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# Effect of parasitism on a nucleopolyhedrovirus amplified in *Spodoptera frugiperda* larvae parasitized by *Campoletis sonorensis*

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**Abstract**

We evaluated the consequences of parasitism by the solitary ichneumonid endoparasitoid *Campoletis sonorensis* (Cameron) towards the replication, genetic composition and virulence of a nucleopolyhedrovirus (Baculoviridae) originating from *Spodoptera frugiperda* (J. E. Smith) larvae. Parasitism by *C. sonorensis* and viral infection of third and fourth instar *S. frugiperda* larvae resulted in reduced growth compared with nonparasitized control larvae. A positive correlation was observed between virus yield and larval instar at the moment of infection. When larvae were virus-inoculated in the fourth instar, parasitism resulted in a significant reduction in mean per capita virus yield compared to the virus yield from nonparasitized larvae. In an experiment involving 10 serial passages of virus in both parasitized and nonparasitized larvae, restriction endonuclease analysis of viral DNA amplified in nonparasitized larvae revealed the presence of the wild-type virus as well as three additional variants (A, B, and C) diagnosed by the presence of novel submolar *Pst*I fragments of different sizes. In contrast, analysis of viral DNA from parasitized larvae showed the presence of the wild-type virus and two other variants (E and F), each characterized by a different submolar *Bgl*II fragment. Southern blot analysis indicated that the submolar fragments of variants E and F contained sequences originating from the viral genome. Bioassay of the different virus variants in *S. frugiperda* larvae indicated that their virulence was equal or less than that of the wild-type virus. We conclude that parasitism can affect the quantity of virus produced in dually infected and parasitized larvae, but no adverse effects were detected in terms of the biological activity of the virus.

## Introduction

Wild-type baculoviruses have already been used extensively as bioinsecticides, and are currently in use at several locations world-wide (Moscardi, 1999). They are particularly suitable for small scale use in developing countries (Jones et al., 1993) since they can be produced *in vivo* locally (Cherry et al., 1997). Their high virulence and narrow host range makes them ideal for use in programs of integrated pest management (Huber, 1986). Furthermore, baculoviruses have the ability to multiply in their target host and, therefore, they may persist or even spread in the host population initiating widescale epizootics (Fuxa, 1991).

The nature of interactions between the entomopathogen and other control agents, including parasitoid species, must be thoroughly evaluated when considering a virus for use in a pest management program. Interactions between parasitoids and baculoviruses are known to occur in simultaneous infections in many host-parasitoid-virus systems (Harper,

1986). The death of the parasitoid can occur within virus-infected hosts due to virus-induced host mortality (Eller et al., 1988; Hochberg, 1991), or due to toxic factors produced during viral replication in the host insect (Hotchkin & Kaya, 1983), or because of a physiological incompatibility of the infected host for parasitoid development (Brooks, 1993).

Studies of host-parasitoid-virus interactions have principally focussed on the impact of the virus on parasitoid fitness components such as survival, body size, longevity and fecundity (Brooks, 1993). There have been very few studies of the effect of parasitism on the quantity and quality of virus produced in simultaneously parasitized and infected hosts. Virus production can be affected due to decreased susceptibility of the parasitized host to viral infection or by direct competition for host resources by the developing endoparasitoid (Santiago-Alvarez & Caballero, 1990). In contrast, parasitoids may also act as virus disseminators: wasps that emerge from virus-infected hosts may vector the virus to other susceptible individuals in the host population (Caballero et al., 1991).

Natural populations of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), suffer parasitism from many species of endo- and ectoparasitoids (Ashley, 1986). The solitary larval endoparasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) is one of the most abundant parasitoids of *S. frugiperda* (Pair et al., 1986). The nucleopolyhedrovirus (Baculoviridae) of *S. frugiperda* (SfMNPV) is a major, natural, biotic control agent in certain areas (Shapiro et al., 1991) and is being developed as a potential bioinsecticide for small scale Latin-American maize farmers (Williams et al., 1999). In the present paper we report the impact of parasitism by *C. sonorensis* on the production of a nucleopolyhedrovirus as well as on the genetic composition and virulence of serially passaged virus in nonparasitized and parasitized hosts.

