BIOLOGICAL CONTROL

Host Stage Structure and Baculovirus Transmission in *Mamestra brassicae* L. (Lepidoptera: Noctuidae) Larvae: a Laboratory Examination of Small Scale Epizootics

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Estrutura Etária do Hospedeiro e Transmissão de Baculovírus em Larvas de *Mamestra brassicae* L. (Lepidoptera: Noctuidae): Uma Análise de Epizootias em Pequena Escala em Laboratório

RESUMO – Os efeitos da estrutura etária e da densidade do hospedeiro na transmissão horizontal de um baculovírus foram investigados em laboratório, utilizando-se populações de lagarta do repolho, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). Os insetos foram mantidos a três combinações de ínstares e três densidades populacionais em recipientes fechados contendo larvas infectadas. As larvas foram observadas diariamente e o número de mortes e o tempo letal foram registrados. Os níveis de mortalidade viral foram marginalmente superiores em recipientes contendo hospedeiros sob maior densidade populacional. As larvas apresentaram um risco de infecção aparentemente maior quando combinações de instares mais avançados foram usadas. Os níveis de mortalidade de larvas mais velhas foram maiores que as de larvas mais jovens. As médias de tempo letal das populações larvais foram maiores para larvas mais jovens, observando-se um declínio mais acentuado na curva de sobrevivência das larvas em estágio mais adiantado de desenvolvimento.

PALAVRAS-CHAVE: Epizootiologia, nucleopoliedrovírus, lagarta do repolho, densidade do hospedeiro, controle biológico.

ABSTRACT - The effects of stage structure and host density on baculovirus horizontal transmission were examined in the laboratory using larvae of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). Insects were reared at three instar combinations and three host densities in closed containers with infected larvae. Insects were observed daily and the number of deaths and time to death were recorded. Levels of virus mortality were marginally higher in the containers where a higher density of hosts was introduced. Larvae appeared to have a greater risk of infection when late instar combinations were used. Final levels of mortality of older larvae were significantly higher than those of younger larvae. Mean times to death of larval populations were longer for larvae at earlier instar combinations, with a faster decrease in survivorship of older larvae over time.

KEY WORDS: Epizootiology, nucleopolyhedrovirus, cabbage moth, host density, biological control.

The dynamics of a disease in insect populations is influenced by properties of the host, pathogen and environment, which determine the changes in host and pathogen numbers over time. A key process in this interaction is pathogen transmission, which varies with several host-related factors, particularly differential susceptibility to infection and behaviour (Dwyer 1991, Fuxa 1995, Alves & Lecuona 1998, Vasconcelos 2001). For example, most lepidopteran larvae show increased mobility and food consumption as they age, even though their susceptibility to infection to some pathogens, such as baculoviruses, seems to decline considerably as larvae develop (Evans 1983, Teakle *et al.* 1986, Sait *et al.* 1994, Sosa-Gomez & Moscardi 2001).

Baculoviruses (Family *Baculoviridae*) are arthropod-specific pathogens that typically infect lepidopteran larvae through the consumption of contaminated food. Progeny virus is usually released after lysis of the infected cadaver, leading to a localised inoculum distribution (Tanada & Kaya 1993).

Virus acquisition is largely dependent on chance encounters between host and inoculum, so that more mobile larvae have, theoretically, higher probability of encountering the patchily distributed virus in the environment. In many lepidopteran species, changes in larval behaviour, such as enhanced mobility and feeding rates, may be triggered by higher host densities or developmental stage, since older (and larger) larvae tend to move further distances than their younger counterparts (see Reavey 1993, for a review). The extent to which these features influence pathogen transmission patterns has received surprisingly little empirical attention (see Webb & Shelton 1990, Goulson *et al.* 1995), despite the importance of this information on the understanding of epizootics and the design of microbial control programmes.

In this study we examined the horizontal transmission of a baculovirus in larval populations of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae) in the laboratory. The objective was to investigate whether a) overall mortality patterns differ for larvae at different stage structures, b) virus transmission is affected by the initial host density and c) the survivorship of the infected population varies with larval density and instar combination.

