Detection of Anticarsia gemmatalis Nuclear Polyhedrosis Virus in Predatory Arthropods and Parasitoids After Viral Release in Louisiana Soybean^{1, 2}

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ABSTRACT Anticarsia gemmatalis Hübner nuclear polyhedrosis virus (AgNPV) was released into field plots; samples were then collected weekly to determine the complex of predatory arthropods and parasitoids contaminated with the virus due to attacks on the infected hosts. No contamination was detected on day 1 after the release or at any time in control samples. In the treated plots, the percentage of contaminated predatory arthropods and parasitoids declined from 79% on day 8 to 20% on day 41. In samples averaging 16 m distance from treated plots, contamination increased from 32% on day 8 to 69% on day 27, then decreased to 21% on day 41. The AgNPV was detected in at least six species of Hemiptera, one Coleoptera, nine species of spiders, one species of hymenopteran parasitoid, and two dipteran parasitoids.

KEY WORDS Baculovirus; nuclear polyhedrosis virus; transmission, viral; predator; parasitoid; *Anticarsia gemmatalis*.

Disease transmission is a process critically important to microbial control of insects (Fuxa 1987). When entomopathogens are used as short-term microbial insecticides, the artificial application of the pathogen must at least partially replace its natural transmission. In the long-term approaches to microbial control, such as introduction-establishment of an entomopathogen, transmission can be the critical factor which determines whether the control attempt will succeed or fail. For example, efficient transmission was a major reason for successful, long-term control of coconut rhinoceros beetle, *Oryctes rhinoceros* (L.), by a nonoccluded baculovirus and of European spruce sawfly, *Gilpinia hercyniae* (Hartig), by a nuclear polyhedrosis virus, or NPV (Harper 1987). In general, transmission of entomopathogens, whether by means of biotic or abiotic agents, is not very well understood.

Transmission has been researched in the Anticarsia gemmatalis Hübner nuclear polyhedrosis virus (AgNPV) system, though many questions remain to be answered. This virus has potential for introduction-establishment in the United States; it has been known to spread as well as persist for at least three years after its release in soybean (Richter and Fuxa 1984). Rainfall, host

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density, and host age structure have been demonstrated to affect transmission of AgNPV, mostly within one or a limited number of host plants (Young and Yearian 1986, 1989a, 1989b, Young et al. 1987). Horizontal spread of AgNPV has been more difficult to study. Predatory arthropods have been implicated as transport agents in laboratory and field cage studies, in which these arthropods attacked infected *A. gemmatalis* larvae and then passed viable AgNPV in their feces (Abbas and Boucias 1984, Young and Yearian 1987, 1989c, Kring et al. 1988, Ruberson et al. 1991, Young and Kring 1991). It was demonstrated in a field cage study that *Nabis roseipennis* Reuter can spread nuclear polyhedrosis in *A. gemmatalis* larvae (Young and Yearian 1992). A field release of AgNPV resulted in a number of predatory arthropods becoming contaminated with the virus (Boucias et al. 1987). However, in this study none of the predators were identified to species and most were not identified to genus. Also, parasitoids were not included, and the study was limited to one location in Florida.

The purpose of this study was to identify the complex of predatory arthropods and parasitoids in Louisiana that become contaminated with AgNPV due to preying on or parasitizing *A. gemmatalis* infected with the virus after a field release. The intention was to include parasitoids as well as predators, and to identify as many of these arthropods as possible to genus and species.

Materials and Methods

The AgNPV was released into a 2.57-ha soybean field on the Louisiana State University Agricultural Center Rice Research Station near Crowley. The AgNPV was released into two plots, each measuring 14.6×14.6 m, situated approximately 100 m apart. Two release plots were used in order to increase the treated area available for sampling, thereby increasing the number of samples and the chance to detect non-target arthropods with low population densities. Also, two release plots were used in order to increase the chance of the AgNPV persisting and spreading after its release, though the final results indicated no difference in disease dynamics between the plots. Control samples were collected in untreated soybeans approximately 55 m from the field where AgNPV was released.

