INTRODUCTION

*Spodoptera frugiperda* (the fall armyworm) is a destructive lepidopteran pest of several economically important crops. Microbial control of *S. frugiperda* has relied on the baculovirus *Spodoptera frugiperda* M nucleopolyhedrovirus, which has a very narrow host range that comprises only *S. frugiperda*, *Spodoptera litura* and *Spodoptera exigua* (Hamm & Styer, 1985; Adams & McClintock, 1991). Although specificity is important for any pesticide, it is economically desirable to control more than three pest species with any single control agent. *Autographa californica* M nucleopolyhedrovirus (AcMNPV) can infect and kill the larvae of at least 32 lepidopteran species, including *S. frugiperda* (Granados & Williams, 1986). Not all host species are highly susceptible to fatal infection by AcMNPV, and the biological basis for resistance in the suboptimal hosts has been explored in only a few species. Almost all host species demonstrate some degree of developmental resistance, i.e. increasing resistance with age (Stairs, 1965; Engelhard & Volkman, 1995). This type of resistance is observed only when virus is administered orally, and in some species it has been linked with the sloughing of infected midgut cells (Engelhard & Volkman, 1995; Kirkpatrick et al., 1998). This midgut cell sloughing can be reduced by the addition of the optical brightener M2R to the viral inoculum (Washburn et al., 1998). In addition, M2R disrupts the integrity of the peritrophic membrane lining the midgut and may enhance oral infectivity in some species by increasing access of occlusion-derived virus (ODV) to target cells (Wang & Granados, 2000). Shapiro & Argauer (1997) and others have demonstrated that M2R enhances virulence in a number of combinations of baculovirus and lepidopteran species when the brightener and occlusions are co-administered orally. These results suggest that midgut cell sloughing and possibly peritrophic membranes constitute major lepidopteran defences against baculovirus infection (Shapiro & Argauer, 1997; Washburn et al., 1998; Wang & Granados, 2000).

Two pest species resistant to AcMNPV infection, *Manduca sexta* and *Helicoverpa zeae*, mount cellular immune responses involving haemocyte encapsulation and the deposition of melanin around viral foci, processes that enhance the clearance of virus infections from the host (Washburn et al., 1996, 2000). For *H. zeae*, however, the major source of resistance is haemocyte absorption of budded virus (BV) from the surrounding haemolymph and their subsequent failure to amplify the virus (Trudeau et al., 2001). These resistance mechanisms result in various degrees of larval susceptibility to mortal infection and impact the utility of AcMNPV as a biological control agent.

We have characterized infection and pathogenesis of an *Autographa californica* M nucleopolyhedrovirus recombinant, AcMNPV-*hsp70/lacZ*, carrying the *lacZ* reporter gene, in penultimate (fifth) instar *Spodoptera frugiperda*. Bioassays revealed that while <0.1 p.f.u. of budded virus was required to generate an LD_{50} by intrahaemocoelic injection, approximately 6000 occlusions were required orally to achieve the same mortality in newly moulted fifth instar (5^0) larvae. In pathogenesis experiments, 78% of the 5^0 larvae inoculated orally with 6000 occlusions of AcMNPV-*hsp70/lacZ* were LacZ-positive at 8 h post-inoculation (p.i.) and 50% had LacZ signals in tracheal cells indicating that in these larvae infection had been transmitted from the midgut to secondary target cells. At 24 h p.i., maximum numbers of midgut and midgut-associated tracheal foci were observed (mean of 35 foci per infected larva), and 88% of the larvae were LacZ-positive. The extremely low foci-per-occlusion ratio (0.006) indicated that successful infection of midgut cells was the primary barrier to fatal infection. A second barrier involved the loss of infected tracheal cells associated with the midgut. At 24 h p.i., 88% of the inoculated larvae had a systemic infection, but in bioassays only 51% succumbed to polyhedrosis disease. The absence of melanized tracheal cells in the insects throughout the time-course suggested that the larvae that cleared their infections (38%) did so by a mechanism other than a classical immune response.

