Gonad-Specific Virus of *Helicoverpa zea* Does Not Affect Infectivity of Nuclear Polyhedrosis Virus[1]

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ABSTRACT A gonad-specific virus (GSV), an enveloped, rod-shaped virus, which prevents the development of normal ovaries and testes of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) has been suggested for use in biological control of *H. zea* (Raina an Adams 1995). Because the GSV resembles the Hz-1 virus which was described as a persistent infection in an *H. zea* tissue culture that inhibited infection by other viruses, this research was designed to determine if the GSV would interfere with infection of *H. zea* larvae by its homologous nuclear polyhedrosis virus (HzSNPV) which is currently being used in biocontrol of *H. zea* and *H. virescens*. In three of four tests, there was no significant difference in LC50 (i.e., the 95% confidence intervals overlapped) for HzSNPV in larvae from normal moths and larvae from moths injected with GSV. Surviving larvae from the GSV-injected moths produced 97.8 to 100% agonadal moths. This indicates that GSV does not protect agonadal larvae from infection by NPV. Thus, release of GSV into the natural population of *H. zea* should not interfere with use of the more virulent NPV for control *H. zea*.

Key Words Corn earworm, *Helicoverpa zea,* virus, gonad-specific virus, nuclear polyhedrosis virus, Elcar, integrated pest management

An enveloped virus with a rod-shaped nucleocapsid, originally described as a gonad-specific virus (GSV) (Raina and Adams 1995) develops in the reproductive tracts of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and inhibits the development of normal ovaries and testes. The virus is transmitted vertically resulting in nearly 100% agonadal progeny from later oviposition days (four and greater) of reproductively normal moths that carry the virus (Hamm et al. 1996). Raina and Adams (1995) postulated that this virus might be used in the biological control of *H. zea* if it can be more widely distributed in the natural population.

The *H. zea* nuclear polyhedrosis virus (HzSNPV) has been used to control various species of *Heliothis* and *Helicoverpa* (Ignoffo and Couch 1981). The HzSNPV is also being tested as part of an areawide suppression program for *H. zea* and *Heliothis virescens* (Fab.) (Bell 1991, Bell and Hardee 1994, Bell and Hayes

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1994). The morphology and size of virions of GSV closely resemble those of the Hz-1 virus (Hamm et al. 1996) that was isolated from a culture of *H. zea* ovarian tissue (Burand et al. 1986). This cell culture was not susceptible to infection by the HzSNPV (Burand et al. 1986). My objective was to determine whether larvae carrying the GSV are susceptible or nonsusceptible to the HzSNPV.

Materials and Methods

Insects and rearing techniques. Newly-emerged female *H. zea* moths were injected with an homogenate of a deformed female reproductive tract containing GSV. Larvae used in the bioassay were from eggs laid four days after the moths were treated. Previous tests indicated that nearly 100 percent of progeny from oviposition day four carried the GSV and developed into agonadal adults (Hamm et al. 1996). All survivors were dissected as moths to determine if they were normal or agonadal as proof that the progeny of the GSV-injected moths were infected with the GSV. Elcar™, a commercial formulation of HzSNPV, (Sandoz, Palo Alto, CA) was bioassayed against larvae from treated and untreated moths by surface treating meridic diet, i.e., pinto bean diet without formalin (Burton 1969). Five-day-old larvae were treated with five concentrations (decimally diluted) ranging from 50 to 0.005 polyhedral occlusion bodies (OB) per mm^2 and a control of sterile deionized water. One larva was placed in each 30-ml cup, and 60 larvae were used per virus concentration. Mortality due to treatment was based on typical symptoms of liquefaction and/or presence of OB in the dead larvae. The concentration-mortality response was analyzed by probit analysis (Polo-PC; LeOra Software 1987).

In a second experiment, three bioassays were conducted on three-day-old larvae. In the first test five virus concentrations (three-fold dilutions) ranging from 9 to 0.1 POB/mm^2 were used with 90 larvae per concentration. In the additional two bioassays six concentrations, three-fold dilutions, ranging from 3 to 0.012 POB/mm^2 were used with 90 larvae per concentration.

Results and Discussion

There was no significant difference in LC50 for Elcar against five-day-old larvae of normal and GSV-injected moths (Table 1). Although the progeny of GSV-injected moths had a slightly higher LC50 than those of the normal moths, the 95% confidence intervals for LC50s overlapped. The concentration-response lines were not parallel. The slope for the normal larvae was 0.912 ± 0.089 and the slope for the GSV-infected larvae was 1.579 ± 0.215. All 164 surviving progeny of the normal moths were normal and all 197 surviving progeny of the GSV-injected moths were agonadal.

There was a significant difference in response of the three day old larvae from GSV-treated moths and the larvae from control moths in the first test (Table 2). The LC50 for the progeny of GSV-injected moths was slightly lower than the LC50 for progeny of normal moths and the 95% confidence intervals did not overlap. The hypothesis that the response lines were parallel was accepted, slope = 2.002 ± 0.163. All 179 surviving progeny of the normal moths

Table 1. Effect of gonad-specific virus (GSV) infection on susceptibility of 5 day old *Helicoverpa zea* larvae to HzSNPV.

GSV*	LC50 OB / mm^2	95% CI of LC50	Slope ± SE	Relative Susceptibility	95% CI
-	1.705	0.693-4.408	0.912 ± 0.089		
+	3.202	1.163-8.884	1.579 ± 0.215	0.53	0.14-2.15

* - Larvae from normal moths, none of survivors were agonadal
+ Larvae from female moths injected with GSV, all survivors were agonadal.

Table 2. Effect of gonad-specific virus (GSV) infection on susceptibility of 3 day old *Helicoverpa zea* larvae to HzSNPV.

GSV*	LC50 OB / mm^2	95% CI of LC50	Slope ± SE	Relative Susceptibility	95% CI
-	0.195	0.153-0.238	1.945 ± 0.201		
+	0.113	0.081-0.143	2.014 ± 0.279	1.8	1.4-2.4
-	0.256	0.160-0.415	1.253 ± 0.094		
+	0.156	0.097-0.250	1.336 ± 0.097	1.6	0.85-3.3
-	0.192	0.150-0.246	1.531 ± 0.109		
+	0.224	0.175-0.287	1.525 ± 0.108	0.86	0.60-1.2

* - Larvae from normal moths, none of survivors were agonadal
+ Larvae from female moths injected with GSV, 97.8 to 99.2% of survivors were agonadal.

were normal and 99.2% of the 131 surviving progeny of the GSV-injected moths were agonadal.

There was no significant difference between LC50 of the virus for larvae of normal and GSV-injected moths in the second and third bioassays of three-day-old larvae (Table 2). In these two tests the concentration-response lines for larvae of treated and untreated moths were parallel, slope = 1.294 ± 0.067 in the second test and slope = 1.528 ± 0.077 in the third test. All 362 surviving larvae of the normal moths produced normal moths in the second test of three-day-old larvae while 97.8% of 317 surviving progeny of GSV-injected moths were agonadal. All 275 surviving progeny of normal moths were normal in the third test of of three-day-old larvae while 98.4% of 305 surviving progeny of GSV-injected moths were agonadal.

In three of four tests, there was no significant difference in LC50 for HzSNPV in larvae from normal moths and larvae of moths injected with the GSV. This indicates that the GSV does not protect agonadal larvae or normal carriers of the virus from infection by NPV. Thus, release of GSV into the natural population of *H. zea* should not interfere with use of the more virulent NPV for control of *H. zea*.

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