The AgNPV was a Brazilian isolate obtained originally from G. R. Carner (Dept. of Entomology, Clemson University). The AgNPV was produced in laboratory-reared A. gemmatalis, semipurified by filtration and table-top centrifugation, and counted with a counting chamber (Petroff-Hausser, Hausser Scientific Partnership, Horsham, PA). The virus was sprayed with a backpack CO_2 sprayer at a spray pressure of 2.1 kg/cm² through TX-4 hollow-cone nozzles (Spraying Systems Co., Wheaton, IL), at a rate of 4.1×10^{12} viral polyhedral inclusion bodies per ha in the two plots. The plots were treated on 30 August 1990. At that time, the number of A. gemmatalis larvae averaged 17.8 per 40-sweep sample with a sweep net; 52% and 46% of the larvae were in the 3-4 and 5-6 instars, respectively; and the soybeans were in the R5 stage of development (Fehr and Caviness 1977).

Insect samples were collected immediately before spraying, on the first day after spraying, and approximately weekly thereafter until the soybean plants became senescent. Each sample consisted of 40 sweeps through the foliage with a 38-cm net. All arthropods with any possibility of being a predator or parasitoid of *A. gemmatalis* were placed in empty, individual 30-ml plastic cups; *A. gemmatalis* larvae were placed individually in similar cups containing velvetbean caterpillar artificial diet (Greene et al. 1976) without formalin. Within each treated plot and a similarly sized plot in the control area, four 40-sweep samples were collected from four different rows randomly selected for each sample date. Additional 40-sweep samples were collected at an average distance of 16.0 m from the plots in order to determine whether infected *A. gemmatalis* or contaminated predatory arthropods or parasitoids could be detected outside treated plots. Sixteen such samples were collected around each treated plot on every sampling date after the plots were sprayed. Additionally, on the last sampling date (10 October 1990), a similar set of samples was collected around the control plot to determine whether the AgNPV had spread to the control field.

The A. gemmatalis returned to the laboratory were examined for NPV infection. They were reared at 27°C, 80% RH, and a 14:10 LD photoperiod until they either died or pupated and emerged as adults. Each insect that died before successful emergence as an adult was examined for NPV. Tissues of the dead insect were smeared on microscope slides and examined for viral polyhedra under phase-contrast microscopy.

The method of detection of AgNPV in predatory arthropods and parasitoids was adapted from that of Young and Yearian (1990). Each 30-ml cup containing a predator or parasitoid was stored at -4°C until the bioassay. The insects were identified by the third author in the Louisiana State University Entomology Department Museum, where voucher specimens were submitted (voucher specimens could not be kept for those insects collected in very low numbers due to the destructive nature of the bioassay and the possibility of losing a single contaminated member of the species). For the bioassay, each individual insect was macerated with a glass rod in a test tube containing 0.1 ml of 2.0% KCl. After the insect was macerated, the homogenate was mixed with another 0.9 ml 2.0% KCl, and 0.1 ml of this mixture was spread onto the surface of the Greene et al. (1976) artificial diet, without formalin, in each of ten 30 ml plastic cups. One neonatal A. gemmatalis was added to each of the ten cups. Each run of the bioassay included a control of ten neonatal A. gemmatalis treated similarly except that the 1.0 ml of 2.0% KCl contained no insect homogenate. The bioassay insects were reared as above for 14 days. Every bioassay larva that died was examined for NPV polyhedra, as above. There was no mortality in the bioassay control insects. Hymenopteran parasitoids were treated identically except that none were sampled by sweep net; every hymenopteran parasitoid in the study emerged from the field-collected A. gemmatalis larvae reared in the laboratory. After their emergence from the host insect, hymenopteran parasitoids were treated the same as the predators and dipteran parasitoids with the exception of the polyembryonic Copidosoma truncatellum (Dalman), for which large numbers of the tiny parasitoid emerging from one host were combined and treated as one insect. The predator or parasitoid was counted as AgNPV-contaminated if at least one of the bioassay larvae became infected with NPV.

