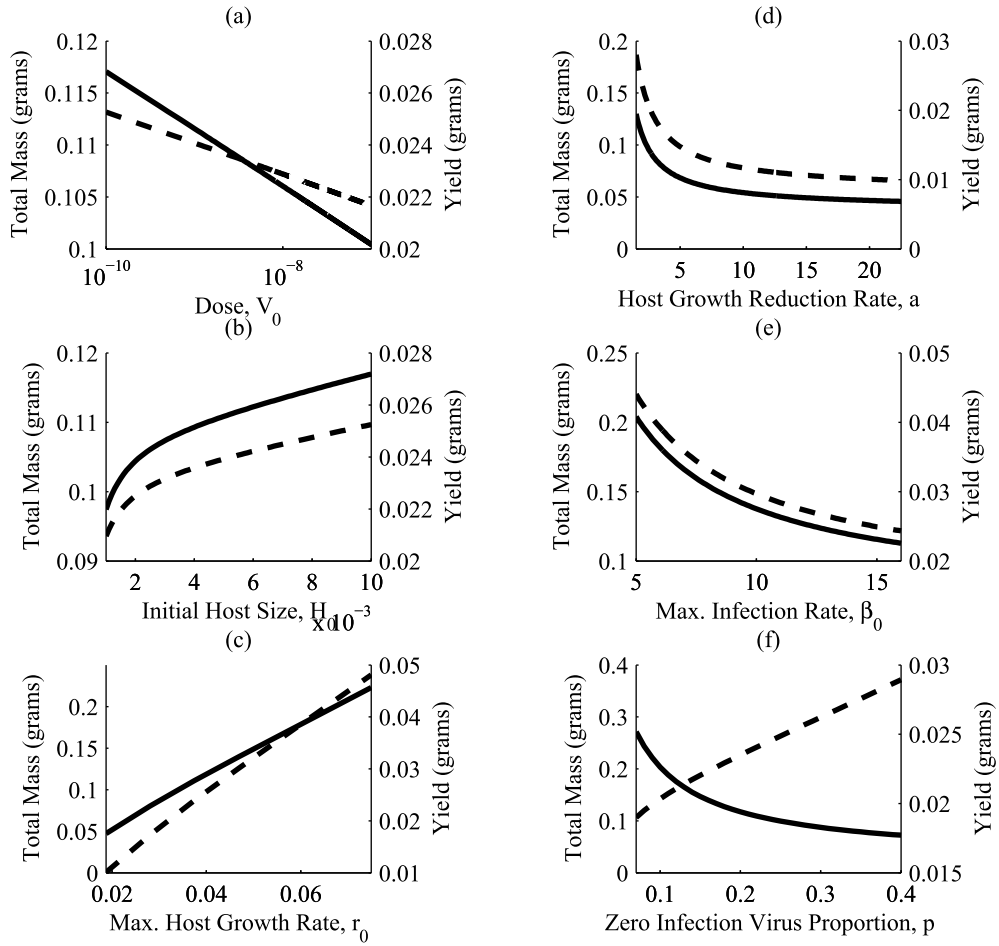


Figure 4



1 Modelling the within-host growth of viral infections in
2 insects: Electronic supplementary material

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11 **1 Insect and virus stocks**

12 *Spodoptera exigua* larvae were obtained from Syngenta (Jeallotts Hill, UK) in 2003 and
13 reared in continuous culture on artificial diet [1]. This population was shown to be free from
14 persistent baculovirus infections by PCR and RT-PCR for the viral polyhedrin gene using
15 total insect DNA as a template.

