Interactions between Nuclear Polyhedrosis Virus and Three Larval Parasitoids of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT Interactions between a *Helicoverpa* nuclear polyhedrosis virus (NPV) and three larval parasitoids of *H. armigera* were recorded in laboratory studies. The parasitoids *Microplitis demolitor*, *Cotesia kazak* and *Hyposoter didymator* required a time advantage of at least 3 d at 25 °C before the host was exposed to NPV to ensure successful completion of development. *Helicoverpa* larvae parasitised by *C. kazak* died from NPV after the parasitoids emerged from the host, and thus could provide a source of virus inoculum for secondary spread of the disease. On the other hand, as the interval between parasitisation and host exposure to NPV increased, the proportion of hosts parasitised by *M. demolitor* which died from NPV after emergence of the parasitoid decreased. It was also shown that as the time interval between host parasitisation by *M. demolitor* and exposure to virus increased, higher virus doses were required to kill parasitised hosts than were required for nonparasitised hosts of the same age.

Introduction

Contarinia sorghicola (Coquillett) (Diptera: Cecidomyiidae) and *Helicoverpa armigera* (Hübner) are the major pests of grain sorghum in Queensland (Passlow *et al.* 1985). With the increasing adoption of *C. sorghicola*-resistant hybrids there will be a reduction in insecticide spraying for midge control (Franzmann 1993). In the past, pyrethroids used for *C. sorghicola* control also controlled *H. armigera*. However, increasing resistance to pyrethroids in *H. armigera* has reduced the efficacy of these products (Forrester and Bird 1992), and for the immediate future there will remain a requirement to control *H. armigera*. Any reduction in the use of disruptive, broad-spectrum insecticide sprays increases the opportunity to take advantage of biological control occurring naturally or by artificially released agents.

Three solitary endoparasitoids are currently being investigated for biological control of *Helicoverpa* spp. in Australia (Murray *et al.* 1992). *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae) is native to Australia and has been recorded from *H. armigera* and *Helicoverpa punctigera* (Wallengren) (Zalucki *et al.* 1986). *Cotesia kazak* (Telenga) (Hymenoptera: Braconidae) and *Hyposoter didymator* Thunberg (Hymenoptera: Ichneumonidae) were introduced to Western Australia from southern Europe in 1983 (Michael 1989), and have been released for establishment in eastern Australia since 1991 (Ridland *et al.* 1993).

Natural disease caused by *Helicoverpa* nuclear polyhedrosis virus (NPV) is commonly recorded in *H. armigera* on sorghum. Preliminary assessments of the use of NPV on sorghum for safe, specific control of *H. armigera* with minimum impact on biological control agents have been encouraging (Teakle *et al.* 1983, 1985b). However, even selective microbial insecticides applied inappropriately can disrupt parasitism. Disease organisms are capable of killing developing parasitoids by destroying host larva biomass (Brown 1983). On the other hand, prior parasitism may suppress NPV disease development (Teakle *et al.* 1985b). It is important to understand the interaction between parasitoids and selective microbial insecticides in order to use them most effectively on grain sorghum. We investigated the interaction between an NPV and three larval parasitoids of *H. armigera*. The dose-mortality responses of NPV in nonparasitised and *M. demolitor*-parasitised *H. armigera* larvae were also investigated.

Materials and methods

Parasitoid and host cultures. A laboratory culture of *M. demolitor* was established from approximately 50 parasitised *H. armigera* larvae collected on cotton and sorghum in south-east Queensland during February/March 1990. Cultures of *C. kazak* and *H. didymator* were established from field collections of parasitised *Helicoverpa* spp. larvae in Western Australia during February-March 1991. All cultures were initially maintained in *H. armigera* larvae, but since November 1991, *M. demolitor* and *C. kazak* have mostly been reared in *Chrysodeixis argentifera* Guenee larvae and *H. didymator* has been reared in *Spodoptera litura* (F.) larvae. Parasitoids were reared at 25 °C, 60-70% RH and natural photoperiod using culture methods similar to those of Powell and Hartley (1987). The *H. armigera* used in these tests had been cultured on artificial diet (modified from Shorey and Hale 1965) for 7 years using the techniques of Teakle and Jensen (1985).

Virus. Elcar (Sandoz Agro Ltd.), a commercial

formulation of NPV from *Helicoverpa zea* (Boddie), with a stated minimum of 4×10^9 polyhedral inclusion bodies (PIB) per gram, was used throughout the experiment. A 1% stock suspension of Elcar was prepared in 0.067 M phosphate buffer saline (PBS), pH 7.0.