Results

The one-time application of AgNPV in the treated plots resulted in a seasonlong epizootic of the virus in the A. gemmatalis population, peaking at a prevalence of 78.3% infection on 26 September (Fig. 1a). Additionally, infected A. gemmatalis larvae were detected in the samples 16 m outside sprayed plots; viral prevalence in these samples was 19.0% as early as 7 September and peaked at 59.3% on 26 September. Not a single infected A. gemmatalis larva was detected on 30 August (before the virus was sprayed) or in any control sample throughout the experiment (Fig. 1a). The overall, season-long infection rate of A. gemmatalis larvae was 71.7% in treated plots and 30.4% in the 16-m samples (Table 1).

The pattern of contamination of predatory arthropods and parasitoids over time (Fig. 1b) was similar to that of infections in *A. gemmatalis*. The most important exception was that no NPV was detected in these arthropods on 31 August, one day after the virus was sprayed in treatment plots. NPV was never detected in control samples. In AgNPV-treated plots, the predators and parasitoids were 65.1% contaminated on 7 September, a percentage which declined to 20-31% in October. In the 16-m samples, contamination increased from 26.2% on 7 September to a peak of 59.8% on 19 September, then decreased to 18.5% on 10 October (Fig. 1b). The overall, season-long contamination of predatory arthropods and parasitoids was 48.8% in the treated plots and 36.1% in the 16-m samples (Table 1).

Contamination by AgNPV was detected in at least 16 species of predatory arthropods and three species of parasitoid (Table 1). In one case, two predatory species [Sinea diadema (F.) and Atrachelus cinereus cinereus (F.)] appeared so similar that they were not identified as separate species until the bioassays were completed. In this case, relatively large numbers of insects (82) were involved; thus, it is likely that 17 species of predators were contaminated with AgNPV. The Hemiptera and the spiders had the greatest numbers of species and individuals contaminated with the virus. The total number of sampled individuals contaminated with AgNPV was 330 for Hemiptera, 5 for Coleoptera, 3 for Diptera, 4 for Hymenoptera, and 126 for spiders. The most important families, in terms of total number of sampled individuals contaminated with AgNPV, were Pentatomidae (165 contaminated individuals), Lygaeidae (65), Nabidae (58), Reduviidae (42), Araneidae (40), Lycosidae (37), Thomisidae (31), and Oxyopidae (16). The AgNPV was not detected in 10 predatory species, including 7 unidentified spiders, or in 2 parasitoid species. In all of these species in which AgNPV was not detected, relatively few individuals (<8) were sampled. In addition to the arthropods, unidentified tree frogs were a common predator in the field where the AgNPV was released, but they were not bioassayed for the virus.

Discussion

These results add to the data implicating predatory arthropods as an agent in the spread of AgNPV from release sites. Spread of AgNPV in Louisiana, and probably in the United States in general, is relatively simple to document, because the virus is not indigenous (Carner and Turnipseed 1977, Fuxa and

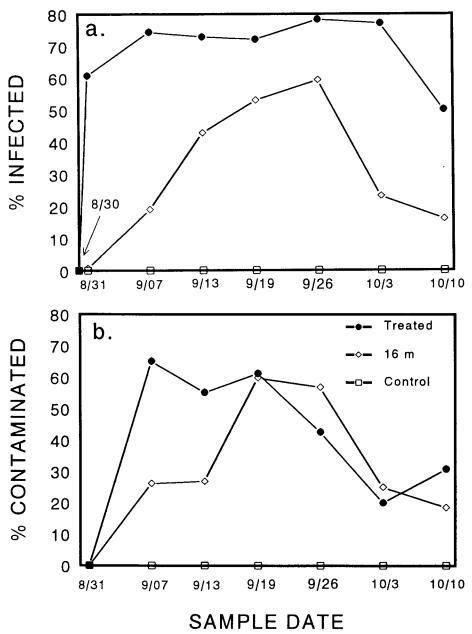


Fig. 1a. Total percentage infection of *A. gemmatalis*, and b. percentage contamination of predatory arthropods and parasitoids by AgNPV in virustreated plots, at an average distance of 16.0 m outside the plots, and in a control area. Treated plots were sprayed with AgNPV on 30 August 1990, when the percentage AgNPV infection of *A. gemmatalis* larvae was zero.