Pathogenesis of *Autographa californica* M nucleopolyhedrovirus in fifth instar *Spodoptera frugiperda*

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We present evidence here for two additional barriers to fatal infection by AcMNPV. Both barriers were associated with infection via the oral portal of entry into fifth instar *S. frugiperda*; they were the inability of AcMNPV ODV to infect midgut cells efficiently and the loss of infected tracheal cells in the absence of a classical immune response. Oral infection was only moderately enhanced by M2R, indicating that most of the midgut barrier was due to lack of initial infection rather than cell sloughing or lack of ODV contact with primary cellular targets. Our results differed from those of Clarke & Clem (2002) who reported that the haemocytes of *S. frugiperda* larvae are refractory to infection. In our hands, both *S. frugiperda* larvae (from the same source used by these investigators) and their haemocytes were highly sensitive to infection by BV within the haemocoele.

**METHODS**

**Maintenance and inoculation of larvae.** *Trichoplusia ni*, *Heliothis virescens* and *S. frugiperda* eggs were provided by the American Cyanamid Corporation (Princeton, NJ, USA) and purchased from Benzon Research (Carlisle, PA, USA) or AgriPest (Zebulon, NC, USA). Larvae were reared in groups on a semi-synthetic diet (Stoneville) at 28˚C under constant illumination until the beginning of the penultimate instar (fourth for *T. ni* and *H. virescens* and fifth for *S. frugiperda*). Under these rearing conditions, *S. frugiperda* had six instars while *T. ni* and *H. virescens* had five. All larvae were inoculated with a plastic syringe fitted with a 32-gauge needle and mounted onto a microapplicator (Burkhard). Larvae were inoculated orally within 15 min of moulting to the penultimate instar or at specific times afterwards by carefully inserting a blunt-tipped needle through the mouth and into the lumen of the anterior midgut where the virus suspension was discharged. Larvae were injected intrahaemocoelically (IH) by inserting a sharp-tipped needle through the planta of one of the prolegs, and a 1:0 µl suspension of the virus was delivered directly into the haemocoele. Following inoculation, all test larvae were maintained individually on a modified Stoneville diet in 25 ml plastic cups in an incubator (strain L1), vHSGFP, which carries an egFP gene under the control of the *Drosophila hsp70* heat shock promoter at a site adjacent to the polyhedrin gene (Clarke & Clem, 2002), was obtained from R. J. Clem (Kansas State University, Manhattan, KS, USA) and used for one of our studies. For all remaining studies, we used AcMNPV-hsp70/lacZ (containing all the wild-type E2 viral genes and the *Escherichia coli* β-galactosidase reporter gene driven by the functionally early *Drosophila hsp70* heat shock promoter). AcMNPV-hsp70/ lacZ generates mortality in larvae at levels identical to wild-type AcMNPV (Engelhard et al., 1994).

**Viruses and virus purification.** Two AcMNPV recombinants were used for the experiments reported here. A recombinant of AcMNPV (strain L1), vHSGFP, which carries an egFP gene under the control of the *Drosophila hsp70* heat shock promoter at a site adjacent to the polyhedrin gene (Clarke & Clem, 2002), was obtained from R. J. Clem (Kansas State University, Manhattan, KS, USA) and used for one of our studies. For all remaining studies, we used AcMNPV-hsp70/lacZ (containing all the wild-type E2 viral genes and the *Escherichia coli* β-galactosidase reporter gene driven by the functionally early *Drosophila hsp70* heat shock promoter). AcMNPV-hsp70/lacZ occlusions were isolated from virus-killed *T. ni* cadavers, partially purified as described previously (Summers & Smith, 1987) and stored in a neutrally buoyant solution of glycerine and water (3:2, v/v). Occlusion numbers were quantified using a haemocytometer and occlusions were maintained at 4˚C until use. For some experiments, ODV was isolated as previously described by Volkman et al. (1976) with the following modifications. The ODV pellets were resuspended in minimal volumes of PBS, the total volumes measured and samples removed to determine the protein concentrations using the BCA protein assay (Pierce). Subsequently, 1 % foetal bovine serum was added for stabilization, and the ODV suspension was aliquoted into microfuge tubes for storage at ~80˚C until use. BV was harvested from the cell culture medium of infected SF-9 cells 3 days post-infection (p.i.), and the titres were determined by immunoplaque assay using SF-9 cells (Volkman & Goldsmith, 1982).

