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Parasitoid–Pathogen–Pest Interactions of *Chelonus insularis, Campoletis sonorensis,* and a Nucleopolyhedrovirus in *Spodoptera frugiperda* Larvae

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In this study we examined interactions between two solitary endoparasitoids, the braconid *Chelonus insularis* and the ichneumonid *Campoletis sonorensis,* and a multiple-enveloped nucleopolyhedrovirus infecting *Spodoptera frugiperda* larvae. We examined whether ovipositing females minimize interference by discriminating amongst hosts and examined the outcome of within-host competition between parasitoid species and between the parasitoids and the virus. The egg–larval parasitoid *Ch. insularis* did not discriminate between virus-contaminated and uncontaminated *S. frugiperda* eggs; all *S. frugiperda* larvae that emerged from surface-contaminated eggs died of viral infection prior to parasitoid emergence. The larval parasitoid *C. sonorensis* also failed to discriminate between healthy and virus-infected *S. frugiperda* larvae or between larvae unparasitized or parasitized by *Ch. insularis.* Host larvae parasitized in the egg stage by *Ch. insularis* were suitable for the development of *C. sonorensis* when they were multiparasitized by *C. sonorensis* as first, second, third, and fourth instars, whereas emergence of *Ch. insularis* was dramatically reduced (by 85 to 100%) in multiparasitized hosts. Nonspecific host mortality was significantly higher in multiparasitized hosts than in singly parasitized hosts. The development time and sex ratio of *C. sonorensis* in multiparasitized host larvae were unaffected by the presence of *Ch. insularis* larval stages. Both *Ch. insularis* parasitized and nonparasitized larvae of the same instar (second, third, or fourth instars) had a similar quantitative response to a challenge of virus inoculum. All host larvae that ingested a lethal dose of virus were unsuitable for *Ch. insularis* development. In contrast, *C. sonorensis* did not survive in hosts that ingested a lethal virus dose immediately after parasitism, but parasitoid survival was possible with a 2-day delay between parasitism and viral infection and the percentage of parasitoid emergence increased significantly as the interval between parasitism and viral infection increased. The development time of *C. sonorensis* was significantly reduced in virus-infected hosts compared to conspecifics that developed in healthy hosts. *C. sonorensis* females that oviposited in virus-infected hosts did not transmit the virus to healthy hosts that were parasitized subsequently. Field applications of virus for biocontrol of *S. frugiperda* may lead to substantial mortality of immature parasitoids, although field experiments have not yet demonstrated such an effect.

Key Words: **fall armyworm; multiple parasitism; parasitism; baculovirus; nucleopolyhedrovirus; interference; interspecific competition.**

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), causes substantial losses in maize and sorghum throughout the Mesoamerican region, and control is usually achieved through the application of a number of common synthetic insecticides (Sparks, 1979; Hruska and Gould, 1997). Such chemicals have been reported to cause widespread chronic poisoning of agricultural workers in this region (McConnell and Hruska, 1993; Tinoco and Halperin, 1998) and are very toxic to insect natural enemies (Croft and Brown, 1975). Insect baculoviruses have shown considerable promise as biological insecticides for control of defoliating lepidopteran pests (Moscardi, 1999). Recently, a series of field trials involving application of a nucleopolyhedrovirus for control of *S. frugiperda* in Mesoamerica indicated that the prevalence of infection

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observed in larvae collected in treated plots of maize at 2 days postapplication was around 40–50%. Emergence of parasitoids from these larvae accounted for an additional 20% mortality, resulting in an overall level of control of 60–70% (Martínez *et al.*, 2000).

The solitary egg–larval endoparasitoid *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) is often the most abundant parasitoid of *S. frugiperda* in the Mesoamerican region (Wheeler *et al.*, 1989; Cave, 1993). This wasp parasitizes the eggs of *S. frugiperda* and the parasitoid develops in the larval stages of the host; parasitized larvae begin metamorphosis in the fifth instar, whereas nonparasitized larvae do so in the sixth instar (Jones, 1985). Likewise, *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) is among the most abundant parasitoids collected from *S. frugiperda* infesting maize in the southern United States (Pair *et al.*, 1986). This species develops as a solitary endoparasitoid of early instar larvae (Isenhour, 1985). The multiple-enveloped nucleopolyhedrovirus of *S. frugiperda* (SfMNPV) is a highly host-specific baculovirus that causes lethal infections in host larvae following ingestion of an infectious dose of virus. The susceptibility of *S. frugiperda* larvae to the virus is stage dependent; early instar larvae are far more susceptible to infection than late instar larvae (Escribano *et al.*, 1999).