Control Area	Treated Plots	Vicinity of Treated Plots†
·		
0	717	30.4
(750)	(367)	(3380)
0	04.0	
-		52.9
(31)	(33)	(259)
0	40.0	31.9
(16)	(25)	(91)
0	46.2	27.7
(10)	(13)	(47)
0	71.4	34.8
(6)	(7)	(69)
-	100	52.6
	(3)	(19)
—	_	$\begin{pmatrix} 0\\(2) \end{pmatrix}$
		(-)
0	31.6	30.1
(42)	(38)	(176)
0	50.0	20.0
-		(10)
(=/	(0)	(10)
_	100	0
	(2)	(2)
		$\begin{array}{c} 25.0 \\ (4) \end{array}$
_	0	0
	(2)	(5)
	_	
-		40.0
	(1)	(10)
	Area 0 (750) 0 (51) 0 (16) 0 (10) 0 (6) - -	Area Plots 0 71.7 (750) (367) 0 84.8 (51) (33) 0 40.0 (16) (25) 0 46.2 (10) (13) 0 71.4 (6) (7) $-$ 100 (3) $ 0$ 31.6 (42) (38) 0 50.0 (2) (6) $ 100$ $ 0$

Table 1. Seasonal* percentage (number examined) of A. gemmatalisinfected with AgNPV and of predatory arthropods and para-sitoids contaminated with AgNPV. All of the insects were sam-pled in or near an AgNPV release site.

Arthropod	Control Area	Treated Plots	Vicinity of Treated Plots†
Hymenoptera			
BRACONIDAE		0	<u>^</u>
Meteorus sp.	—	0 (1)	0 (5)
ENCYRTIDAE			
Copidosoma truncatellum (Dalman)		-	0 (2)£
Spiders			
ARANEIDAE			
Neoscona sp. A	0 (2)	-	-
Neoscona sp. B	0	43.8	37.5
-	(9)	(16)	(88)
LYCOSIDAE			
Unidentified species	0	37.5	25.3
(Schizocosa or Lycosa)	(2)	(32)	(99)
OXYOPIDAE			
Oxyopes sp.	-	62.5 (8)	39.3 (28)
SALTICIDAE		.,	. ,
Phidippus sp.	-	_	33.3 (3)
THOMISIDAE			(2)
Misumenops sp. A.	0	100	27.5
	(5)	(3)	(40)
Misumenops sp. B	_	33.3	22.2
		(3)	(18)
Misumenops sp. C	0	22.2	12.5
	(1)	(9)	(16)
Misumenops sp. D		66.7	54.5
		(3)	(11)
Unidentified spiders	_	25.0	0
(Probably 8 spp.)		(4)	(11)
GRAND TOTAL	0	48.8	36.1
(Excluding A. gemmatalis)	(146)	(209)	(1015)

Table 1. Continued.

* August 31 through October 10, 1990.

 \dagger Mean distance of sample from treated plot = 16.0 m.

‡ Two species, not distinguished while data were being collected.

£ The parasitoids from two lepidopteran, mummified larvae were bioassayed. In each case, large numbers of the parasitoid were combined in the bioassay.

Richter unpublished data). The AgNPV has spread outside a release site at least twice previously, a distance of at least 69 m in one case (Richter and Fuxa 1984) and approximately 18 m in another (Boucias et al. 1987). In previous research, one predator, the nabid N. roseipennis, has been demonstrated experimentally to be a disseminator of AgNPV in a caged field population of A. gemmatalis (Young and Yearian 1992). Laboratory experiments have demonstrated that N. roseipennis, the pentatomid Podisus maculiventris (Say), and the lynx spider Oxyopes salticus Hentz can defecate infectious AgNPV after feeding on infected A. gemmatalis (Abbas and Boucias 1984, Young and Yearian 1987, Kring et al. 1988). A field survey of AgNPV-contaminated predators in Florida implicated the hemipteran genera Podisus and Spangonicus and the coleopteran genera Oecanthus and Notoxus in AgNPV transport (Boucias et al. 1987). Our results, in addition to supporting some of these earlier results, add the following predators to the list of possible transport agents: Nabis capsiformis Germar, S. diadema, A. cinereus cinereus, Zelus cervicalis Say, Geocoris punctipes (Say), Calleida decora Say, Neoscona sp., Phidippus sp., and Misumenops spp. (Table 1).