Material and Methods

Insect and Virus Characteristics. *Mamestra brassicae* larvae were obtained from a culture reared on semi-synthetic diet (Hunter *et al.* 1984) at the Centre for Ecology and Hidrology, Oxford. The multiply-enveloped nucleopolyhedrovirus (NPV, *Baculoviridae*) used in this study was isolated in Germany in 1973 and is well characterised biochemically and in terms of biological activity (Vlak & Gröner 1980, Brown *et al.* 1981, Evans 1983, Doyle *et al.* 1990). Experimental units consisted of 27.5 x 15.5 x 9 cm clear acrylic boxes containing a 1 cm layer of semi-synthetic diet (Hunter *et al.* 1984). Box lids were ventilated and had a sheet of tissue paper under the lid to absorb humidity and provide substrate for larvae prior to pupation.

The inoculum consisted of a moribund primary infected larva (referred to as PIL hereafter), at three instars: second, third and fourth. To obtain PIL at the desired instar, larvae were infected at one instar younger. Neonate larvae were infected with a suspension of 400 polyhedral inclusion bodies (PIBs)/µl using the droplet feeding method (Hughes *et al.* 1986). Second and third instar larvae were infected individually with 5.0×10^3 and 3.2×10^4 PIBs respectively, using the diet plug method (Doyle *et al.* 1990). PIL were used at six (2^{nd} and 3^{rd} instar) or seven days post-infection (4^{th}instar). Mean weights of PIL were (± SEM): 2.83 ± 0.07 mg for 2^{nd} instar, 15.75 ± 0.34 mg for 3^{rd} instar and 53.76 ± 1.26 mg for 4^{th}instar larvae (N = 100 for each instar).

Experimental Procedure. Three instar combinations of infected and healthy larvae were used: a) 2^{nd} instar PIL + 3^{rd} instar healthy; b) 3^{rd} instar PIL + 4^{th} instar healthy, and c) 4^{th} instar PIL + 5^{th} instar healthy. Because infection delays host development/moulting, all larvae in each treatment were at the same age, although at different stadia. Each instar combination was tested using three densities of healthy larvae/box: low (4), medium (10) or high (16). Twelve replicates were made; a replicate consisting of a set of all instar combinations and at all host densities. Control treatments consisted of larvae at each instar combination reared at the highest density (16/box) without introduction of inoculum, and were assembled first.

One moribund PIL was placed in each container and death occurred within 24h, followed shortly (<12h) by lysis. Based on previous data by Evans *et al.* (1981), the inoculum introduced into the containers (the polyhedra yield of a PIL) was *ca.* 8.4×10^6; 2.0×10^8, and 6.3×10^8 PIBs for 2^{nd}, 3^{rd} and 4^{th} instar PIL respectively. Healthy larvae were introduced six hours after the PIL. Following assembly, boxes were inspected daily, and the number of deaths recorded. All dead larvae were kept in the containers, allowing transmission resulting from secondary deaths to occur. Survivors that reached the pre-pupal stage were then removed, since only larval mortality was examined.

Statistical Analysis. Analysis was performed using the Generalized Linear Interactive Modelling program (GLIM). The significance of each treatment was assessed using *F* - tables (data with normal sampling errors) or c^2 tables (binomial errors). To estimate a mean time to death of the population in each treatment, the period between introduction in the container and death by virus for each larva was recorded. The means of each treatment were analysed using the Weibull distribution, which allows the instantaneous risk of infection to vary with time (Crawley 1993). This parameter is important in characterising survivorship curves, as populations with similar final mortalities may show different times of start and duration of the mortality wave.

Results

Epizootic Development. Proportions of virus-killed larvae were analysed using treatment (control *vs* virus) and instar combination ($2^{nd}/3^{rd}$, $3^{rd}/4^{th}$ and $4^{th}/5^{th}$) as factors. Host density was introduced into the model as a continuous variable, so that regression lines were obtained. Mortality in virus treatments was clearly significant when compared to controls ($c^2_1 = 521.7$; $P<0.0001$) and was higher for larvae at older instar combinations ($c^2_2 = 31.11$; $P<0.0001$) (Fig. 1). Viral deaths in control treatments were negligible (<0.01%) throughout the experiments.