16 Four different baculoviruses were used in this study; the Oxford strain of *Mamestra bras-*
17 *sicae* nucleopolyhedrovirus (*MbNPV*) [2], *Panolis flammea* nucleopolyhedrovirus (*PafNPV*)
18 variant 4 [3], *Autographa californica* nucleopolyhedrovirus (*AcNPV*) strain C6 [4] and *Spodoptera*
19 *exigua* nucleopolyhedrovirus (*SeNPV*) [5]. Stocks of each virus were made by dosing third
20 instar *S. exigua* larvae with 10^8 occlusion bodies (OBs) by diet plug feeding [6], and puri-
21 fying the virus by density gradient centrifugation [1]. The titre of the purified virus stock
22 was estimated using an Improved Neubauer haemocytometer (B.S. 748, Weber, UK) and
23 the virus stored at -20°C . Virus stocks were re-counted before each use.

24 **2 Statistical Methods**

25 The data were analysed using generalised linear modelling techniques (GLIM version 3.77,
26 Royal Statistical Society, 1985). For the analysis of mortality all explanatory variables
27 (virus concentration, virus, block) and their interactions were fitted to the mortality data.
28 A binomial error structure was assumed, which was substantiated by subsequent inspection
29 of the scale parameter [7]. The contribution of each term was tested for significance and non-
30 significant terms removed to leave the minimal adequate model. Box-Cox transformations
31 indicated an inverse transformation was required for data on time to larval death.

32 **3 DNA Extraction & Quantification**

33 DNA (insect and viral) was extracted from the frozen larvae by first thawing them and
34 then disrupting them using a manual tissue grinder. Total DNA was then extracted from
35 this material using a DNEasy mini kit (Qiagen). The DNA was eluted from the column into

36 200 μ l of elution buffer and quantified by spectrophotometry at 260nm and 280nm. Extracted
37 DNA was stored at -20°C. DNA was extracted from 5 of the larvae harvested at each time
38 point.

39 Viral DNA was quantified by real-time PCR using a Rotor Gene RG-3000 (Corbett
40 Research) and a CAS-1200 liquid handling system (Corbett Research). Primer pairs were
41 designed, specific to the sequence of each virus, to amplify a region of approximately 200
42 base pairs (bp) from the viral *iel* gene (*AcIE1-1* AAGGTGTGGTGGGCCAGTTT, *AcIE1-*
43 *2* TGGTTCGGAGAACCTGTTGGA, *MbIE1-1* TTGCTTCCGAAGGACCACAA, *MbIE1-2*
44 ATCCCGTGTGCGAGCAAATGA, *PfIE1-1* CGTCAACGGCATCAACAACA, *PfIE1-2* TG-
45 GCAGCTCCTTTTCCAACA, *SeIE1-1* TCGACAACAGCGGCATCTTT, *SeIE1-2*
46 CGGTAGCGTTCGATGGTGAC).

47 Each real-time PCR reaction mixture consisted of Platinum SYBR Green qPCR SuperMix-
48 UDG (Invitrogen) (10 μ l), sterile distilled water (6.2 μ l), BSA (1 μ l), and the appropriate
49 primers (10pmol/ μ l, 0.4 μ l of each primer) to which was added 2 μ l of the extracted total
50 DNA. The reaction profile was a single cycle of 50°C for 2 minutes, followed by 40 cycles of
51 95°C for 15 seconds, 57°C for 15 seconds and 72°C for 15 seconds. This was followed by a
52 stage in which the temperature was raised from 57°C to 99°C in 1°C intervals to allow for
53 subsequent melt curve analysis.

54 For each sample duplicate real-time PCR reactions were run and each PCR run included
55 duplicate negative controls in which the template DNA was replaced by 2 μ l of sterile distilled
56 water. For the quantification of the samples, genomic DNA from the appropriate virus was
57 used to generate a standard curve. Viral genomic DNA was purified by caesium chloride
58 gradient purification of DNA released from virus particles [6]. For each set of quantification
59 reactions a series of five decimal dilutions of the viral genomic DNA was set up using the
60 CAS-1200 system. This dilution series was made from an initial sample of the virus DNA
61 which had been quantified by spectrophotometry at 260nm and 280nm. Standard samples
62 were also run in duplicates. A standard curve was generated based on this dilution series
63 using the software associated with the RG-3000, which also quantified the samples based on

64 this curve. Standard curves with an R2 value of less than 0.99 were rejected. Samples were
65 only regarded as giving a positive real-time PCR result if the take-off point of the reaction
66 was before that seen with any primer dimers produced in the negative control reactions and a
67 product with the appropriate denaturation temperature was seen on the melt-curve analysis.

