COMPATIBILITY OF TWO FALL ARMYWORM PATHOGENS WITH THE PREDACEOUS BEETLE, *CALOSOMA SAYI* (COLEOPTERA: CARABIDAE)

Orrey P. Young[1] and John J. Hamm[2]
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ABSTRACT

Adult carabid beetles *(Calosoma sayi* DeJean) were allowed to feed on fall armyworm larvae *[Spodoptera frugiperda* (J. E. Smith)] infected with either a nuclear polyhedrosis virus or a microsporidian protozoan *(Vairimorpha* sp.). Subsequent longevity of the beetles was monitored and fecal material was bioassayed to determine pathogen infectivity. Beetles fed infected larvae lived at least as long as beetles fed noninfected larvae. Both pathogens were highly infective when voided in beetle feces within 24 hours of ingestion. Concentration of pathogens and infectivity declined in subsequent fecal samples, with some infectivity detected in feces voided 13 - 15 days after consumption of infected larvae.

Key Words: *Spodoptera frugiperda,* NP virus, *Vairimorpha* sp., predation, feces.

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INTRODUCTION

Investigators working with entomopathogens have been optimistic for many years that organisms lethal for economically important insect pests can be found and developed for control purposes. Among the important constraints, however, are suitable methods for disseminating pathogens and studying the effects of these pathogens on non-target organisms. For several years USDA-ARS scientists at the Southern Grain Insects Research Laboratory have been evaluating the potential of a nuclear polyhedrosis virus (NPV) and a microsporidian protozoan *(Vairimorpha* sp.) as control agents for the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Both pathogens are usually transmitted to FAW by larvae feeding on contaminated host plants. Previous studies indicated that the *Vairimorpha* sp. was less deleterious than the NPV to hymenopteran parasitoids of FAW (Hamm and Hare 1982; Hamm et al. 1983). The objectives of this study were to determine the effect of two pathogens on the adult life-span of a predator, measure the passage interval of pathogens through a predator digestive system, and determine the effect of that passage on subsequent pathogen infectivity.

METHODS AND MATERIALS

Calosoma sayi DeJean (Coleoptera: Carabidae), a large (25 - 30 mm) and abundant beetle commonly known as the "caterpillar hunter," was chosen for initial testing and method development. Adult *C. sayi* were collected from a walk-in black-light trap near Tifton, Tift Co., GA, during July and August of 1981. This trap was surrounded by fields planted in field corn, peanuts, and grain sorghum.

[1] Southern Field Crop Insect Management Laboratory, ARS, USDA, Stoneville, MS 38776.
[2] Southern Grain Insects Research Laboratory, ARS, USDA, Tifton, GA 31793.

Sixty beetles were maintained on FAW larvae in the laboratory at ambient conditions (ca. 25°C, 75% RH, local photoperiod) in individual cages for 2 - 3 weeks prior to testing. Clear plastic cages (17 × 12 × 6 cm) contained a sheet of moistened paper toweling on the floor. Each caged beetle was offered three live FAW larvae (laboratory-reared, 4th - 5th instar, 25 - 30 mm) every 2 - 3 days, and at the same time paper toweling and debris were removed and fresh moistened toweling was added. These conditions appeared to be satisfactory for *C. sayi,* as adults could survive for as long as 170 days (Young 1985). The experiment with *Vairimorpha* was initiated in mid-July, whereas due to virus unavailability the experiment with NPV began in mid-September. Both experiments were terminated in mid-October, at a time when field collections by UV-light traps and pitfall traps indicated that active *C. sayi* adult population densities were close to zero (Young, unpublished data).

Laboratory-reared FAW larvae were infected with *Vairimorpha* sp. originally isolated from the cotton leafworm *[Alabama argillacea* (Hubner)] obtained in Bolivia. Neonates were allowed to feed for ca. 24 h on modified pinto bean diet (Burton 1969), without formalin, containing 2.5×10^6 spores/ml of diet. Eleven days post-treatment, larvae were weighed individually and segregated into groups of 4, with a mean total weight/group of 0.2687 gm and a range of weights within each group of $< 5\%$. This grouping technique minimized variation in the amount of food, and presumably pathogens, subsequently available to each beetle. Three groups were triturated and the number of *Vairimorpha* spores were counted on a haemocytometer, obtaining an estimate of 1.5×10^9 spores/group of 4 FAW larvae. Material from these same three groups was bioassayed for infectivity by placement on the surface of bean diet, without formalin, with subsequent feeding by laboratory-reared FAW larvae. Infectivity was demonstrated at all concentrations from 10^1 to 10^4 spores/ml, with an extrapolated LD_{50} of ca. 10^5.