Initial host numbers were marginally associated with higher mortality ($c^2_1 = 3.85$; $P \gg 0.05$). The effect of host density on virus transmission was similar for all instars (interaction instar x density: $c^2_2 = 1.80$; $P>0.30$). Final levels of virus mortality are described by the following equations (on a logit scale):

(I) $y = -0.040 + 0.043$ *density* (at the $2^{nd}/3^{rd}$ instar combination);

(II) $y = 0.801 + 0.043$ *density* ($3^{rd}/4^{th}$ instar combination), and

(III) $y = 1.285 + 0.043$ *density* (for the $4^{th}/5^{th}$ instar combination).

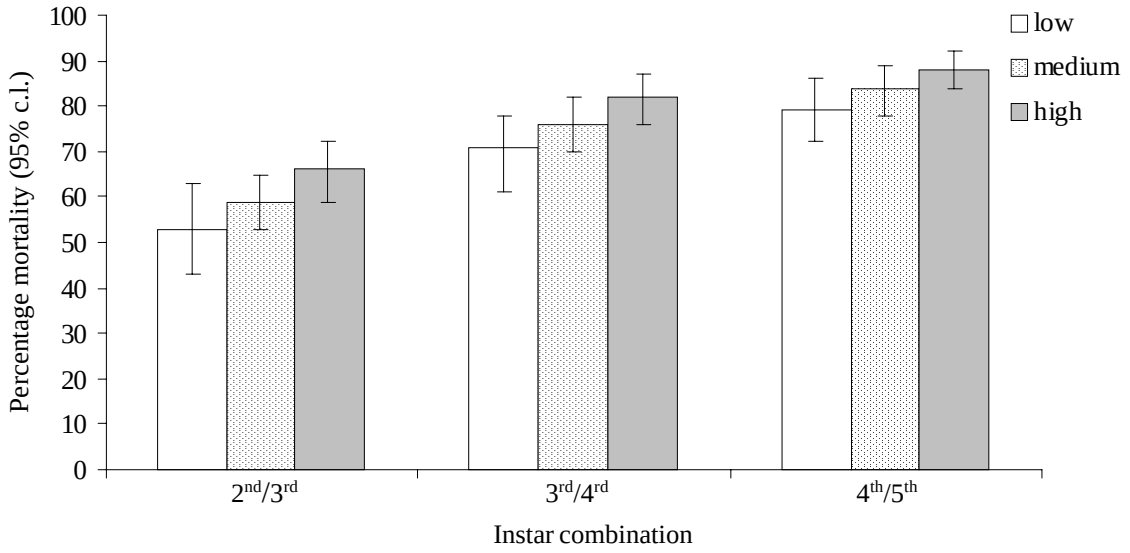

Figure 1. Mortality caused by baculovirus in *M. brassicae* larvae reared at three densities (low = 4, medium = 10 and high = 16 larvae/container) and three stage structures. Values represent the mean of 12 replicates and are shown with 95% confidence limits.

Host density did not affect the mean time to death of the population ($F_{(1,96)}$ = 0.24; P>0.20) (Fig. 2). Larval stage, however, influenced the pattern of mortality, as the mean time to death of the population was significantly shorter for larvae at older instar combinations ($F_{(4,97)}$ = 2.54; P<0.05). Survivorship curves are shown in Fig. 3. Because host density did not affect the time to death, the three densities were combined to produce one curve for each instar. The curves indicate stage-related differences in the survival of larvae; the short plateau observed at day 8-10 suggests that the decline in the proportion of survivors was less pronounced for younger larvae than for their older conspecifics.

Discussion

Like other pathogens, baculovirus transmission is believed to act in a density-dependent manner, with the rate of successful infections being proportional to the number of encounters between healthy and infected hosts - or infective stages (Anderson & May 1981). However, this assumption has been questioned using empirical evidence indicating that the transmission coefficient may decline with increasing host and virus density (D'Amico *et al.* 1996). Other insect pathogen systems have also reported non-linearities in the transmission coefficient (Knell *et al.* 1996).