This virus is currently being developed for the management of *S. frugiperda* populations in Mesoamerican maize crops (Williams *et al.*, 1999). One advantage of the virus over conventional pesticides is that it is not toxic to other natural enemies of the target pest. However, this does not mean that applications of virus will not have adverse effects on parasitoid populations. The potential for interspecific competition exists between parasitoids and the virus within a larval host because of the extensive overlap in the resource requirements of parasitoid immature stages and the replicating virus in dually infected and parasitized hosts. The consequences of such interspecific competition should be considered when viruses are evaluated as potential bioinsecticides. Powerful interspecific competition between natural enemies is clearly undesirable if we wish to develop a sustainable system in which both virus and parasitoids contribute to host mortality.

The strength and outcome of interspecific competitive effects will be determined in part by parasitoid behavior. If ovipositing parasitoids are able to detect and avoid hosts which are infected with virus or that have already been parasitized by other species, then interference will be minimized. If this does not occur and hosts become simultaneously or sequentially infected by two natural enemies, then the relative competitive abilities of the parasitoids/pathogen will determine which survives. An understanding of these interspecific interactions may enable us to predict the environmental effects of nucleopolyhedrovirus applications on parasitoid populations.

In this study we examine interactions between the two parasitoids, *Ch. insularis* and *C. sonorensis*, and the virus SfMNPV. We examine whether ovipositing females minimize interspecific competition by discriminating among hosts. We also examine the outcome of within- host competition between parasitoid species and between the parasitoids and the virus.

MATERIAL AND METHODS

The Insects

Larvae of *S. frugiperda* were obtained from the 16th generation of a culture established in 1997 at the Universidad Pública de Navarra, Spain, and maintained on an artificial diet (Poitout and Bues, 1974) in a growth chamber at 26 ± 1°C, 85% RH, and with a 16-h day length. The braconid egg–larval parasitoid *Ch. insularis* came from the 4th generation of a culture started with field-collected parasitized *S. frugiperda* larvae from El Zamorano, Honduras. The ichneumonid *C. sonorensis* was obtained from the 6th generation of a culture established at Texas A & M University, College Station, Texas. Both parasitoids were maintained continuously using *S. frugiperda* from the laboratory culture.

The Baculovirus

The virus used was the *S. frugiperda* multiple-enveloped nucleopolyhedrovirus (SfMNPV) originally isolated in Nicaragua and recently characterized (Escribano *et al.*, 1999). A stock of virus was obtained by feeding early fourth instar (L_4) *S. frugiperda* larvae with virus-contaminated artificial diet; larvae were subsequently reared on a formaldehyde-free diet until death. Viral occlusion bodies (OBs) were extracted and purified by filtration and differential centrifugation as described elsewhere (Caballero *et al.*, 1992). The concentration of the resulting suspension was determined in triplicate using a Thoma counting chamber (Hawksley) under phase-contrast microscopy at 400X magnification.

Experimental Procedures with Ch. insularis

Parasitoid ovipositional behavior. Because ovipositional experience can affect parasitoid host discrimination responses (van Lenteren, 1981), *Ch. insularis* females were used in this experiment that were 4 to 5 days old and had prior experience of oviposition in clean host eggs. Fall armyworm eggs 24 to 48 h old laid on the surface of paper cylinders were obtained from insect cultures. The papers to which the eggs adhered were cut into small sections, each containing 25 eggs. These paper sections were virus-contaminated by im-

mersion in a virus suspension of 8.5×10^6 OBs/ml in a 0.1% (v/v) solution of the wetting agent Agral. Uncontaminated (control) paper sections were obtained by treatment with the wetting agent in sterile distilled water. Paper sections were allowed to air dry for 2 h and then simultaneously exposed to a single mated *Ch. insularis* female in a plastic petri dish (115 mm in diameter, 45 mm in height) for 1 h. The behavior of parasitoid females to both virus-contaminated and uncontaminated eggs was observed continuously and the number of contacts and ovipositor insertions with each type of egg was recorded. Host eggs were considered to have been examined if they were touched by the parasitoid antennae. Probing or oviposition was recorded when the female inserted her ovipositor into an egg. This test was replicated five times, using a different female each time.

Parasitoid survival in infected hosts. Experiments were carried out using 24- to 48-h-old eggs as well as early second, third, and fourth instar larvae that had been exposed as eggs to mated *Ch. insularis* females. Host eggs were surface contaminated by dipping in virus suspension as described previously, whereas larvae were inoculated using the droplet feeding techniques described below. In all cases a virus concentration representing twice the calculated LC_{90} of the treated insects was used: 1.7×10^7 OBs/ml for second instars, 6.6×10^7 OBs/ml for third instars, and 1.3×10^9 OBs/ml for fourth instar larvae. This test was replicated three times using 20 individuals/replicate. Larvae were checked every 12 h until death from viral infection or parasitoid emergence or until survivors pupated. The virus-killed larvae were dissected to ascertain whether they were parasitized.