Laboratory-reared FAW larvae were infected with a strain of FAW NPV from Ohio (Loh et al. 1982) by allowing 7-day-old larvae to feed for ca. 24 h on bean diet, without formalin, containing 3.27×10^8 polyhedral inclusion bodies (PIB) per ml of diet. Five days post treatment, larvae were weighed individually and segregated into groups of 2, with a mean total weight per group of 0.4117 gm and a range of weights within each group of $< 15\%$. Three groups were triturated and the PIB counted on a haemocytometer, obtaining an estimate of 4.28×10^8 PIB per group of 2 larvae. Material from these same three groups was tested for infectivity by mixture of four concentrations of polyhedra with bean diet without formalin and feeding to laboratory-reared FAW larvae. Infectivity was demonstrated at all concentrations from 10^3 to 10^6 polyhedra/ml, with an LD_{50} of ca. 10^4.

On the initial day of the beetle-FAW larva test, 32 *C. sayi* individuals were removed from their cages and cleaned. Each beetle was briefly immersed in water, removed and rubbed with a wet piece of cloth, immersed again in water, placed in a container with crumpled paper toweling and allowed to dry for 2 h. Beetles were then weighed individually on a Mettler A-30 balance scale, examined for gender, and placed in clean and separate containers (17 × 12 × 6 cm) with a moistened filter paper (Whatman No. 3) substrate. Infected or noninfected groups of larvae were placed in the appropriate container (1100 h EDST) with beetles that had been deprived of food for the previous 48 h. Consumption of larvae by beetles occurred within one h, after with the substrate paper with debris was replaced. Previous experiments had indicated that ingested food required 7 - 24 h for

passage through the beetle alimentary system (Young and Hamm 1985), thus the substrate paper was removed and fresh paper added to the cage 24 h after feeding.

The following method was used to monitor the infectivity of pathogens excreted by *C. sayi.* Substrate papers were processed from a sub-set of 2 male and 2 female beetles which had consumed *Vairimorpha*-infected larvae and 3 male and 3 female beetles which had consumed NPV-infected larvae. A fecal spot produced by each beetle was cut from the contaminated paper and placed in a vial with 5 ml of 0.1 M phosphate buffer, pH 7.0, containing 100 mcg/ml of gentamicin sulfate, and the vial was agitated on a Vortex mixer. The resulting suspensions were divided into two portions and treated in the following manner: one portion was examined under a compound microscope and the spores *(Vairimorpha* sp.) or PIB (NPV) were counted with the aid of a haemocytometer and the number of spores or PIB per fecal sample calculated. The other portion was dispensed in 0.1 ml aliquots onto the surface of bean diet, without formalin, in 30 ml plastic cups. After one h, 40 - 50 FAW neonate larvae were placed in each cup and allowed to feed for 24 h. At the end of this period, 36 of the larvae were individually isolated in cups of bean diet, with formalin, and held for observation until death or adult emergence. All larvae or pupae that died were examined for spores or PIB. This general procedure for bioassaying fecal spots was repeated at 1 - 7 d intervals after the initial exposure.

Four beetles involved in the *Vairimorpha* experiment were allowed to feed on a second group of infected FAW larvae 24 h after the first feeding, with subsequent treatment the same as for other beetles. After the initial exposures to pathogens, all beetles were fed FAW larvae 2 - 3 times each week and observed for mortality, as was the control (nonexposed) group. Dead beetles were examined for pathogens and characteristic pathology.

Two types of controls were utilized for the bioassays. A fecal control to monitor mortality due to beetle excreta consisted of FAW larvae exposed to fecal material from two untreated beetles. An untreated control consisted of FAW larvae retained for 24 h on untreated diet, without formalin, then isolated on diet with formalin to monitor mortality due to possible contamination of the bioassay system by pathogens in the laboratory.