## Materials and methods

*The insects.* Larvae of *S. frugiperda* came from a culture maintained at the Universidad Pública de Navarra, Spain, which was initiated in 1997 and reared on semi-synthetic diet (Poitout & Buès, 1974) at 26 ± 1 °C, 85% r.h., and with a L16:D8 h regime. A culture of the ichneumonid parasitoid *C. sonorensis* was started from pupae received from M. D. Summers (Texas A and M University, College Station, Texas), and maintained continuously on *S. frugiperda*. Emerging wasps were held in plastic containers (115 mm diameter; 45 mm height) under constant light and allowed to feed on droplets of a 30% v/v honey solution in water for 6 days, after which four mated females were transferred to new plastic containers containing 40 third instar larvae of *S. frugiperda* and oviposition was allowed to occur overnight resulting in a high prevalence of parasitism.

*The baculovirus.* The virus used was a *S. frugiperda* multiply enveloped nucleopolyhedrovirus (SfMNPV) which was originally isolated in Nicaragua and shown to have a high degree of infectivity toward a culture of *S. frugiperda* larvae originating from Mexico and Honduras (Escribano et al., 1999). This virus was amplified by feeding early fourth instar ($L_4$) *S. frugiperda* larvae and the viral occlusion bodies (OBs) were purified and the concentration of the resulting suspension was determined as described elsewhere (Caballero et al., 1992).

*Larval growth and virus production.* Thirty newly-moulted third and fourth instar larvae were inoculated with $LC_{90}$ concentrations of $3.32 \times 10^7$ and $6.83 \times 10^8$ OBs $ml^{-1}$, respectively, using the droplet-feeding method of Hughes & Wood (1981). All these larvae were previously starved for 8 h and half of them were simultaneously exposed to *C. sonorensis* females to obtain dually infected and parasitized larvae. Then, larvae were individually reared in 25 ml diet cups at 26 ± 1 °C, 85% r.h., and a 16 h daylength and weighed daily to an accuracy of ± 0.1 mg. Groups of twenty respectively, second, third, fourth, and fifth instar larvae were infected as above, with their corresponding $LC_{90}$'s (Escribano et al., 1999), and individually reared under the same conditions. Moribund virus-infected larvae were transferred to individual 1.5 ml microfuge tubes and weighed prior to death. These larvae usually died and liquefied in less than 12 h. Larvae that died before being transferred to the microfuge tube were not used for yield measurements. Virus-killed larvae were homogenized in 300 $\mu$l double-distilled water, and the OBs were purified by filtration through cheesecloth to remove the larval debris and then through a 30% (wt/vol) sucrose layer by centrifugation at $6800 \times g$ for 10 min. Yields of OBs per larva were determined by a Thoma counting chamber (Hawksley) under phase-contrast microscopy. Each sample was counted a minimum of three times.

In vivo *passaging of virus.* The serial passage experiment involved inoculating 30 third-instar *S. frugiperda* larvae nonparasitized or parasitized 8 h previously by *C. sonorensis* with a virus suspension containing $3.32 \times 10^7$ OBs $ml^{-1}$ which represented the $LC_{90}$ concentration. Larvae were then reared individually on semi-synthetic diet as described above. All larvae succumbing to virus infection in each treatment (parasitized and nonparasitized) were collected, pooled and the virus was extracted as described above. A second passage was made following the same procedures and using the same virus concentration. This *in vivo* passaging was repeated ten times in total. A sample of purified OBs from each passage was stored at $-20$ °C and later used for viral DNA purification and analysis (see below). The experiment was replicated three times.

*Isolation of viral DNA, REN analysis, and gel electrophoresis.* Viral DNA extraction and purification was performed according to Caballero et al. (1992). Virions were released from OBs by incubation with DAS buffer (30 mM $Na_2CO_3$, 50 mM NaCl, 30 mM EDTA [pH 10.5]) and purified by centrifugation in a continuous sucrose gradient. Purified virions were incubated with proteinase K (200 $\mu$g $ml^{-1}$) at 45 to 50 °C for 2.5 h and then with 1% sodium dodecyl sulfate for an additional 0.5 h. After phenol extraction, the aqueous suspension containing the DNA was dialysed against three to four changes of 10 mM TE buffer at 4 °C for 48 h. For restriction endonuclease analysis 1 $\mu$g of DNA was incubated with 10 units of the restriction enzymes *Pst*I, or *Bgl*II (Amersham, UK), as described in the supplier's instructions, at 37 °C for 4 h. Reactions were stopped by the addition of one-sixth volume of 6×loading buffer (0.25% bromophenol blue, 40% sucrose). Electrophoresis was performed using horizontal 0.8% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA [pH 8.0]) containing 0.25 $\mu$g of ethidium bromide per ml. Gels were placed on a UV transilluminator (302 nm) and photographed.