**Bioassays.** To quantify the relative susceptibilities of *S. frugiperda* and *H. virescens* to AcMNPV-hsp70/lacZ, newly moulted or feeding penultimate instar larvae were inoculated orally or IH with increasing concentrations of occlusions or BV, respectively, and maintained until pupation or death as described above (*n=25–32* larvae per treatment). Regression lines for dose–mortality relationships were fitted by the least-squares method. To assess the degree to which optical brightener could enhance mortal infection of *S. frugiperda* by the oral portal of entry, 1 % M2R (solubilized in DMSO) was incorporated into viral inocula containing occlusions. Identical volumes of DMSO without M2R were added to inocula to generate control inocula (Washburn et al., 1998). Newly moulted and 16 h post-moult fifth instar *S. frugiperda* larvae (designated 5° and 5°, respectively) were inoculated orally with either M2R or control inocula and maintained until pupation or death (*n=32* larvae per developmental stage and treatment).

**Midgut lumen pH.** The pH values of the midgut lumena of uninfected fourth instar *H. virescens* and *T. ni* larvae and fifth instar *S. frugiperda* larvae (Benzon strain) were determined by dissecting eight feeding larvae per species and rinsing each haemocoele three times with double-distilled (dd) H2O. After the final rinse was removed by vacuum aspiration, a longitudinal cut was made along the midgut and the contents of each midgut lumen removed and placed into a single microfuge tube containing 200 µl ddH2O, then centrifuged at 2000 g for 10 min. The pH of the supernatant was measured with a microtip pH probe (Corning, G-P Micro Combo).

**Time-course.** A time-course experiment was conducted to elucidate the timing and pattern of lacZ expression in 5° *S. frugiperda* larvae (Benzon strain) inoculated orally with 6000 occlusions of AcMNPV-hsp70/lacZ. At specific times post-inoculation, cohorts of 23–25 larvae were sacrificed and processed for lacZ expression (see Washburn et al., 1998). Subsequently, larvae were examined microscopically to quantify the number and cellular composition of viral foci. For time-points between 2 and 30 h p.i., only midguts were examined for a LacZ signal, but for the 48, 60 and 84 h p.i. time-points, whole mounts of entire larvae were prepared and examined. Larvae with greater than 200 midgut foci were not included in analyses of foci number and composition for technical reasons. For the *S. frugiperda* time-course shown, this included one larva each at 18 and 24 h p.i., two at 60 h p.i. and three at 84 h p.i. Two cohorts of 32 larvae each were held until death or pupation for an internal control (LD50).

**Analysis of haemocyte infection.** *S. frugiperda* larvae (5° stage Benzon and AgriPest strains) were inoculated IH with 70 p.f.u. AcMNPV-hsp70/lacZ BV. At 24 h intervals, haemocytes were collected from 10 randomly selected larvae of each strain and processed for lacZ expression as described by Trudeau et al. (2001). The percentage of LacZ-positive haemocytes was determined by counting 200 randomly selected cells per sample. For some samples at the 24 h p.i. time-point, the entire well was examined for the presence of LacZ-positive haemocytes because they were so rare. By 96 h p.i., some larvae of both strains had died of polyhedrosis disease, in which case only live larvae (those responding to physical stimuli) were bled. The cadaver from each larva bled for haemocytes was fixed in 2 % paraformaldehyde, processed for LacZ expression and examined using light microscopy.
RESULTS

Relative susceptibility of *S. frugiperda* and *H. virescens* larvae to oral infection

We compared virulence of AcMNPV-hsp70/lacZ in 4° *H. virescens* and 5° *S. frugiperda* larvae in oral bioassays and found a 1000-fold difference between the two species in their sensitivity to mortal infection. Only nine occlusions were required to generate 50 % mortality in 4° *H. virescens*, whereas over 9000 occlusions were required to obtain the same mortality level in orally inoculated 5° *S. frugiperda* (Fig. 1A). In view of the low oral infectivity, we compared the pH values of the midgut lumena fluids of fifth instar *S. frugiperda* and those of two hosts susceptible to oral infection by AcMNPV in order to determine whether the pH of *S. frugiperda* midgut fluid was sufficiently alkaline to dissolve occlusions efficiently. The pH values were 9-5 for *S. frugiperda*, 8-7 for *H. virescens* and 9-2 for *T. ni*. Although pH of lumenal fluids did not appear to be a causative factor for low oral infectivity in *S. frugiperda*, we were able to rule out any occlusion dissolution problems conclusively by conducting oral bioassays with ODV. Similar to occlusions, AcMNPV-hsp70/lacZ ODV was over 1000-fold more virulent in 4° *H. virescens* than in 5° *S. frugiperda* (Fig. 1B).