Parasitized host larvae susceptibility. Bioassays were carried out on early second, third, and fourth instar fall armyworm larvae that had been exposed as eggs to *Ch. insularis* or unparasitized larvae of the same instar. Prior to treatment, larvae were starved overnight as molting first, second, and third instar larvae. Five different concentrations of virus were administered to second (9.6×10^3, 4.8×10^4, 2.4×10^5, 1.2×10^6, and 6×10^6 OBs/ml), third (6×10^4, 6×10^5, 6×10^6, and 6×10^7 OBs/ml), and fourth (1.2×10^5, 1.2×10^6, 1.2×10^7, and 6×10^7 OBs/ml) instar larvae, using the droplet feeding method of Hughes and Wood (1981). These concentration ranges were found to kill between 5 and 95% of the test larvae in previous bioassays (Escribano *et al.*, 1999). The virus suspensions were colored using 0.1% Fluorella blue (Hilton-Davis, Cincinnati, OH) and larvae that ingested the suspension within 10 min were transferred to individual cells of a 25-compartment culture dish with a formaldehyde-free diet plug. Control larvae were treated with a solution of sterile water and food coloring. Twenty-five larvae were used per virus concentration and for the controls. Each bioassay was replicated three times over time. Bioassays were conducted at a constant temperature of 25 ± 2°C, and virus-induced larval mortality was recorded every 12 h until larvae had either died or pupated.

Experimental Procedures with C. sonorensis

Parasitoid ovipositional behavior. Ovipositional behavior of *C. sonorensis* females was evaluated in two different experiments to determine whether ovipositing females were able to discriminate hosts that are already parasitized or infected with virus. Experimental *C. sonorensis* mated females 6 to 8 days old had prior oviposition experience of healthy second instar *S. frugiperda* larvae. The first experiment was conducted to determine if *C. sonorensis* females showed any ovipositional preferences when offered hosts parasitized by *Ch. insularis* and unparasitized hosts. Second instar newly molted unparasitized or parasitized fall armyworm larvae were simultaneously exposed in groups of 7 (14 larvae in total) to a single mated *C. sonorensis* female in a plastic petri dish (115 mm in diameter, 45 mm in height) for 20 min. A second experiment was performed to determine whether parasitoid females are able to discriminate among hosts that had been virus-infected 12, 24, 36, 48, and 60 h earlier. A combination of 7 healthy and 7 infected larvae were exposed to parasitism by individual *C. sonorensis* females as described above. In both experiments, healthy larvae were marked with a small spot of white correcting paint to distinguish them from parasitized or diseased larvae. Prior experiments had demonstrated that the paint spot had no effect on the probability of parasitism of marked larvae (data not shown). The experiments were replicated between four and six times over time using a different female on each occasion. Following exposure to parasitoid females, larvae were dissected to determine the presence of parasitoid eggs.

Parasitoid survival in parasitized or infected hosts. C. sonorensis survival was also evaluated in two different experiments. The first experiment was conducted to determine whether *C. sonorensis* can survive in fall armyworm larvae already parasitized by *Ch. insularis.* Host larvae, which had been exposed as eggs to *Ch. insularis,* were subsequently exposed to *C. sonorensis* females as newly molted first, second, third, and fourth instars.

A second experiment was conducted to determine the survival of *C. sonorensis* in virus-infected hosts. *S. frugiperda* larvae were parasitized as newly molted third instars and fed, as indicated above, with a virus concentration representing double the LC_{90} (3.32×10^7 OBs/ml) at 0, 2, 4, and 6 days following parasitism. Larvae were checked daily for death due to parasitoid emergence, viral infection, or other causes. Larvae that

TABLE 1

Comparative Susceptibility to Viral Infection of Second, Third, and Fourth Instar *Spodoptera frugiperda* Larvae, Nonparasitized and Parasitized by *Chelonus insularis*

Host instar at infection	Larval condition[a]	Regression line	LC_{50} (OBs/ml)	Relative potency	95% Fiducial limits	
					Lower	Upper
L_2	Parasitized	$y = 0.61x + 1.80$	1.93×10^5	1		
	Nonparasitized	$y = 0.61x + 1.87$	1.46×10^5	1.32	0.527	3.374
L_3	Parasitized	$y = 0.76x + 0.38$	1.15×10^6	1		
	Nonparasitized	$y = 0.76x + 0.60$	6.03×10^5	1.92	0.799	4.674
L_4	Parasitized	$y = 0.62x + 0.79$	5.34×10^6	1		
	Nonparasitized	$y = 0.62x + 0.92$	3.24×10^6	1.65	0.135	4.231

Note. Parameters were obtained from the POLO-PC program. All χ^2 were not significant at $P = 0.05$ when measuring the goodness-of-fit of each regression line independently as well as when testing the hypothesis that slopes are the same for each host instar.