*Southern blot hybridization.* DNA, respectively, from nonparasitized *S. frugiperda* larvae, parasitized *S. frugiperda* larvae, parasitized *S. frugiperda* larvae from which the parasitoid larvae had been removed, and adult female *C. sonorensis* was isolated using the procedure described by Heckel et al. (1995), and digested with the restriction endonuclease *Bgl*II. Agarose gel electrophoresis and transfer to membrane filters (Hybond-N+, Amersham, UK) were carried out using standard procedures (Southern, 1975; Sambrook et al., 1989). DNA probes were labelled with [$\alpha$-$^{32}$P]dCTP by random priming. Labelled probe was allowed to hybridize at 42 °C in 5×SSC (1×SSC is 0.18 M NaCl, 10 mM $NaH_2PO_4$, and 1 mM EDTA, pH 7.7), 50% formamide, 20 mM $NaPO_4$ (pH 7.0), 1×Denhardt's solution, and 0.1 mg of herring-sperm DNA. After hybridization, the blots were washed twice, 15 min each, at 42 °C in 2×SSC containing 0.1% SDS and exposed to X-ray film (Biomax, Kodax, Rochester, N.Y.) for 3 to 48 h at $-80$ °C with an intensifying screen.

*Bioassays.* The median lethal concentrations ($LC_{50}$s) and the mean times to death of the original wild type isolate and the virus samples recovered from the *in vivo* passages were determined by insect bioassay using the droplet-feeding method of Hughes & Wood (1981). First-instar *S. frugiperda* larvae, selected when they began to moult, as determined by head capsule slippage, were starved for 8 h at 25 °C to encourage them to take up virus droplets, and were allowed to drink from an aqueous suspension containing virus at concentrations of $9.6 \times 10^3$, $4.8 \times 10^4$, $2.4 \times 10^5$, $1.2 \times 10^6$, and $6 \times 10^6$ OBs $ml^{-1}$. This concentration range was found to kill between 5 and 95% of the test larvae in bioassays previously performed with the original wild-type isolate (Escribano et al., 1999). Larvae that ingested the solution within 10 min were transferred to individual cells of a 25-compartment Petri dish with a formaldehyde-free diet plug. Bioassays, using 25 larvae per virus concentration and 25 larvae as controls, were replicated three times. Bioassays were conducted at a constant temperature of $25 \pm 2$ °C, and larval mortality was recorded every 12 h until larvae had either died or pupated.

*Data analysis.* The effects of host instar at the time of virus infection and larval condition (nonparasitized or parasitized by *C. sonorensis*) on virus yield were determined by a factorial analysis of variance using the generalized linear modelling of the program SPSS (v. 7.5). Model-checking showed that the data satisfied the assumption of normality required by analysis of variance. Significant differences among means were determined by Fisher's least significant difference (LSD). Concentration-mortality regressions for the different virus inocula bioassayed were calculated with the probit option (Finney, 1971) of the computer program POLO-PC (Le Ora Software, 1987).