Time-course

To investigate whether the barrier to mortal infection by the oral portal of entry involved midgut cells and/or primary targets of BV generated from them, we performed time-course experiments with 6000 occlusions of AcMNPV-hsp70/lacZ inoculated orally into 5° *S. frugiperda* larvae. The first LacZ-positive viral foci were detected at 4 h p.i. (none was apparent at 2 h p.i.), and the seven foci observed consisted of infected midgut epithelial cells distributed among only four of 23 larvae examined (17 %; Fig. 2A, B). By 8 h p.i., 78 % of the inoculated larvae were LacZ positive, and 50 % of these had foci involving tracheal cells (Fig. 2A). This result showed that in 50 % of the larvae, BV had already initiated infection in the tracheal epidermis within the first 4 h after primary infection had been established in the midgut (Fig. 2A). In addition, 18 % of the larvae sampled at 8 h p.i. contained viral foci consisting of tracheal cells only, demonstrating that the underlying infected midgut cells had been sloughed (Fig. 2B). The maximum percentage of lacZ-expressing larvae (96 %) occurred at 10 h p.i. (Fig. 2A), when 83 % of infected larvae contained midgut-associated foci with LacZ-positive tracheal cells. By 24 h p.i., the proportion of lacZ-expressing larvae was slightly lower (88 %), but all of these larvae had infected tracheal cells (Fig. 2A). The maximum number of midgut and midgut-associated tracheal foci per infected larva [35 ± 24 (SD), n = 20 larvae] occurred at the 24 h p.i. time-point (Fig. 2B). By 30 h p.i., both the percentage of lacZ-expressing larvae and the average number of foci per infected larva had dropped precipitously and reached 50 % and 8, respectively (Fig. 2A, B). Moreover, there was no indication of melanization in any larva; hence, it appeared that the viral foci had been cleared by a mechanism other

![Fig. 1. Larval mortality levels of *S. frugiperda* (Benzon strain) and *H. virescens* inoculated orally with increasing concentrations of AcMNPV-hsp70/lacZ occlusions (A) or ODV (B) as newly moulted penultimate instars. The regression equations for (A) are: *S. frugiperda* (solid line), y = 19·8log(x)−28·5 (r² = 0·98), LD₉₀ = 9200 occlusions; for *H. virescens* (dashed line), y = 47·1log(x)+5·6 (r² = 0·91), LD₉₀ = 9 occlusions. Each point represents between 27 and 32 larvae. The regression equations for (B) are: *S. frugiperda* (solid line), y = 26·9log(x)−72·6 (r² = 0·98), LD₉₀ = 3·6 × 10⁴ pg of ODV; for *H. virescens* (dashed line), y = 30·0log(x)+20·4 (r² = 0·94), LD₉₀ = 9·6 pg of ODV. Each point represents between 30 and 32 larvae.](http://vir.sgmjournals.org)
than a phenoloxidase-mediated immune response. Although the percentage of larvae expressing lacZ at 24 h p.i. was equivalent to the final mortality level (51%), at 84 h p.i. only 29% of the larvae had non-midgut-associated viral foci (Fig. 2A).

The most striking result of the time-course experiment was the remarkably low efficiency with which ODV was able to establish primary infection within the midgut epithelia of *S. frugiperda* larvae. The efficiency of primary infection can be estimated by the ratio of the number of occlusions administered per larva and the maximum average number of midgut foci established per infected larva. For 5° *S. frugiperda*, 6000 occlusions generated a maximum of 35 midgut and midgut-associated tracheal foci per infected larva (Fig. 2B); thus, on average 171 occlusions were required to establish each midgut focus of infection. In comparison, only 0·5 occlusions were required in 4° *H. virescens* (data not shown), a 342-fold difference between the two species.

**Developmental resistance and M2R rescue**

To determine whether developmental resistance in fifth instar *S. frugiperda* could contribute significantly to the lack of ODV infectivity, cohorts of newly moulted fifth instar larvae and larvae at 3, 6, 10, 16 and 22 h post-moult were inoculated orally with 4000 occlusions of AcMNPV-hsp70/lacZ, and subsequent larval mortality levels were determined. Compared with the newly moulted cohort in which mortality was 54%, in the 3 h post-moult cohort, 16% of the inoculated larvae died of polyhedrosis. Mortality levels for larval cohorts inoculated even later during the fifth instar were not much lower and ranged between 14 and 4% (Fig. 3). To determine the extent to which the low oral infectivity of AcMNPV-hsp70/lacZ occlusions in
S. frugiperda could be rescued by M2R (and hence point to midgut cell sloughing or obstruction by the peritrophic membrane as potential causes of oral resistance), we conducted dose–response experiments with and without M2R in the inoculum (final concentration 1%, v/v) in two developmental stages (5^0 and 5^16) of S. frugiperda. Incorporation of M2R into the inoculum increased the susceptibility of both developmental stages of larvae to fatal infection, but the level of M2R rescue was not sufficient to account for the extreme oral resistance to AcMNPV infection observed in S. frugiperda (Fig. 4).