68 An average of two duplicates was taken to be the quantification for a given sample. As
69 the total amount of DNA in the PCR reaction was known ($2\mu\text{l}$ of known concentration in
70 each reaction) the proportion of this which was viral could therefore be calculated.

71 4 Consequences of Censoring Technique

72 One drawback of our sampling method is that data points towards the end of the time
73 series are censored. Some insects died before the final time point, so those censored at the
74 final time point are selected from those that survived. There are likely to be yield differences
75 depending upon time of death, and therefore the final sub-sample will be biased. It is unclear
76 how this affects our results, but it is most likely to affect host-pathogen systems where one
77 compares a virus with a high degree of variance in the speed of kill to a virus with a low
78 degree of variance (which does not apply here) as this will influence the degree of bias. To
79 combat this, the only solution would be to monitor the growth of virus in individual larvae
80 by subsampling from the same insect throughout the course of infection. However, there
81 are a number of technical issues with sampling tissue and accurately estimating total virus
82 abundance within the host without killing the insect.

83 5 Virus Growth Rate

84 By equation (B.3), the model predicts that the initial growth rate is double exponential. This
85 is faster than the single exponential growth rate that is common in many other infection
86 models. Indeed, using an approximation to equation (B.3) such that

$$V(t) \approx V_0 \exp\{\beta_0 H_0 t\} \quad (1)$$

87 equation (1) underpredicts the growth of virus (see Figure S2).

88 **6 Prescribing r_0**

89 In the main text we show the results of the model fitting whereby all model parameters are
90 fitted to the data from infected individuals simultaneously. This is done so that we account
91 for stochastic differences between treatments and to allow the value to be an emergent
92 property of the simultaneous fitting. However, r_0 , the maximum host growth, is the innate
93 parameter of host growth and should be independent of the infection. Hence, an alternative
94 fitting strategy could be to fit r_0 from the initial control data (i.e. before any pupation effects
95 occur), fix this parameter and fit the remaining parameters as described by the previous
96 method. In this section, we carry out this fitting and discuss the implications.

97 The results of prescribing r_0 are shown in Table S1. Comparing this result to our previous
98 result (Table 1 in the main text), we see that the biggest effect is on the host growth reduction
99 rate, a . Here we see a large increase in this parameter value compared to the previous fitting.
100 This difference would suggest that, by not fitting fixing the maximum host growth rate to
101 the control data, the fitting method underestimates the host growth slow-down caused by
102 the virus.

103 **7 Dependence on the Speed of Kill**

104 In Figures S3 and S4 we have further explored the impacts of the speed of kill on the host
105 mass (left hand column) and yield (right hand column) for all 6 parameters (rows) in the
106 model for two contrasting virus strains: *AcNPV* and *SeNPV*. The results are discussed in
107 the main text Discussion.

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Table S1: Fitted parameter values for the infection model using the method outlined in Section 3.2 with the r_0 prescribed by fitting it to the first 7 census points of the control data. See Table 1 in the main manuscript for a comparison.

Parameter	<i>Ac</i> NPV	<i>Paf</i> NPV	<i>Mb</i> NPV	<i>Se</i> NPV
Initial Host Mass (g), H_0	4.437×10^{-3}	3.512×10^{-3}	5.5512×10^{-3}	3.763×10^{-3}
Virus Dose (g), V_0	1.25×10^{-9}	4.56×10^{-9}	9.72×10^{-9}	5.19×10^{-10}
Max. Host Growth Rate (h^{-1}), r_0	3.642×10^{-2}	3.642×10^{-2}	3.642×10^{-2}	3.642×10^{-2}
Zero Infection Virus Proportion, p	2.439×10^{-1}	4.058×10^{-2}	2.726×10^{-2}	1.709×10^{-1}
Max. Infection Rate ($\text{g}^{-1}\text{h}^{-1}$), β_0	19.859	18.130	6.881	16.354
Host Growth Reduction Rate (h^{-1}), a	1.116	26.775	37.104	1.281