Effect of time advantage of interactions between parasitoids and NPV. Two groups of about 250 second-instar *H. armigera* larvae were placed in 18 cm diameter × 20 cm high plastic containers for each parasitoid species. Twenty pairs of 3-4 d old mated parasitoids without oviposition experience were placed in each container for 2 h. Larvae were then placed in groups of 60 in 1 L cylindrical plastic containers lined on the bottom and sides with a thin layer of artificial diet. On each of days 2, 3, 4 and 5 after stinging (days 3, 4 and 5 only for *M. demolitor*), 48 parasitised larvae (nonreplicated) were dosed using the diet-surface contamination method of Ignoffo (1966), at 6,000 PIB/mm^2. This dose rate had been shown to give >95% mortality (Teakle *et al.* 1985a). Control treatments comprised larvae only exposed to PBS, to NPV, and parasitised, respectively. Larvae were incubated at 25 °C and 55% RH. Two days after initial exposure, larvae were transferred from the tissue culture trays to 28 mL clear plastic cups containing uncontaminated diet. The cups were ventilated with four pin holes in the plastic cap. Larvae which were judged, on the basis of their frass production, to have consumed no contaminated diet, were discarded. Larvae were monitored for mortality daily. Mortality due to NPV was confirmed by phase-contrast microscopic examination of the body contents. Successful emergence of the parasitoid larva from its host was recorded and observations of each pupa (usually formed on the side of the rearing cup) continued until adult parasitoid emergence.

Susceptibility to NPV of parasitised and nonparasitised larvae. The dose-mortality responses to the NPV of parasitised and nonparasitised *H. armigera* larvae were compared by bioassay (Ignoffo 1966). Early second-instar *H. armigera* larvae were parasitised by *M. demolitor*. Parasitised and nonparasitised larvae were dosed on the same day as parasitisation occurred (Day 0), and 2 and 4 d after parasitisation. Five-fold doses ranged from 2 to 6,000 PIB/mm^2 on Days 0 and 2, and from 48 to 156,250 PIB/mm^2 on Day 4. Control larvae received diet surface-treated with PBS only. The bioassays were incubated at 25 °C and 55% RH. After 2 d, larvae were transferred from the contaminated diet to uncontaminated diet in 28 mL clear plastic cups. Larvae which were judged, on the basis of their frass production, to have consumed no contaminated diet, were discarded. Mortality was recorded daily and virus infection confirmed microscopically, and the successful emergence of the parasitoid from the host recorded. The dosage-mortality data were analysed by probit analysis (Finney 1971).

Results

Time advantage interactions between parasitoids and NPV. The percentage of parasite emergence was dependent on the length of the interval between host parasitisation and exposure to NPV (Table 1). In preliminary tests, *M. demolitor*, *C. kazak* and *H. didymator* failed to complete development in hosts exposed to NPV before or on the same day as parasitisation. For *C. kazak* and *H. didymator*, only a few (<25%) parasitoids completed development when there was a 2 d period between parasitisation and exposure to NPV. 62-88% of parasitoids successfully completed development when the interval was 3 d, and 98-100% when the interval was 4 or 5 d. For each of the three parasitoid species, there was no significant difference ($P > 0.05$) in the time required for successful completion of parasitoid development in hosts treated with or without NPV. Also there was evidence of parasitoids successfully pupating if the host larva died before the parasitoid had started spinning its cocoon.

After parasitoid emergence, some parasitised hosts subsequently died from NPV (Table 1). With *M. demolitor*, the percentage of host larvae dying from NPV after parasitoid emergence decreased as the interval between parasitisation and exposure to NPV increased. With *C. kazak* there was a high percentage (90-100%) of parasitised hosts that died from NPV after parasitoid emergence, irrespective of the interval between parasitisation

Table 1. Mortality of *Helicoverpa armigera* parasitised by *Microplitis demolitor*, *Cotesia kazak* and *Hyposoter didymator* and subject to delayed exposure to nuclear polyhedrosis virus, and developmental times[1] of parasitoids.

Days from parasitisation to exposure to virus	*M. demolitor*			*C. kazak*			*H. didymator*		
	% parasitism	% infected with virus	Parasitoid developmental time (days) mean ± s.e.	% parasitism	% infected with virus	Parasitoid developmental time (days) mean ± s.e.	% parasitism	% infected with virus	Parasitoid developmental time (days) mean ± s.e.
Parasitised only	93 (0)[2]	0	13.4 ± 0.10	91 (0)	0	14.2 ± 0.16	100	0	16.1 ± 0.05
2	—[3]	—	—	23 (90)	77	13.8 ± 0.16	19	81	16.0 ± 0.00
3	88 (95)	12	13.2 ± 0.08	73 (97)	27	13.9 ± 0.14	62	38	16.8 ± 0.09
4	88 (63)	2	13.4 ± 0.08	100 (100)	0	14.0 ± 0.10	100	0	16.0 ± 0.03
5	100 (16)	0	13.3 ± 0.10	100 (100)	0	13.8 ± 0.08	100	0	16.0 ± 0.03

[1] Developmental time for all species is the period from oviposition to emergence of adult parasitoid.
[2] Number in brackets is the percentage of parasitised host larvae dying from virus after parasitoid emergence.
[3] No data available.

and exposure to NPV. Host mortality could not be attributed to NPV when *H. didymator* completed development because the host was consumed by the parasitoid.