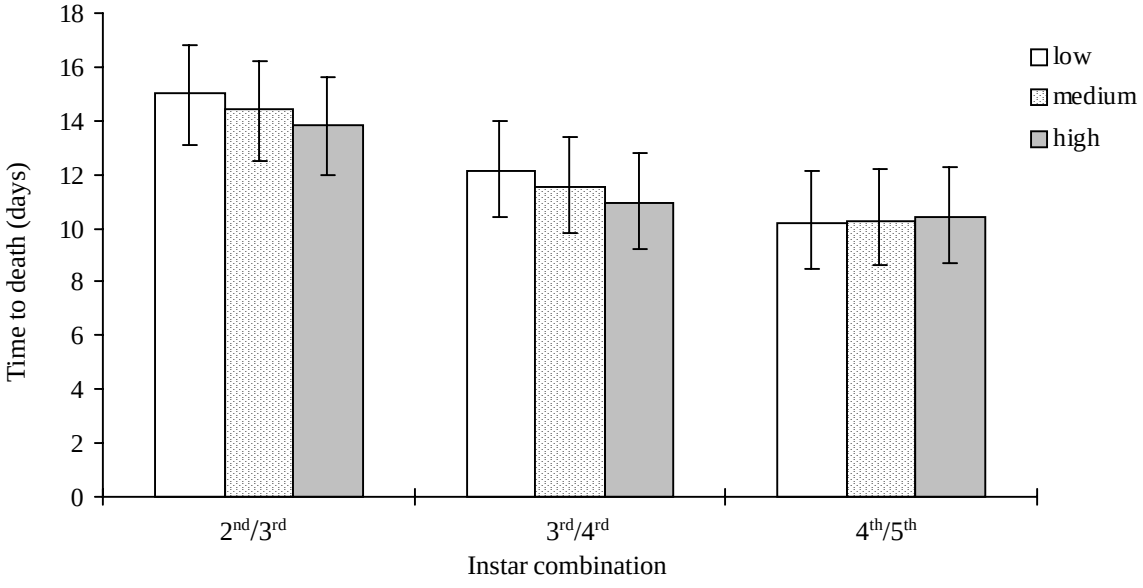

Figure 2. Mean time to death, in days, of *M. brassicae* larvae exposed to a baculovirus at different combinations of host density and age structure. Values represent the mean of 12 replicates and are shown with 95% confidence limits.

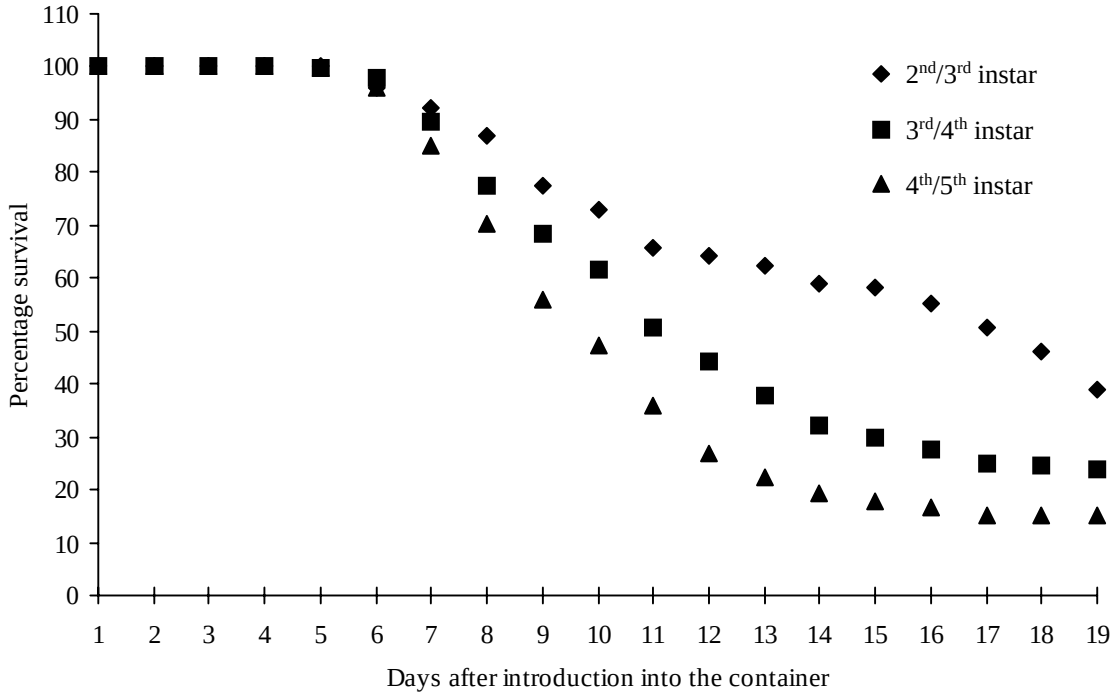

Figure 3. Survivorship curves for *M. brassicae* exposed to a baculovirus at different stage structures. Each curve represent a combination of all host densities tested (N = 36).