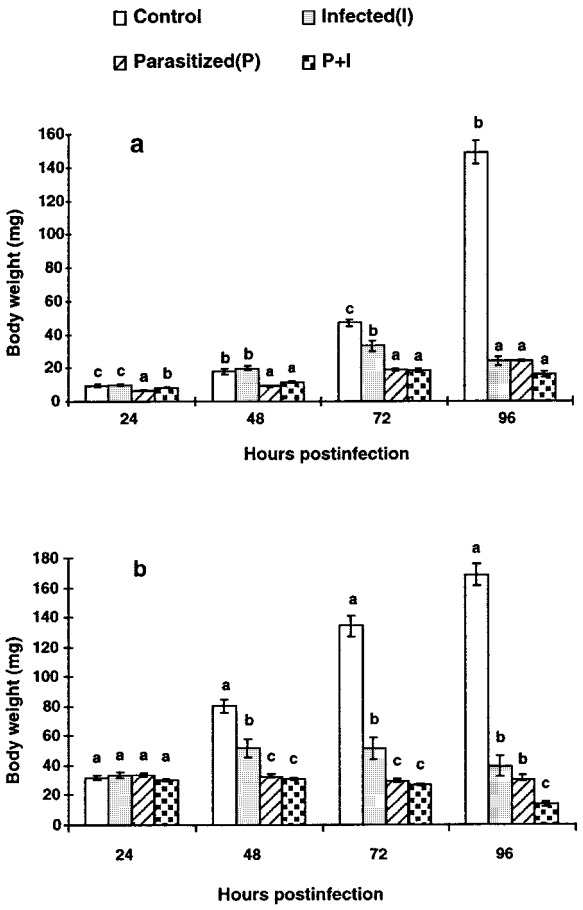


*Figure 1.* Body weight of *Spodoptera frugiperda* third (a) and fourth (b) instar larvae nonparasitized or parasitized by *Campoletis sonorensis* and standard error bars at various times postinfection with an $LC_{90}$ virus concentration of $3.32 \times 10^7$ OBs $ml^{-1}$. Columns with the same letter at each hour postinfection are not significantly different ($P > 0.05$; Fisher's least significant difference).

## Results

*Larval weight and virus yield.* Parasitism by *C. sonorensis* and viral infection of *S. frugiperda* larvae resulted in significantly lower larval weights compared with nonparasitized mock-infected control larvae (Figures 1a and 1b). The effect of the parasitism on larval weight was detected at 24 h after the start of the experiment in third instar, and at 48 h in fourth instar larvae. In virus infected larvae, this effect was detected from respectively 72 h and 48 h after infection in third and fourth instars. In third instar larvae, no significant difference was found between the mean body weights of nonparasitized virus-infected larvae and parasitized virus-infected larvae by 96 h postinfection (p.i.) when the peak virus-induced mortality occurred.

*Table 1.* Mean occlusion body yields (± S.E.) in respectively, second, third and fourth instar *Spodoptera frugiperda* larvae nonparasitized or parasitized by *Campoletis sonorensis* following infection with an $LC_{90}$ of nucleopolyhedrovirus

| Host condition | Larval instar and production (OBs$\times 10^8$/larva) | | |
|---|---|---|---|
| | $L_2$ | $L_3$ | $L_4$ |
| Nonparasitized | $0.31 \pm 0.04$a | $1.26 \pm 0.15$b | $3.38 \pm 0.47$c |
| Parasitized by *C. sonorensis* | $0.30 \pm 0.04$a | $1.19 \pm 0.07$b | $1.62 \pm 0.09$b |

Means in the same column followed by the same letter are not significantly different ($P > 0.05$: Fisher's least significant difference).

In contrast, in fourth instar larvae the average body weight of virus-infected nonparasitized larvae by 96 h p.i. was significantly higher than that of virus-infected parasitized larvae (Figures 1a and 1b).

The virus yield per larva increased significantly with the larval instar at the time of infection ($F_{2,55} = 101.38$, $P<0.05$). Parasitism resulted in a significant reduction in mean virus yield ($F_{1,55} = 22.36$, $P<0.05$), and the interaction between these factors was also significant ($F_{2,55} = 20.51$, $P<0.05$). In nonparasitized virus-killed larvae the instar at the time of infection had a significant effect on the number of viral OBs produced per larva (Table 1). In virus-killed larvae parasitized by *C. sonorensis*, the number of OBs produced in larvae infected during the second instar was significantly lower than that produced in larvae infected in the third instar, whereas no difference was found between larvae infected in the third or fourth instars. No significant differences were found among the mean OBs produced in nonparasitized or parasitized larvae when the larvae were infected in the second or third instar. However, nonparasitized larvae infected as fourth instars produced more than twice the number of OBs per larva compared with those parasitized by *C. sonorensis*.

*Virus amplified in nonparasitized and parasitized larvae.* Restriction endonuclease analysis of viral DNA amplified in nonparasitized larvae revealed the presence of the wild-type virus as well as three additional variants. The existence of such variants, which were named A, B, and C, was diagnosed by the presence of novel submolar fragments of about 6.1 kb, 8.1 kb, and 6.5 kb, in the *Pst*I digested viral DNA (Figure 2). These variants were indistinguishable from wild-type viral DNA when digested *with Bgl*II (data not shown). The wild-type DNA restriction profile remained unchanged until passage 3 in replicates I and II, and