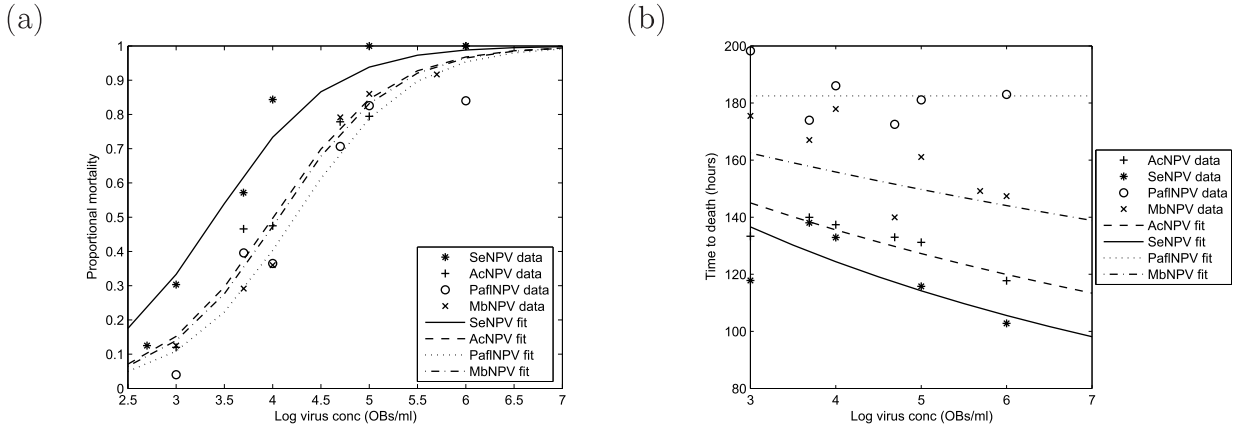


Figure S1: In (a) dose-mortality curves for *AcNPV*, *MbNPV*, *PaflNPV* and *SeNPV*. The lines show the fitted values for *AcNPV* and *MbNPV* ($\text{logit} = -6.948 + 1.725 \times \log(\text{virus conc})$), *PaflNPV* ($\text{logit} = -7.2812 + 1.725 \times \log(\text{virus conc})$) and *SeNPV* ($\text{logit} = -5.802 + 1.725 \times \log(\text{virus conc})$) and proportional mortality is given by $p = 1/(1 + (1/e^{\text{logit}}))$. In (b) mean time to death vs dose curves for *AcNPV*, *MbNPV*, *PaflNPV* and *SeNPV*. The lines show the fitted values for *AcNPV* (time to death = $1/(0.005454 + 0.0004807 \times \log \text{dose})$), *MbNPV* (time to death = $1/(0.00537152 + 0.0002615 \times \log \text{dose})$), *PaflNPV* (time to death = 182.48) and *SeNPV* (time to death = $1/(0.0051692 + 0.0007172 \times \log \text{dose})$). The analysis carried-out was inverse transformed with normal errors.