RESULTS AND DISCUSSION

Nine *Calosoma sayi* adults maintained at laboratory conditions and fed noninfected mature FAW larvae three times a week lived an average of 58 d (Table 1). Five beetles fed on group of FAW larvae infected with *Vairimorpha* sp., then maintained on noninfected larvae, lived an average of 77 d. Four beetles fed two groups of infected FAW larvae, then maintained on noninfected larvae, survived an average of 63 d. Examination of individual beetles at death provided no evidence of *Vairimorpha* sp. infection, nor were spores present. Our data indicate that consumption of FAW larvae infected with *Vairimorpha* sp. did not shorten the life span of adult *C. sayi* beetles.

The ingestion by *C. sayi* adults of FAW larvae infected with NPV also did not shorten the life span of the beetles (Table 2). Four beetles fed noninfected larvae lived an average of 17.8 d and 10 beetles fed NPV-infected larvae survived an average of 19.9 d. The longevity periods are shorter than in the *Vairimorpha*

Table 1. Longevity of *Calosoma sayi* after consuming *Vairimorpha*-infected fall armyworm larvae.

Number of feedings of *Vairimorpha*-infected FAW larvae	Number of individual beetles	Mean longevity* (days)	Range of longevity* (days)
Twice in 24 hours	4	63.5	37 - 90
Once	5	77.0	52 - 94†
[Both categories]	[9]	[71.0]	[37 - 94]
None	9	58.1	13 - 83

* Experiment terminated at 94 days. For computational purposes individuals alive on day 94 assumed to die within 7 days.
† One beetle alive at 94 days.

Table 2. Longevity of *Calosoma sayi* after consuming NPV-infected fall armyworm larvae.

Number of feedings of *NPV*-infected FAW larvae	Number of individual beetles	Mean longevity* (days)	Range of longevity* (days)
Once	10	19.9	4 - 32†
None	4	17.8	9 - 32‡

* Terminated after 32 days. For computational purposes, individuals alive on day 32 assumed to die within 7 days.
† Two beetles alive at 32 days.
‡ One beetle alive at 32 days.

experiment, probably because of the lateness of the season when these tests were performed and the associated natural mortality of older individuals. Although the experiment was terminated after 32 d, which would seem to guarantee a shorter longevity, over 80% mortality had occurred by the end of the period. By mid-October most laboratory beetles in both experiments were dead, as well as colony adults. Regardless of these extenuating circumstances, it still remains that the two FAW pathogens tested did not shorten the survival period of *C. sayi* adults.

The fate of the pathogen spores and PIB after ingestion by *C. sayi* was monitored by examining beetle fecal material. When ca. 10^9 spores of *Vairimorpha* were fed to a beetle, ca. 10^8 spores were passed in the feces within 24 h (Table 3). These spores were highly infective when bioassayed on FAW larvae. No mortality occurred in the untreated controls or the controls exposed to feces from untreated beetles. Preliminary experiments demonstrated that passage of food through the beetle alimentary systems slows down during starvation, such that in the male beetles no detectable spores were obtained 2 d after feeding. A feeding on day 2 functioned to "flush" the alimentary tract, however, again producing detectable numbers of spores. The threshold of detection for the counting procedure was ca. 10^5 spores, but even though the day 2 count was below that value, the fecal material was still highly infective. A decline in infectivity and number of spores subsequently occurred. The pattern was not as straight-forward for the female beetles monitored (Table 3). Even after 13 d, detectable spores were passed in the feces and these were still infective. This infective material was probably not newly replicated, but merely spores from the original feeding that had survived in the alimentary tract for 13 d.

Table 3. Bioassay of *Calosoma sayi* feces for *Vairimorpha*.

Day of testing, after consumption of infected FAW larvae	Day of consumption of non-infected FAW larvae	♂♂ (2) Mean no. of spores per fecal sample*	♂♂ (2) Infectivity (mean % mortality of FAW larvae and pupae)	♀♀ (2) Mean no. of spores per fecal sample*	♀♀ (2) Infectivity (mean % mortality of FAW larvae and pupae)
1		2.1×10^8	100	2.0×10^8	100
2		ND†	90	0.8×10^6	6
	2				
3		6.9×10^6	60	4.7×10^6	6
5		No Test	0	No Test	0
	5				
8		ND	0	5.4×10^6	0
	9				
13		ND	0	2.8×10^6	16

* Potential inoculum (in FAW larvae) = 1.5×10^9 spores/group of 4 larvae.
† ND = not detectable (less than 10^5 spores).