[a] *S. frugiperda* larvae were parasitized in the egg stage by *Ch. insularis.*

died from causes other than parasitoid emergence were dissected to determine the presence of parasitoid immature stages.

Virus transmission by the parasitoid. Five to ten newly molted third instar *S. frugiperda* larvae were fed with a lethal virus concentration of 3.32×10^7 OBs/ml and 2 to 3 days later were offered to individual mated parasitoid females placed in plastic petri dishes (115 × 45 mm). Each female was observed to parasitize an infected larva. These parasitoids were individually transferred to a cup containing 10 healthy first instar host larvae and parasitic activity was allowed to occur for 1 h. These larvae were then placed individually in plastic cups (30 mm in diameter), supplied with artificial diet, and checked daily for death due to viral infection or parasitoid emergence.

Data Analysis

The data were subjected to analysis of variance followed by means separation by Fisher's least significant difference using the general linear models of the program SPSS 7.5 (Norusis, 1995). An inverse transformation was necessary for the time-to-death values and percentage of parasitism values were arcsine transformed prior to analysis. Model checking indicated that transformed data satisfied the assumption of normality required by analysis of variance. Concentration–mortality data were subjected to probit analysis (Finney, 1971) using the POLO-PC program (LeOra Software, 1987).

RESULTS

Interactions of Ch. insularis

The number of antennal contacts made by female *Ch. insularis* was similar for *S. frugiperda* eggs treated with virus (mean ± SE, 4.0 ± 0.7) compared to untreated eggs (3.4 ± 0.8; $F = 0.12$, $df = 3,16$, $P > 0.05$). The number of ovipositor insertions was 7.8 ± 1.5 (mean ± SE) for contaminated eggs and 6.6 ± 1.3 for untreated eggs and was not significantly affected by viral contamination ($F = 0.22$, $df = 3,16$, $P > 0.05$). Eclosion of *S. frugiperda* larvae from virus-treated and untreated eggs was invariably high (>93%) and was not affected by virus treatment.

All *S. frugiperda* larvae that emerged from surface-contaminated eggs died of viral infection prior to parasitoid emergence, whereas *Ch. insularis* emerged from 85.0 ± 2.9% of *S. frugiperda* larvae that developed from uncontaminated eggs. Parasitized *S. frugiperda* larvae that were subsequently virus-infected in the second, third, or fourth instars all died from viral infection prior to parasitoid eclosion. Dissection of these larvae indicated that the percentage of parasitism in healthy (81.7 to 90%) and virus-infected larvae (85.0 to 91.7%) did not differ significantly among instars ($F = 0.46$, $df = 7,16$, $P > 0.05$).

The percentage of virus-induced mortality of second, third, and fourth instar parasitized and nonparasitized larvae increased with virus concentration. No systematic heterogeneity was detected in the larval response for each instar, as indicated by the χ^2 values. The hypothesis that slopes and intercepts are the same for parasitized and nonparasitized larvae was accepted for the second ($\chi^2 = 2.15$, $df = 8$, $P = 0.976$), third ($\chi^2 = 4.13$, $df = 6$, $P = 0.659$), and fourth ($\chi^2 = 5.63$, $df = 6$, $P = 0.467$) instar larvae, indicating that both larval conditions (parasitized and nonparasitized) of the same instar had a similar quantitative response to viral infection (Table 1). When the regression lines for second, third, and fourth instars were fitted in parallel ($\chi^2 = 9.87$, $df = 9$, $P = 0.361$), a significant decrease in susceptibility to viral infection between successive instars was observed. Second and third instar larvae were about 27 and 5 times more susceptible than