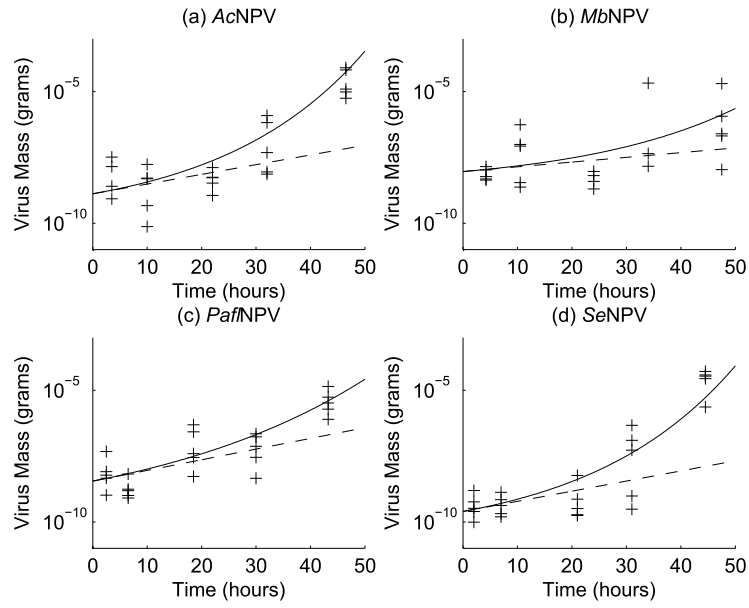


Figure S2: Comparisons of the two approximations for the initial growth of virus. Solid lines denote the double exponential approximation function (B.3); dashed lines denote the exponential approximation function (1). All parameters used are taken from the full ODE model for each virus strain.

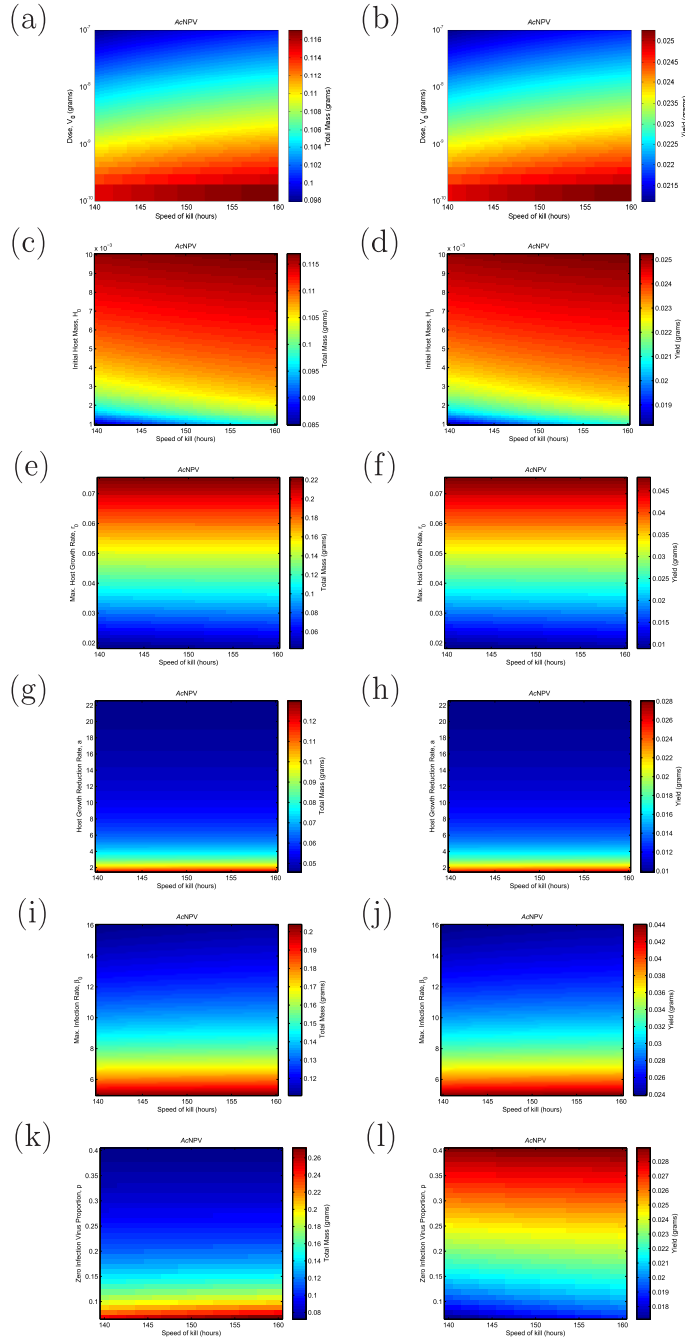


Figure S3: Quantifying the effects of the speed of kill on host mass and yield of virus for *AcNPV*. Here we run simulations of Model (1) using the parameters in Table 1 for *AcNPV*. We have plotted the total host mass (left hand column) and viral yield (right hand column) for all 6 parameters (rows). The colours indicate the masses for each parameter and speed of kill combination.