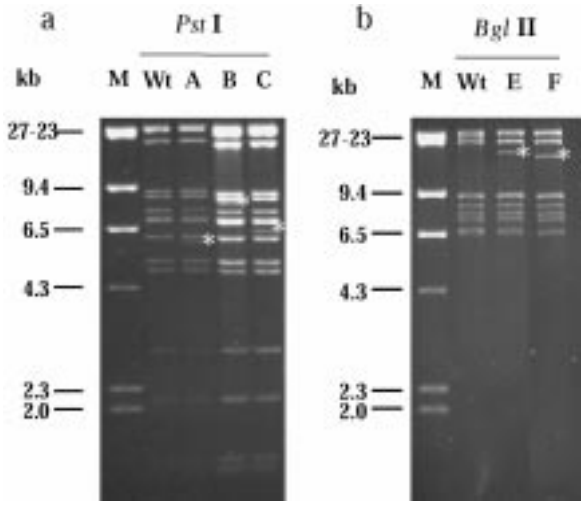


*Figure 2.* Restriction endonuclease profiles of viral DNA, amplified in *Spodoptera frugiperda* larvae nonparasitized or parasitized by *Campoletis sonorensis*, following digestion with *Pst*I (a) and *Bgl*II (b) and electrophoresed in a 0.8% agarose gel. M (Marker), wt (original wild-type virus inoculum), A, B, and C (virus variants amplified in nonparasitized larvae), E and F (virus variants amplified in parasitized larvae). A *Hind*III digest of λ DNA was used as molecular marker (fragment molecular sizes of 27.49, 23.13, 9.42, 6.56, 4.36, 2.32 and 2.03 kbp). Asterisks indicate submolar fragments indicative of virus genotypic variants.

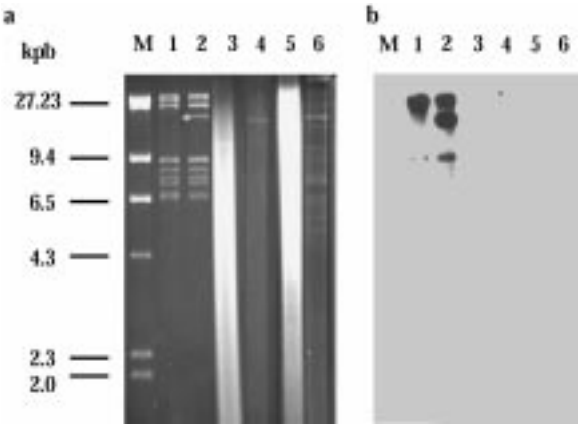


*Figure 3.* (a) Restriction endonuclease profiles of *Bgl*II-digested DNA from: wild-type virus (lane 1), virus variant E (lane 2), *S. frugiperda* larvae (lane 3), *S. frugiperda* parasitized larvae (lane 4), *S. frugiperda* parasitized larvae with parasitoid larva removed (lane 6) and adult female *C. sonorensis* (lane 5). (b) Southern blot analysis of gel shown in (a) using the 14.2 kb submolar fragment of restriction profile E as probe. Lane λ, lambda DNA digested with *Hind*III (fragment sizes as per figure 2). Asterisks indicate submolar fragments indicative of virus genotypic variants.

until passage 6 in experiment III. Profile variant A was detected in experiment III after passage 10 and in experiment I after passage 3, but in this experiment it was replaced by profile variant B detected after passage 10. Profile C was detected in experiment II after passage 6 and remained unchanged thereafter.

Restriction endonuclease analysis of viral DNA amplified in parasitized larvae revealed the presence of the wild-type isolate and two other variants which were called E and F. Profile variant E was observed in experiment I after passage 6 and remained unchanged for all subsequent passages. DNA profile variant F was only detected in experiment II after passage 10. These variants were characterized by a submolar *Bgl*II fragment at 14.2 and 13.8 kb, respectively, but they were indistinguishable from the wild-type profile when treated with *Pst*I. Hybridization to the radioactively-labelled fragments of 14.2 kb (Figure 3b) or 13.8 kb (Figure 4b) indicated that both fragments contained sequences from the viral genome since signals were detected in DNA isolated from the virus variants E and F and from the wild-type virus. Probe hybridization was not detected to DNA isolated from nonparasitized *S. frugiperda* larvae, *S. frugiperda* larvae parasitized by *C. sonorensis* with or without the parasitoid larva, and adult female *C. sonorensis*. Strong hybridization was observed to the submolar fragment at 14.2 kb of virus variant E (Figure 3b) and the submolar fragment at 13.8 kb of virus variant F (Figure 4b), but no signals were detected in the wild-type virus profile at either 14.2 and 13.8 kb indicating that these submolar fragments were not present in detectable quantities in the wild-type isolate. Hybridization signals were also observed with various equimolar restriction fragments of virus variants E and F and the wild-type virus genome, suggesting some degree of homology.