Susceptibility of parasitised and nonparasitised larvae to NPV. Dose-mortality responses were similar for both nonparasitised host larvae and those parasitised by *M. demolitor* when *H. armigera* exposure to NPV occurred on the same day as parasitisation (Table 2). When exposure to NPV occurred 2 d after parasitisation, there was a 5-fold increase in the LC_{50} for the parasitised hosts relative to the LC_{50} for nonparasitised hosts. There was insufficient mortality to determine relative dose-mortality responses when there was a 4-day period between parasitisation and exposure to NPV.

Discussion

For each parasitoid species an interval of at least 3 d at 25 °C between parasitisation and exposure to NPV was required to allow a high percentage of parasitoids to complete development. A similar interval was required by *Glabromicroplitis croceipes* (Cresson) in *Heliothis virescens* (F.) exposed to NPV (Brown *et al.* 1989). The responses of each of the larval parasitoid species to subsequent NPV infection were similar. As each of the parasitoids used in this study readily accept early second to late third instar hosts for parasitisation (Tillman and Powell 1989), it is reasonable to expect similar pathogen-parasitoid-host interactions. Parasitism by *C. kazak* caused least disruption to NPV infection, and secondary spread of the disease could occur, as most parasitised larvae ultimately died from NPV irrespective of the time between parasitism and virus infection. For *M. demolitor*, the percentage of parasitised larvae which died from NPV after parasitoid emergence decreased as the interval between parasitisation and exposure to NPV increased. Consequently, increasing *M. demolitor* parasitisation in the host population could reduce the virus inoculum available for secondary spread. Further studies are required to explain the suppression of NPV infection in larvae parasitised by *M. demolitor*.

Studies in the USA have shown that NPV can be transmitted by parasitoids that have previously oviposited in infected *H. virescens* larvae (Brown *et al.* 1989; Young and Yearian 1990). Hosts infected with NPV during contact with parasitoids invariably died before the parasitoid completed development. Thus NPV-transmission by parasitoids would not reduce the host population beyond that by parasitism alone unless some parasitoid-host interactions did not result in parasitism.

The results of our study suggest that NPV sprays would jeopardise parasitoid survival if sprays were applied within 3 d of parasitisation. A 3-d delay in application of NPV could help to conserve developing parasitoids, but such delays would result in larger larvae requiring bigger doses of NPV (Teakle *et al.* 1985a). However, as NPV infection levels of *H. armigera* on sorghum were largely independent of the age of the larvae treated during the first three instars, presumably because decreasing susceptibility is compensated for by increased feeding (Teakle *et al.* 1985b), less precision in timing of NPV sprays would be required. Although the majority of *H. armigera* oviposition in sorghum occurs at the pre-flowering stage (Teakle *et al.* 1985b), plants do not develop uniformly in most sorghum crops, and consequently there will be a spread of *H. armigera* larval ages present. This diversity of host larval age would make it very difficult to conserve the larval parasitoids while maintaining the efficacy of NPV applications. Thus mortality from NPV sprays will be at the expense of the naturally occurring larval parasitoids in sorghum especially where *H. armigera* larval age is uniform. A 3-d delay in application of NPV would not adversely affect *H. armigera* larval susceptibility, providing larvae were still less than fourth instar.

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Table 2. Dose-mortality responses of *Microplitis demolitor*-parasitised and nonparasitised *Helicoverpa armigera* larvae dosed with a nuclear polyhedrosis virus.

Days post-parasitisation	Condition	LC_{50} (95% C.L.)[1] (PIB/mm^2)	Dosage-mortality regression line ($y = bx + a$)[2]
0	Parasitised	9 (4-15)	$y = 1.1x + 3.9$
	Nonparasitised	6 (3-12)	$y = 1.1x + 4.1$
2	Parasitised	180 (104-311)	$y = 1.2x + 2.2$
	Nonparasitised	35 (19-58)	$y = 1.3x + 3.0$
4	Parasitised	—[3]	
	Nonparasitised	270 (135-482)	$y = 1.2x + 2.3$

[1] based on 46-48 larvae per dose, 5 doses per bioassay.
[2] y, mortality (in probits); x, log dose (polyhedra/mm^2 diet surface).
[3] insufficient mortality to calculate dose-mortality response.

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