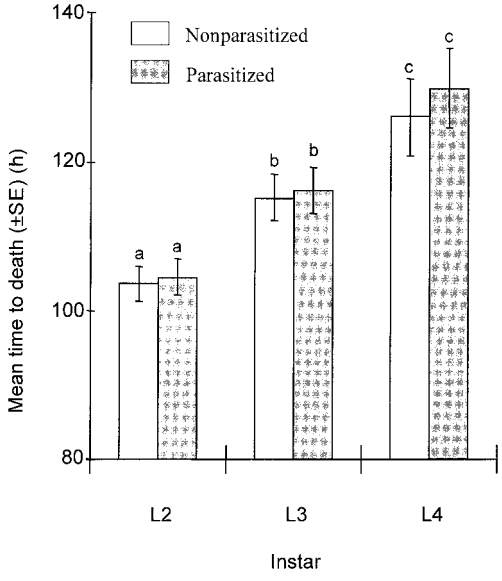

FIG. 1. Mean time to death of second, third, and fourth instar *Spodoptera frugiperda* larvae nonparasitized or parasitized by *Chelonus insularis* following inoculation with double the LC_{90} concentration of nucleopolyhedrovirus.

fourth instar larvae to viral disease, respectively. Mean time-to-death values induced by viral infection did not differ significantly between parasitized and nonparasitized *S. frugiperda* larvae of the same instar, but a significant increase in virus-induced time-to-death with increasing larval instar was observed ($F = 44.7$, $df = 3,204$, $P < 0.001$) (Fig. 1).

Interactions of C. sonorensis

Percentages of parasitism by *C. sonorensis* (as determined by dissection of hosts) did not differ significantly when individual *C. sonorensis* females were offered unparasitized fall armyworm larvae (mean ± SE, 32.1 ± 6.8%) and larvae already parasitized by *Ch. insularis* (53.6 ± 9.0%) ($\chi^2 = 0.75$, $df = 4$, $P > 0.05$). The percentage of emergence of *C. sonorensis* did not differ significantly between unparasitized hosts and those parasitized by *Ch. insularis* when they were subsequently parasitized by *C. sonorensis* as first ($F = 5.87$, $df = 1,6$, $P > 0.05$), second ($F = 2.56$, $df = 1,6$, $P > 0.05$), or third ($F = 0.82$, $df = 1,6$, $P > 0.05$) instar larvae (Table 2). However, the percentage of eclosion of *C. sonorensis* was significantly higher in fourth instar hosts parasitized by *Ch. insularis* than in unparasitized larvae ($F = 34.7$, $df = 1,6$, $P < 0.005$). In contrast, the emergence of *Ch. insularis* from singly parasitized hosts varied between 81.3 and 86.3%, whereas emergence of *Ch. insularis* from hosts subsequently parasitized by *C. sonorensis* was dramatically reduced in larvae multiparasitized in the first ($F = 121.5$, $df = 1,6$, $P < 0.001$), second ($F = 61.8$, $df = 1,6$, $P < 0.001$), third ($F = 176.2$, $df = 1,6$, $P < 0.001$), and fourth ($F = 146.9$, $df = 1,6$, $P < 0.001$) instars (Table 2).

Although fall armyworm mortality in the absence of parasitoid emergence occurred in all treatments, host mortality was significantly more prevalent in hosts parasitized by *Ch. insularis* in the egg stage and subsequently parasitized by *C. sonorensis* as first ($F =$

TABLE 2

Mean Percentage of Emergence of *Campoletis sonorensis* and *Ch. insularis* in Singly and Multiparasitized Hosts Following Multiparasitism in Different Host Instars

Host instar	Larval treatment	Percent emergence			*S. frugiperda* larval mortality[a]
		C. sonorensis	*Ch. insularis*	Moth	
L_1	*C. sonorensis*	57.5 ± 6.3a		5.0 ± 5.0a	37.5 ± 2.5b
	Ch. insularis		82.5 ± 4.8b		10.5 ± 4.8c
	Multiparasitized	30.0 ± 9.1a	2.5 ± 2.5a		67.5 ± 11.1a
	Control			87.5 ± 2.5b	12.5 ± 4.8c
L_2	*C. sonorensis*	40.0 ± 7.1a		10.0 ± 4.1a	50.0 ± 8.2a
	Ch. insularis		85.0 ± 5.4b		10.0 ± 4.1b
	Multiparasitized	25.0 ± 6.4a	15.0 ± 5.0a		60.0 ± 4.1a
	Control			92.5 ± 3.2b	7.5 ± 4.8b
L_3	*C. sonorensis*	70.0 ± 7.1a		15.0 ± 6.5a	15.0 ± 8.7b
	Ch. insularis		86.3 ± 3.8b		12.5 ± 2.5b
	Multiparasitized	62.5 ± 4.8a	0.0 ± 0.0a		37.5 ± 4.8a
	Control			92.5 ± 2.4b	7.5 ± 3.4b
L_4	*C. sonorensis*	12.5 ± 2.5a		67.5 ± 6.3a	20.0 ± 4.1b
	Ch. insularis		81.3 ± 4.3b		5.0 ± 2.9c
	Multiparasitized	35.0 ± 2.8b	2.5 ± 2.5a		62.5 ± 2.5a
	Control			97.5 ± 2.3b	2.5 ± 2.5c

[a] Mortality of *S. frigiperda* larvae from unknown causes. Larvae were dissected to ascertain whether they were parasitized. In all cases, comparisons were made only between treatments in the same host instar. For a given instar, means in the same column followed by the same letter are not significantly different ($P > 0.05$; Fisher's least significant difference).