Behavioural changes associated with higher host densities, such as enhanced levels of mobility could increase the probability of encounter between host and inoculum. In this study, the effect of host density was only marginally detectable. This may be partially due to the small, homogeneous nature of the experimental arenas or to the moderate range of host densities used (as lower densities would compromise statistical analysis and higher densities resulted in non-specific host mortality). The more complex environment of the plant may reduce contact between the inoculum and the insect and enhance age-related differences in virus acquisition.

Virus transmission varied with host stage structure, as larvae at older instar combinations appeared to have a greater risk of acquiring infection, shown by the higher mortality. This agrees with Dwyer's (1991) findings that virus-killed *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae) cadavers at late instars were somehow more infectious than at earlier ones. He suggested that the decrease in susceptibility of older larvae is surpassed by their greater likelihood of acquiring virus, due to higher mobility and food consumption, and to the greater yield of larger cadavers. Contrarily, virus transmission in *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) (Young & Yearian 1988) and *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Woods & Elkinton 1987) was more effective in causing epizootics when hosts died at earlier instars. In both cases, it was suggested that, by the time older larvae died, most surviving individuals had low susceptibility to infection. Also, in the case of *A. gemmatalis*, lysis of infected corpses of older larvae may be incomplete, thus reducing inoculum availability (Young & Yearian 1988).

Two variables are crucial when investigating host stage effects on epizootic development: the yield of infected cadavers and the population susceptibility. In this experiment, the higher initial virus density in the arena containing older larvae is counterbalanced by their lower susceptibility to infection (LD_{50} around 5.4×10^3; 5.9×10^4 and 2.4×10^5 PIBs for 3rd, 4th and 5th instar larvae respectively) (Evans 1981). Therefore, it is unlikely that virus availability influenced transmission, as the inoculum introduced was sufficiently high to lethally infect all larvae in the container. In a field study on host stage structure and virus transmission in *M. brassicae*, Goulson *et al*. (1995) found that transmission rates were similar for larvae at 2nd, 3rd and 4th instar exposed to the same inoculum (3rd instar PIL). However, using the yield/LD_{50} rate, it appears that younger larvae were exposed to a higher dose of virus than older ones in that experiment, since instar combinations were not used.

The shorter time to death recorded for larvae at older instar combinations in this study contradicts most data on dose-response on baculovirus infection, which assume that larvae at earlier instars die more rapidly than their older counterparts (Ignoffo 1966, Abbas *et al.* 1991, Tanada & Kaya 1993). In such studies, however, virus was administered as a discrete dose in small leaf discs, diet plugs or droplets, and larvae had a limited period to feed. The protocol used here allowed behavioural differences to affect the probability of virus acquisition. *Mamestra brassicae* larvae at older instars move at least three times further than their younger counterparts (Vasconcelos *et al*. 1996) and food consumption rates increase up to 30 times as larvae age (Goulson *et al*. 1995). Since higher virus doses

accelerate host death (Teakle *et al.* 1986, Van Beek *et al.* 1988), the shorter time to death could have resulted from older larvae encountering virus more frequently, ingesting higher amounts of inoculum or ingesting a lethal dose earlier than younger larvae.

The period between infection and death can influence the secondary cycling of inoculum, depending, among other factors, on the susceptibility of the remaining hosts and the rate of virus production *in vivo* (see Vasconcelos 2001, for a review). In this study, a secondary wave of mortality may have contributed to the dynamics of infection, since the survival of 3rd instar larvae showed a steadier decrease when compared to other instars. Thus, it is possible that some larvae that died at the end of the experiment acquired virus resulting from earlier deaths.

Unfortunately, field data on the effect of host parameters on the success of viruses as biopesticides - or as agents for natural regulation of insect populations - still remain scarce. Information on epizootiological aspects of the insect-virus interaction can aid in designing strategies for microbial control, such as different timing, rate and techniques of application, if the pathogen is aimed at insects at different stadia. The results presented here indicate that the stage structure of the host population can affect the pattern of virus epizootics and stress the importance in fitting larval stadium into quantitative studies of insect-pathogen interactions.

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