*Activity of OBs from nonparasitized and parasitized larvae.* The insecticidal activities of viral OBs from the various restriction profiles (A, B, C, E, F and wild-type) were determined based on their median lethal concentration ($LC_{50}$) towards second-instar *S. frugiperda* larvae. For all variants, viral mortality increased with the virus concentration and in all cases the $\chi^2$ value was not significant at P=0.05 when measuring the goodness-of-fit of each regression line independently. The regression line slopes were not significantly different from one another ($\chi^2 = 8.74$, df=5, P=0.121) which allowed us to fit all these lines in parallel, with a common slope of 0.76 (Table 2). No significant differences were found between the virus profiles A and B compared with the wild-type isolate.

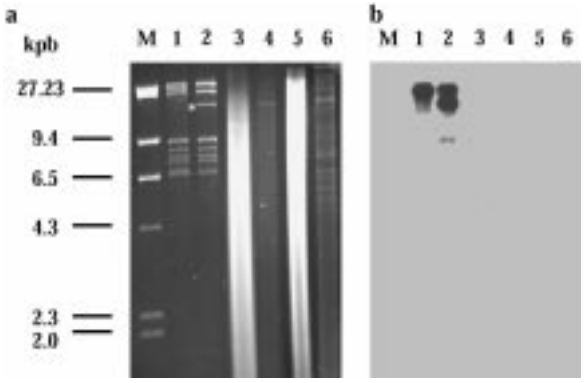


*Figure 4.* (a) Restriction endonuclease profiles of *Bgl*II-digested DNA from: wild-type virus (lane 1), virus variant F (lane 2), *S. frugiperda* larvae (lane 3), *S. frugiperda* parasitized larvae (lane 4), *S. frugiperda* parasitized larvae with parasitoid larva removed (lane 6) and adult female *C. sonorensis* (lane 5). (b) Southern blot analysis of gel shown in (a) using the 13.8 kb submolar fragment of restriction profile F as probe. Lane λ, lambda DNA digested with *Hind*III (fragment sizes as in Figure 2). Asterisks indicate submolar fragments indicative of virus genotypic variants.

Viral OBs of profile C were significantly less infective than the wild-type virus. The relative potencies and their corresponding confidence limits indicated that the infectivity of OBs produced in parasitized larvae (virus profiles E and F) were not significantly different from those observed in nonparasitized larvae (virus variants A and B) or compared with the wild-type virus.

## Discussion

The possibility that insect parasitism might affect both the quantity and quality of virus produced in dually infected and parasitized hosts has not been previously studied. The mean number of OBs produced in virus-killed larvae parasitized by *C. sonorensis* was not significantly different from that produced in nonparasitized *S. frugiperda* larvae when they were infected in second or third instar. The similar body weight of nonparasitized and parasitized infected larvae at the moment of death is the most likely explanation for this result since host weight gain during the period of virus replication is a highly influential factor in virus yield (Shapiro, 1986). A positive correlation between log body weight and log virus yield has also been reported in other host-virus systems (Evans et al., 1981; Kunimi et al., 1996). It appears from our results that the presence of an endoparasitoid larva in second or third instar larvae does not adversely affect virus production, suggesting that in these instars the virus is the superior competitor and dominates the use of host resources.

In contrast, virus production in nonparasitized fourth instar larvae was greater than in parasitized hosts, indicating direct competition for host resources. Such a negative interaction may not be important in viral insecticide based biocontrol programmes in the field since virus applications are usually directed against the most susceptible early larval instars, which are also the preferred hosts for C. sonorensis (Isenhour, 1985; Moscardi, 1999).