Susceptibility of larvae to infection by IH-administered BV

Next we compared the virulence of AcMNPV-hsp70/lacZ in S. frugiperda and H. virescens larvae by IH injection of BV, thereby bypassing the midgut. We found that both species were exquisitely sensitive and equally susceptible to fatal infection (Fig. 5A; LD_{50} = 0.03 and 0.02 p.f.u. for S. frugiperda and H. virescens, respectively). These results suggested that AcMNPV-hsp70/lacZ BV virulence was much greater in S. frugiperda by this portal of entry than had been reported previously for another strain of AcMNPV (Clarke & Clem, 2002). To determine whether the differences between experimental results could be attributed to differences in strains of AcMNPV or S. frugiperda, we exchanged viruses with these authors and conducted bioassays using S. frugiperda obtained from the same source (AgriPest) used by Clarke & Clem for their study (2002). We found that both viral strains were highly infectious and equally virulent by IH injection of AgriPest larvae (Fig. 5B). These results call into question the conclusion that haemocytes of S. frugiperda are not involved in the spread of infection (Clarke & Clem, 2002).

To examine haemocyte infection, we IH injected 5^24 S. frugiperda obtained from both AgriPest and Benzon with 70 p.f.u. AcMNPV-hsp70/lacZ BV. Immediately after injection (0 h) and at 24 h intervals, 10 randomly selected larvae of each strain were bled, and the cadavers and their haemocytes were examined for the presence of LacZ.

No haemocytes were LacZ positive at 0 h p.i., but by 24 h p.i. some of the haemocytes of both strains were positive, although the percentages were very low (Fig. 6; AgriPest strain 0.6%; Benzon strain 6.5%). The percentage of LacZ-positive haemocytes increased progressively thereafter and for both strains reached nearly 100% by 96 h p.i. (Fig. 6). By 48 h p.i., viral occlusions were present within the nuclei of many LacZ-positive haemocytes (data not shown). The distribution and amount of LacZ staining of the cadavers correlated well with the proportion of LacZ-positive haemocytes. For example, at 24 h p.i. there were only a few viral foci in each cadaver, but by 96 h p.i. the host tissues of the larvae of both S. frugiperda strains were replete with viral infections (data not shown).

DISCUSSION

In contrast to a recent report by Clarke & Clem (2002), we found that S. frugiperda larvae were as susceptible to fatal
infection as the highly permissive host, *H. virescens*, following IH injection of either AcMNPV-*hsp70/lacZ* or vHSGFP BV. Moreover, we found that the haemocytes of *S. frugiperda* larvae were highly susceptible to BV infection, a result consistent with the extreme sensitivity of *S. frugi-perda* larvae to mortal infection by intrahaemocoelic BV. Because neither the source of *S. frugiperda* larvae nor the strain of AcMNPV affected the outcome of our results, we conclude that the differences between our findings and those of Clarke & Clem (2002) are likely due to differences in insect handling techniques.

Results from our dose–response studies using orally administered AcMNPV-*hsp70/lacZ* occlusions revealed that *S. frugiperda* larvae were over three orders of magnitude more resistant to fatal infection than larvae of *H. virescens*.

Pathogenesis studies revealed that, unlike the resistant hosts *M. sexta* and *H. zea* (Washburn et al., 1995, 2000, 2001), most of the resistance of *S. frugiperda* larvae stemmed from the failure of AcMNPV-*hsp70/lacZ* to establish infection in midgut cells.

One of the primary mechanisms of larval resistance to fatal infection by the oral portal of entry is host sloughing of infected midgut cells (Washburn et al., 1995, 1998, 2000). In a number of baculovirus–host systems, administration of the optical brightener M2R with viral occlusions increases the susceptibility of larvae to fatal infection. For example, the inclusion of 1 % M2R (also known as Tinopal LPW) in the inocula of *Lymnantria dispar* MNPV occlusions orally administered to *L. dispar* larvae resulted in an increase in mortal infectivity of 800- to 1300-fold compared with control inocula without M2R (Argauer & Shapiro, 1997).