Passage of NPV polyhedra and their subsequent infectivity for FAW larvae present a clear picture in both sexes of *C. sayi* (Table 4). Most of the ingested polyhedra passed through the alimentary tract in the first 24 h and were highly infective. From days 2 through 15, polyhedra were passed in the feces in low but relatively constant numbers. During this period, however, the infectivity of the fecal material steadily declined to ca. zero, suggesting a cumulative deleterious effect of the alimentary tract environment on the polyhedra.

Table 4. Bioassay of *Calosoma sayi* feces for fall armyworm NPV.

Day of testing, after consumption of infected FAW larvae	Day of consumption of non-infected FAW larvae	♂♂ (2) Mean no. of PIB per fecal sample*	♂♂ (2) Infectivity (mean % mortality of FAW larvae and pupae)	♀♀ (2) Mean no. of PIB per fecal sample*	♀♀ (2) Infectivity (mean % mortality of FAW larvae and pupae)
1		1.8×10^8	100	1.6×10^8	100
2		4.4×10^6	89	3.2×10^6	90
	2				
3		1.4×10^6	76	0.6×10^6	92
	3				
4		2.0×10^6	55	4.3×10^6	81
	4, 7				
8		0.7×10^6	19	1.0×10^6	22
	9, 11, 14				
15		2.0×10^6	3	1.2×10^6	0

* Potential inoculum (in FAW larvae) = 2.57×10^9 PIB/group of 2 larvae.

Both experiments demonstrate that a beetle predator of FAW larvae can defecate pathogens that retain some degree of infectivity for FAW larvae for at least 13 - 15 d after ingestion of diseased larvae. This period of time is considerably longer than that documented for other entomopathogens and adult insect predators. Two previous studies have attempted to document the period of time that NPV polyhedra are excreted from insect predators and the subsequent infectivity of the feces. Beekman (1980) demonstrated NPV polyhedra in excreta of an adult hemipteran *(Nabis* sp.) up to 4 d after feeding on diseased lepidopteran larvae, but infectivity of the excreta by that time had decreased to 0.7%. Cooper (1981) allowed adult hemipterans *(Oechalia* sp.) to feed on diseased lepidopteran larvae and subsequently detected polyhedra in the predator feces 4 d later, with an infectivity level at that time of 27%. Different studies have demonstrated the transmission of NPV by predatory insects to other insects, but have not monitored the periods of infectivity of predator feces (Capinera and Barbosa 1975; Hostetter 1971; Stairs 1966; Smirnoff 1959). Microsporidian spores have also been demonstrated to retain infectivity after passage through the digestive tract of a hemipteran *[Zelus exsanguis* (Stal)] predatory on lepidopteran larvae, but only for a period of 3 d post-feeding (Kaya 1979).

Calosoma sayi, with such a long period of voiding infective excreta and with no obvious deleterious effect to itself, may be a possible candidate for utilization as a dissemination agent for pathogens in biological control programs. Parameters that would need to be investigated further, however, are the dispersal abilities of the beetle, the likelihood of target species coming in contact with the infective feces, and the stability of the pathogen in the feces. Carabid beetles in general are strong fliers/walkers and actively disperse both locally and long distance (Baars 1979). Studies with *C. sayi* indicate rapid colonization of crop fields with abundant prey (Price and Shepard 1978a), and a relatively high recovery rate (6.3%) at sites at least 100 m from release sites (House and All 1981). *Calosoma sayi* is also distributed throughout the southeastern United States (Gidaspow 1959), occurs in Georgia as an adult during the period of March to November (Fattig 1949), can produce one or two generations in the summer season (Price and Shepard 1978a), and may have an adult longevity longer than 1 year (Burgess and Collins 1917), all seemingly important characteristics for a predatory pathogen-disseminator. Adults of *C. sayi* are active both on the soil surface and in vegetation above the soil surface (Price and Shepard 1978b) and presumably defecate on leaf surfaces that may subsequently be eaten by pest lepidopterans.

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