The present study also shows the appearance of virus variants in repeated passages of a wild-type nucleopolyhedrovirus in parasitized *S. frugiperda* larvae. Submolar fragments observed in the restriction endonuclease profile of these virus variants typically indicate the presence of genotypic variants (Lee & Miller, 1978; Smith & Crook, 1988) which have been positively selected during the serial passage experiments, since they were not visible in the wild-type virus profile. The lack of hybridization signals indicates that the submolar fragments of the different virus variants were not present in detectable quantities in the wild-type isolate. However, *in vivo* cloning of genotypic variants from a virus population, in which they were present as minor genotypes undetected by hybridization, has been previously reported for different nucleopolyhedrovirus field isolates (Muñoz et al., 1998, 1999), and therefore this possibility cannot be discarded. Alternatively, these genotypic variants may have been generated during the serial passage experiment via point mutation of restriction enzyme recognition sites, by recombination between variants produced after repeated passage, or by other genomic alterations such as additions and duplications. Such phenomena have been identified in genotypic variants cloned from wild-type isolates of the nucleopolyhedrovirus of *Anticarsia gemmatalis* (Hübner) (Croizier & Ribeiro, 1992; García-Maruniak et al., 1996; Maruniak et al., 1999), and *Spodoptera exigua* (Hübner) (Muñoz et al., 1998; 1999). These variants may also be generated by insect transposable elements as has been demonstrated for the granulovirus of *Cydia pomonella* (L.) (Jehle et al., 1995). It appears from our probe hybridization results that the variability observed in the virus population cannot be explained by transposable elements described in lepidopteran species (Salvado et al., 1994) or by genetic entities from the polydnavirus associated with *C. sonorensis* (Stoltz, 1993).

*Table 2.* Response of second instar *Spodoptera frugiperda* larvae to virus variants amplified in *Spodoptera frugiperda* larvae nonparasitized or parasitized by *Campoletis sonorensis*

| Virus variant | Slope±SE | Intercept±SE | $LC_{50}$ (OBs $ml^{-1}$) | $\chi^2$ | df, P | Relative potency | 95% confidence limits | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| wt | $0.94 \pm 0.11$ | $-0.14 \pm 0.62$ | $2.83 \pm 10^5$ | 5.98 | 3, 0.113 | 1 | | |
| A | $0.76 \pm 0.10$ | $0.74 \pm 0.57$ | $3.64 \pm 10^5$ | 2.77 | 3, 0.428 | 0.79 | 0.29 | 2.12 |
| B | $0.82 \pm 0.10$ | $0.77 \pm 0.54$ | $1.39 \pm 10^5$ | 4.33 | 3, 0.228 | 2.08 | 0.79 | 5. 52 |
| C | $0.67 \pm 0.05$ | $0.96 \pm 0.28$ | $11.26 \pm 10^5$ | 3.38 | 3, 0.337 | 0.31 | 0.14 | 0.67 |
| E | $0.77 \pm 0.10$ | $0.84 \pm 0.52$ | $2.72 \pm 10^5$ | 2.87 | 3, 0.412 | 1.05 | 0.39 | 2.78 |
| F | $0.92 \pm 0.11$ | $0.32 \pm 0.54$ | $1.31 \pm 10^5$ | 5.27 | 3, 0.153 | 2.14 | 0.81 | 5.64 |

Parameters obtained from the POLO-PC program (Le Ora Software, 1987). The relative potencies of the virus variants (A-F) were estimated respect to the wild-type (wt) virus after fitting all the regression lines in parallel ($\chi^2 = 8.74$, df $= 5$, P $= 0.121$). $\chi^2$, goodness-of-fit chi-square; df, degrees of freedom for $\chi^2$; P, probability of a greater $\chi$ value.

In serial passage experiments within-host competition drives the evolution of pathogen virulence; the strain with the highest replication rate will achieve the greatest numerical representation in the inoculum used to infect the following generation of insects. The increased virulence observed in such experiments is frequently associated with a reduction in pathogen infectivity because pathogen transmission is performed by the experimenter rather than via a natural process subject to selection (Ebert, 1998). In parasitized hosts infected by virus, the situation is more complicated as all strains of the pathogen experienced interspecific competition for host resources with the developing parasitoid larvae. Of the three virus variants that appeared in nonparasitized hosts in our study, one did show reduced infectivity, whereas the activity of virus variants that appeared in parasitized *S. frugiperda* larvae did not differ significantly from the wild-type inoculum after 10 passages.

Our results indicate that parasitism can affect the quantity of virus produced in dually infected and parasitized larvae, but no adverse effects were detected in terms of the biological activity of the virus. Although viral infection can result in the premature death of parasitized hosts, parasitoids that do manage to complete their development and emerge from virus infected insects can act as efficient vectors for virus dispersal (Caballero et al., 1991). This supports the assertion that, generally, baculoviruses and insect parasitoids are compatible as control agents for lepidopteran defoliators in biorational pest control programmes (Roberts et al., 1991).

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