TABLE 3

Mean Percentages of Emergence of *C. sonorensis* and Adult *S. frugiperda* at Different Time Intervals between Parasitism and Viral Infection and Percentages of Mortality of *S. frugiperda* Larvae from Viral Infection or Other Causes

Time interval (days)	Number of larvae used	Percentage of emergence		Percent of *S. frugiperda* mortality	
		C. sonorensis	Moth	Viral infection	Unknown causes
0	80	0a	0	100.0 ± 0.0a	0
2	75	22.3 ± 5.6b	0	69.7 ± 5.8b	8.0 ± 4.1
4	82	52.0 ± 6.1c	0	34.3 ± 5.8c	12.7 ± 2.5
6	72	62.0 ± 3.5cd	0	27.7 ± 2.9c	10.3 ± 2.9
Control	82	74.7 ± 6.1d	14.9 ± 3.0	0d	10.6 ± 3.0

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

11.7, $df = 3,12$, $P < 0.05$), second ($F = 21.6$, $df = 3,12$, $P < 0.05$), third ($F = 5.6$, $df = 3,12$, $P < 0.05$), or fourth ($F = 85.2$, $df = 3,12$, $P < 0.001$) instars than in any other treatment (Table 3). Dissection of all such larvae indicated the presence of juvenile stages of both parasitoids. The mean development time of *C. sonorensis* in host larvae parasitized by *Ch. insularis* ranged from 9.2 to 11.2 days and was not significantly different from that observed in hosts of any instar parasitized by *C. sonorensis* alone (9.6 to 10.5 days).

No significant differences were found in the mean percentages of parasitism of healthy and virus-infected *S. frugiperda* larvae when simultaneously offered to female *C. sonorensis* at 12 ($F = 1.28$, $df = 1,10$, $P > 0.05$), 24 ($F = 1.40$, $df = 1,10$, $P > 0.05$), 36 ($F = 0.13$, $df = 1,10$, $P > 0.05$), 48 ($F = 0.99$, $df = 1,10$, $P > 0.05$), or 60 ($F = 0.59$, $df = 1,6$, $P > 0.05$) h postinfection (Fig. 2).

C. sonorensis did not survive in third instar *S. frugiperda* larvae that ingested a lethal virus dose immediately after oviposition by the parasitoid (Table 3). A 2-day interval between parasitism and viral infection was sufficient to permit the development and emergence of a small number of *C. sonorensis;* parasitoid survival increased significantly with increasing intervals between parasitism and viral infection ($F = 39.4$, $df = 4,10$, $P < 0.02$) (Table 3). The developmental period (egg to pupa) of *C. sonorensis* in virus-infected hosts was significantly reduced compared to that of conspecifics that developed in uninfected hosts ($F = 40.7$, $df = 3,160$, $P < 0.001$). The duration of the pupal stage of *C. sonorensis* in virus-infected hosts was on average 7.7 ± 0.4 days and did not differ significantly from that of *C. sonorensis* in noninfected larvae (7.9 ± 0.1; $F = 1.4$, $df = 3,115$, $P = 0.24$). The adult *C. sonorensis* sex ratio was also not significantly affected by viral infection of the host, ranging from 63 to 86% male emerging from virus-infected hosts compared to 62 to 74% male emerging from noninfected hosts ($\chi^2 = 0.72$, $df = 1$, $P > 0.05$). Female *C. sonorensis* that had oviposited in virus-infected *S. frugiperda* larvae did not transmit the virus to healthy *S. frugiperda* larvae in subsequent ovipositions.