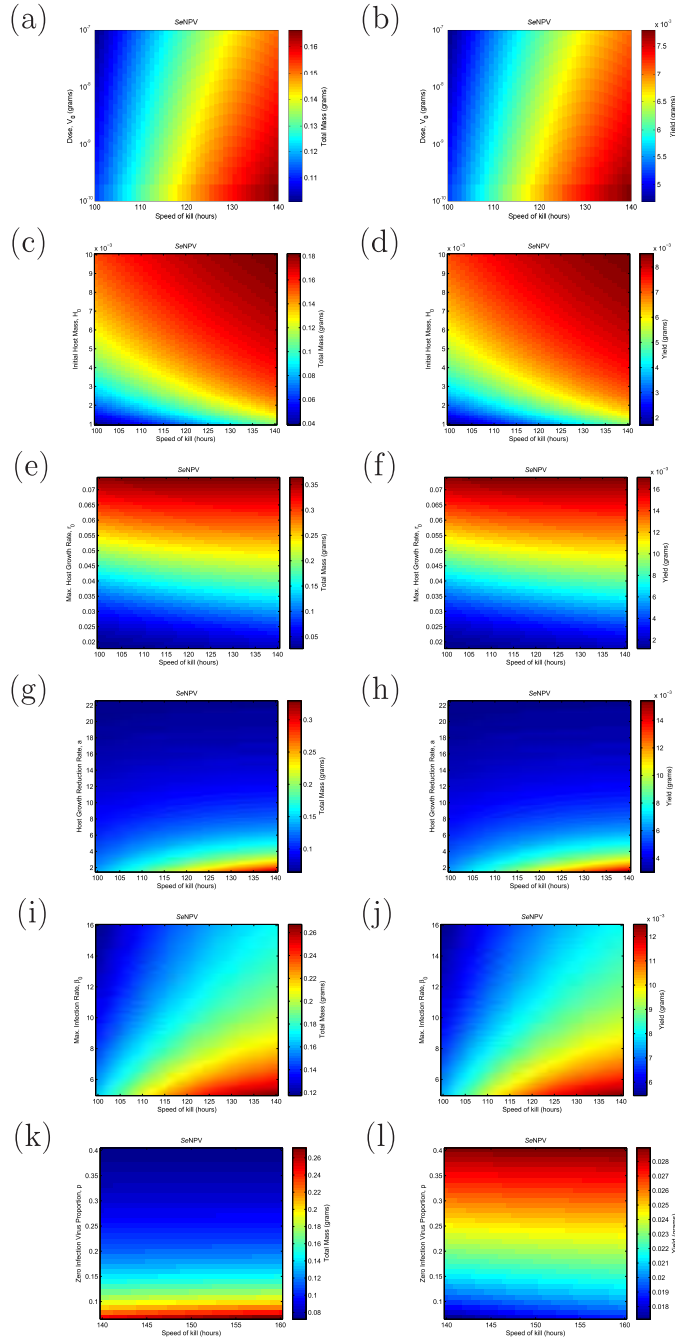


Figure S4: Quantifying the effects of the speed of kill on host mass and yield of virus for *SeNPV*. Here we run simulations of Model (1) using the parameters in Table 1 for *SeNPV*. We have plotted the total host mass (left hand column) and viral yield (right hand column) for all 6 parameters (rows). The colours indicate the masses for each parameter and speed of kill combination.