In addition to these predators, our results implicate three parasitoids as possibly involved in AgNPV transport: the chalcid *Brachymeria ovata* (Say), and two tachinids, *Archytas apicifer* (Walker), and an unidentified species. Parasitoids previously have not been implicated in AgNPV transport. However, hymenopteran parasitoids have been implicated in the transmission of baculoviruses in at least seven other species of Lepidoptera (Thompson and Steinhaus 1950, Laigo and Tamashiro 1966, Irabagon and Brooks 1974, Beegle and Oatman 1975, Raimo et al. 1977, Levin et al. 1979, Caballero et al. 1991). A tachinid parasitoid has been implicated in the transmission of NPV in *Trichoplusia ni* (Hübner) (Vail 1981).

Based on our data, Hemiptera and spiders are the two groups of predatory arthropods or parasitoids that are most likely to be important agents of AgNPV transport. Assuming that all of these arthropods were equally likely to be sampled by the sweep-net method, the greatest numbers of AgNPV-contaminated arthropods were in these two groups (Table 1). In the Florida study (Boucias et al. 1987), the greatest number of contaminated arthropods was in the Hemiptera. The number contaminated in the overall sampling is probably the best measure of importance to viral transport in the current study, because number contaminated is a function of the arthropod's population density as well as its percentage contamination. Other variables beside number of arthropods carrying NPV that affect viral transport, not investigated in the current research, would include the amount of viable NPV defecated by an individual predator as well as the likelihood that the viable virus would be defecated at a place and time such that A. gemmatalis larvae would ingest it. In laboratory experiments, P. maculiventris, N. roseipennis, and O. salticus excreted amounts of AgNPV sufficient to infect A. gemmatalis larvae (Abbas and Boucias 1984, Young and Yearian 1987, Kring et al. 1988).

Our results indicate that the predatory arthropods and parasitoids became contaminated with AgNPV due to their predation or parasitism of *A. gemmatalis.* If these arthropods were becoming contaminated with AgNPV due to being hit with spray droplets or due to contact with contaminated vegetation, the highest percentage contamination should have occurred immediately after spraying and then quickly decreased. Most, if not all, NPV viability on exposed surfaces is generally lost in less than 8 days (Fuxa 1989), primarily due to ultraviolet radiation. However, the pattern of contamination in the current research was just the opposite. AgNPV contamination was not detected in a single natural enemy on 31 August, the first day after the virus was sprayed (Fig. 1b), even though NPV was detected in the A. gemmatalis population (Fig. 1a). Contamination of non-target arthropods then steadily increased until 19 September. It is possible that the predatory arthropods and dipteran parasitoids could have become contaminated when infected A. gemmatalis larvae died and deposited AgNPV on foliage, which would begin approximately one week after the plots were sprayed. However, if this were an important mechanism for contamination, AgNPV should have been detected in at least a few arthropods on 31 August, when foliage contamination was extensive due to the viral spray. Thus, it is possible that application of NPVs could be used in field studies as a sensitive method to identify field predators of various Lepidoptera or sawflies. Possible contamination from foliage would have to be ruled out experimentally, and it would have to be kept in mind that infected insects might be weakened and more susceptible to attack than uninfected hosts.

The likelihood of rapid spread of AgNPV by an extensive complex of predatory arthropods and parasitoids is a promising feature for future microbial control with this virus. Such transport might allow spot or lattice introductions of AgNPV that will then spread throughout a soybean field. Further research on the dynamics of such spread is warranted.

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