DISCUSSION

Parasitoids and pathogens experience scramble competition for host resources in dually infected and parasitized hosts, and this is often highly detrimental to the reproduction of both parasitoid and pathogen (Caballero *et al.*, 1991; Hochberg, 1991a). It might therefore be expected that wasps would avoid parasitism of virus-infected hosts. Indeed, insect hosts infected by baculoviruses show extreme pathological changes that are likely to be detectable by an endoparasitoid evaluating a host for possible oviposition (Brooks, 1993). In addition to virus-induced disease of host tissues and

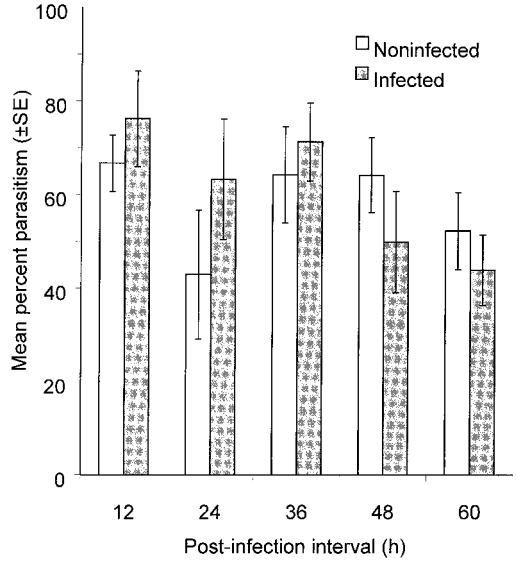

FIG. 2. Percentages of parasitism of virus-infected and healthy larvae exposed simultaneously to individual *C. sonorensis* females at various intervals postinfection. No significant differences between parasitism of healthy and diseased larvae were observed at any time point.

marked endocrinological changes (O'Reilly, 1995) the production of virus-induced toxic factors has been reported in several baculoviruses and these toxins have a direct impact on the viability of a host for parasitoid development (Kaya and Tanada, 1973). There exists no evidence, however, of direct infection of parasitoids by baculoviruses and these two types of natural enemies are often claimed to be compatible for use in programs of biorational insect pest control (Huber, 1986; Hochberg *et al.,* 1990).

In the case of *C. sonorensis,* the apparent lack of discrimination between virus-infected and healthy hosts seems peculiar given that the probability of successful progeny development in infected hosts was low. Recently, Sait *et al.* (1996) observed that the solitary ichneumonid endoparasitoid *Venturia canescens* (Gravenhorst) examined and probed granulovirus-infected *Plodia interpunctella* (Hübner) larvae to the same degree as healthy larvae, although oviposition behavior was significantly reduced in heavily infected hosts.

The situation differed for the experiments with *Ch. insularis,* as hosts were offered as surface-contaminated eggs that became infected following parasitoid oviposition. The mechanism of infection is most likely to have been at the moment of eclosion as *S. frugiperda* larvae chew their way out of the chorion, consuming a lethal dose of virus in the process, an established mechanism of transmission in nature (Clark, 1958; Doane, 1969). The polyhedrin protein that occludes the enveloped nucleocapsids of these viruses is, in essence, an inert structural protein that protects viral particles from environmental degradation and may therefore not be readily detectable by *Ch. insularis.*

The results of the experiments addressing interference between parasitoids and virus were probably intimately related to the developmental biology of these micro- and macroparasites. The koinobiont parasitoid *Ch. insularis* oviposits in host eggs and the parasitoid progeny develop slowly during the larval stage, whereas the virus replicates so quickly that it kills the host in the instar following infection. Thus, scramble competition in dually infected and parasitized hosts led to death of the parasitoid. In contrast, *C. sonorensis* was able to develop fast enough to complete its development in many of the virus-infected hosts, especially those in which parasitism occurred 4–6 days following viral infection.

The development time of *C. sonorensis* was reduced in virus-infected hosts, suggesting that the parasitoid responds to the diseased host environment by speeding up its development. We might predict a cost in reduced development time in terms of adult parasitoid size but this was not evaluated in the present study. Similarly, McCutchen *et al.* (1996) evaluated the impact of recombinant nucleopolyhedroviruses on the development of the braconid endoparasitoid *Microplitis croceipes* (Cresson) in larvae of *Heliothis virescens* (F.). Parasitoids that developed in hosts infected by AcMNPV expressing a juvenile hormone esterase or an insect-selective scorpion neurotoxin emerged earlier and were smaller than conspecifics that developed in uninfected hosts or hosts infected with the wild-type virus.

The rapid development of *C. sonorensis* probably also explains why it was frequently able to outcompete *Ch. insularis* in multiparasitized hosts. Oviposition by *C. sonorensis* in hosts already parasitized by *Ch. insularis* was not associated with high costs for *C. sonorensis* reproduction. Pupating *C. sonorensis* consistently emerged from hosts parasitized by *Ch. insularis,* independent of the degree of development of the *Ch. insularis* larva, and the development time of *C. sonorensis* was not reduced in hosts already parasitized by *Ch. insularis,* suggesting that *C. sonorensis* has a very high intrinsic competitive ability in multiparasitized hosts.