Two different modes of action for M2R have been proposed. First, Washburn et al. (1998) demonstrated that M2R blocks the host from sloughing ODV-infected midgut cells. Secondly, Wang & Granados (2000) have shown that M2R alters the structure of the peritrophic membrane lining the midgut, thereby providing the ODV greater access to the midgut cells. These two mechanisms are not mutually exclusive, as both would increase the number of primary foci within host larvae and could greatly enhance larval mortality. Our results showed that for *S. frugiperda*, larval mortality levels were not increased in the presence of the optical brightener sufficiently to account for the degree of resistance encountered following oral inoculation with AcMNPV occlusions. Therefore, neither infected midgut cell sloughing nor effects of the peritrophic
membrane appear to be the primary mechanisms of resistance to oral infection by AcMNPV in S. frugiperda.

The pattern of extremely low susceptibility of larvae to oral infection with AcMNPV occlusions coupled with their high susceptibility to systemic infection by IH injection with BV is similar to that reported by Faulkner et al. (1997) for an AcMNPV p74 mutant in T. ni. It has been postulated that P74 is involved in the binding and/or fusion of AcMNPV to midgut cells (Faulkner et al., 1997). Thus, the resistance we observed in S. frugiperda to oral infection by AcMNPV-hsp70/lacZ may be due to inefficient binding and/or fusion of AcMNPV ODV to primary cellular targets within the midgut epithelium.

Interestingly, when midgut cell infection did occur, lacZ expression was observed as early as 4 h p.i., similar to the timing observed in T. ni and much more rapidly than in H. virescens, two susceptible hosts (Washburn et al., 1998, 2003). In S. frugiperda, infected tracheal cells were first observed at 8 h p.i., 4 h after the onset of primary midgut infections. Because 4 h is insufficient for AcMNPV replication, these results are consistent with the hypothesis that in S. frugiperda larvae AcMNPV ODV nucleocapsids can be repackaged as BV and establish tracheal infection much more rapidly than can BV that arises by de novo synthesis (Washburn et al., 2003).

Although the incompatibility of AcMNPV-hsp70/lacZ ODV with the midgut cells of S. frugiperda is the first and probably most important barrier to fatal viral infection, we also found a second mechanism of resistance: the loss of virally infected tracheal cells associated with the midgut. For susceptible hosts such as H. virescens and T. ni inoculated orally with AcMNPV-hsp70/lacZ occlusions, final larval mortality levels are equivalent to the maximum proportion of larvae expressing lacZ in the tracheal epidermis (Washburn et al., 1995). For S. frugiperda, the cohort sacrificed at 24 h p.i. had the greatest proportion of larvae with tracheal infections, but the predictive time-point occurred at 30 h p.i. By 30 h p.i., all viral foci were eliminated from 38% of the larvae that were LacZ-positive at 24 h p.i. Moreover, unlike in the resistant hosts M. sexta and H. zea, the S. frugiperda we examined showed no evidence of a phenoloxidase-mediated immune response. While IH inoculation of less than 1 p.f.u. of AcMNPV-hsp70/lacZ BV into the haemocoel of S. frugiperda invariably led to the death of the insect, the establishment of tracheal foci via the oral portal of entry did not. In view of the high susceptibility of S. frugiperda haemocytes to IH injected BV, infected midgut-associated tracheal cells of S. frugiperda evidently fail to release progeny BV into the haemocoel where it can infect circulating haemocytes. Apoptosis of AcMNPV-infected cells is reported to limit the spread of infection in S. litura larvae (Zhang et al., 2002) and apoptosis of the midgut-associated tracheal cells could be the mechanism that prevents the dissemination of systemic infection of orally infected S. frugiperda larvae.

In summary, our results have shown that there are two barriers to fatal infection of S. frugiperda by orally administered occlusions of AcMNPV. First, AcMNPV ODV appears to be unable to infect cells within the midgut epithelium efficiently; secondly, after primary infection of the midgut has been established, secondary infections within midgut-associated tracheal cells can rapidly ensue, but a high proportion of these viral foci are eliminated. The process by which this occurs, however, remains a mystery.

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