Fall armyworm larvae parasitized by either species of endoparasitoid did not show increased susceptibility to infection by nucleopolyhedrovirus in any instar tested. The mean time to death from virus infection was also not affected by parasitism. This finding is more interesting given that both *C. sonorensis* and *Ch. insularis* inject a polydnavirus at oviposition (Stoltz *et al.,* 1995). Polydnaviruses are segmented DNA viruses that induce host hemocyte apoptosis and dramatically reduce the incidence of encapsulation of parasitoid progeny (Stoltz, 1993; Beckage, 1998). The concentration of monophenlyoxidase and a number of antimicrobial proteins may also be greatly reduced following injection of the parasitoid polydnavirus and associated calyx fluid proteins (Webb, 1998). However, our results indicate that host immune suppression in parasitized larvae did not appear to affect their susceptibility to an invading pathogenic baculovirus. In other comparative studies of the susceptibility of parasitized and unparasitized hosts to baculoviruses, the entire spectrum of responses has been found in different host–parasitoid–virus systems (Brooks, 1993). Parasitized hosts may show increased susceptibility (Fuhrer, 1976), reduced susceptibility (Beegle and Oatman, 1975; Santiago-Alvarez and Caballero, 1990), or susceptibility to baculoviruses similar to that of nonparasitized hosts (Hochberg, 1991a). Increased susceptibility is the most disruptive outcome for the parasitoid population but appears to be less common than the other responses.

The compatibility of virus- and parasitoid-induced mortality in programs of integrated pest management that utilize baculovirus bioinsecticides has received theoretical and empirical attention. Hochberg *et al.* (1990) developed a population model and reported that the coexistence of parasitoids and pathogens may be enhanced when there exists asymmetry in the intrinsic and extrinsic competitive abilities of each type of natural enemy, e.g., a greater host discovery capacity by the parasitoid and a greater competitive capacity by the virus in simul-

taneously infected and parasitized hosts. Begon *et al.* (1996), however, observed that host–pathogen and host–parasitoid systems were destabilized to the point of extinction following the introduction of an additional natural enemy, i.e., a switch to a host–parasitoid–pathogen system. The distribution of attacks by each type of natural enemy during the larval stage of the host appeared to be insufficiently asymmetrical in their simple experimental laboratory system.

In an analysis of eight separate field trials involving the application of SfMNPV for control of *S. frugiperda* larvae in maize in southern Mexico and Honduras, Martínez *et al.* (2000) detected no adverse effect of increasing virus-induced mortality on the prevalence of parasitoid emergence from fall armyworm larvae collected in virus-treated plots. This is perhaps to be expected since we found no differences in susceptibility to virus between parasitized and nonparasitized hosts; thus, applications of virus presumably result in mortality of equal proportions of parasitized and nonparasitized larvae.

In contrast, the prevalence of parasitism of *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) larvae by *Cotesia melanoscela* (Ratzeburg) (Hymenoptera: Braconidae) was reduced in *Ld*MNPV-treated plots due to very high virus-induced host mortality (Webb *et al.*, 1989), whereas under enzootic conditions, a positive relationship between virus disease and parasitism was detected (Reardon and Podgwaite, 1976). Braconid and tachinid parasitoids apparently avoid parasitism in granulovirus-infected hosts in the field, which reduces the degree of interference between populations of these natural enemies (Hochberg, 1991b; Stark *et al.*, 1999).

As in our study, Sait *et al.* (1996) reported that transmission through oviposition was not observed following parasitism of virus-infected hosts, although parasitoid activity was responsible for contamination of the feeding environment of host larvae. In contrast, efficient parasitoid vectoring of insect viruses has been observed in a number of other studies, although almost all of these were performed under laboratory conditions (Irabagon and Brooks, 1974; Beegle and Oatman, 1975; Levin *et al.*, 1979; Caballero *et al.*, 1991). Field studies, although sparse, have tended to confirm the role of parasitoids as vectors for baculovirus dispersal in agricultural habitats (Hochberg, 1991b; Fuxa and Richter, 1994).

In summary, we found no evidence for avoidance of virus-infected hosts by ovipositing females of two parasitoid species, despite the low viability of eggs laid in such hosts. Within-host competition between parasitoids and virus usually proved fatal to the parasitoid. *C. sonorensis* females did not avoid hosts already parasitized by *Ch. insularis.* Since within-host competition between parasitoid species was highly asymmetrical, with *C. sonorensis* the dominant competitor, discrimination by *C. sonorensis* females would not be expected. These results suggest that application of virus for biocontrol of *S. frugiperda* is likely to lead to substantial mortality of developing parasitoids. Of course, this mortality is still likely to be much less than would result from use of a conventional pesticide (Croft and Brown, 1975).

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