Microsporidan Spores: Retention of Infectivity after Passage through the Gut of the Assassin Bug, *Zelus exsanguis* (Stål)[1]

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Numerous examples of insect predators disseminating insect pathogens through excreta are known. For example, *Calasoma* beetles after feeding on nuclear polyhedrosis virus (NPV), or microsporidan-infected hosts, excrete polyhedra or spores which are infectious to susceptible hosts (Capinera and Barbosa 1975, Weiser 1957). The predatory orthopteran, *Ephippiger bitterensis* Finot, also excretes infectious polyhedra after feeding on NPV-infected hosts (Vago *et al.* 1966). Predatory wasps and beetles feeding on diseased hosts excrete polyhedra or microsporidan spores which are still infectious (Smirnoff 1959, Weiser 1957). Sarcophagid flies which have fed on NPV-killed hosts also excrete virulent polyhedra (Stairs 1966, Hostetter 1971). These examples involve insects which have chewing or lapping mouthparts.

Two insects with piercing-sucking mouthparts have been shown to disseminate insect pathogens through excreta. These are the hemipteran predators, *Pilophorus uhleri* (Knight) and *Rhinocoris annulatis* L., transmitting NPV to sawflies (Franz *et al.* 1955, Smirnoff 1959). Two predatory mite species are also known to disseminate a microsporidia in bark beetle galleries and fall webworm (*Hyphantrai cunea* (Drury)) nests (Weiser 1957). I report herein the retention of infectivity of 2 species of microsporidia, *Vairimorpha (Nosema) necatrix* (Kramer) and a *Pleistophora* sp., after passage through the gut of the assassin bug, *Zelus exsanguis* (Stål), a predator with piercing-sucking mouthparts.

MATERIALS AND METHODS

General rearing of Z. exsanguis. Egg masses of the assassin bug were collected from oak leaves in a Connecticut forest. After the nymphs hatched, they were fed daily with young larvae of the saltmarsh caterpillar, *Estigmene acrea* (Drury). When the bugs reached the fourth instar, they were reared individually in a plastic Petri dish (60 × 15mm). These bugs were used for the tests.

Infection of hosts. Third instar saltmarsh caterpillars were fed diet contaminated with *V. necatrix* spores at the rate of 300 spores/mm^2 of diet surface and second instars were fed diet contaminated with *Pleistophora sp.* at the rate of 500 spores/mm^2. *V. necatrix* spores were originally isolated from *Spodoptera frugiperda* (J. E. Smith) and the *Pleistophora* sp. from *Dasychira basiflava* (Packard).

Feeding of infected hosts to assassin bug. Microsporidan-infected hosts were killed 12-14 days after exposure to the spores by placing them in hot water (55°C)

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for 2 minutes. The hosts were killed to prevent movement and possible contamination of the surface area of the Petri dish. Prior to placing an infected host with the assassin bug, each bug was transferred into a new Petri dish and starved for 24 hours. After this 24 h period, each bug was placed into a new Petri dish with a dead infected host and feeding observed. After feeding, the bug was transferred to a new dish and fed a healthy live host. Each bug was transferred one or two more times into new dishes, each transfer being made after feeding on a healthy host. Excreta of *Z. exsanguis* were collected from each dish.

Bioassay of spores in excrement. Two kinds of excreta are produced by assassin bugs. One contains primarily uric acid crystals and is whitish in color; the other is primarily fecal material which is dark brown in color. Two feces were scraped with a sterilized scalpel from each dish and placed into 1 ml of sterile water. If feces were not present, then uric acid droppings were scraped. The feces (or uric acid) were collected from starved bugs which served as controls (prior to being fed infected hosts) and from bugs which had been fed on infected hosts after various time intervals. Each suspension was checked microscopically and hemacytometer counts made. Surface of artificial diet in a 24 ml plastic vial (filled with ca 5 ml of media) was contaminated with 0.1 ml of the fecal suspension and air dried. Fourth instar saltmarsh caterpillars were placed individually into each vial for *V. necatrix* tests, while second instars were used for the *Pleistophora* tests. Larvae were maintained at 25° ± 2°C and 18 h photoperiod. Mortality was checked at 8, 10, 12, and 14 days after exposure to the pathogens and the test terminated at the end of 14 days. All larvae were dissected and fat and midgut tissues were examined for microsporidan spores with phase contrast optics.

Results and Discussion

Spores of *V. necatrix* and *Pleistophora* sp. were still infectious after passage through the gut of the assassin bug (Table 1). Nine of 13 bugs in the *V. necatrix* test fed on infected hosts (Table 1). Those which fed had spores in the excreta. These spores were infectious to the test insects. None of the test insects became infected which were fed *Z. exsanguis* excrement collected before the bugs had been exposed to infected hosts.

Excreta deposited during the second collection (see Table 1) from all bugs (except bug 11) which fed upon hosts infected with *V. necatrix* were examined microscopically. Both urine and feces contained spores but a higher frequency was found in the latter (Table 2). Apparently the viability of spores was not adversely affected in urine or feces because test insects fed spores from either source became infected (*i.e.* bug 7, second collection for urine and bugs 8 and 12, second collection for feces).

Excreta of seven out of seven bugs fed *Pleistophora*-infected hosts contained spores (Table 1). However, *Pleistophora* spores were not as infectious as *V. necatrix* spores. For example, spores from bug 16 did not infect any test insect. On the other hand, spores from bugs 15, 18, and 19 infected some of the larvae. Whether there was a loss of viability after passage through the gut remains to be seen.

Microsporidan spores were ingested regardless of which host tissues were infected (*V. necatrix* infects fat tissue, and *Pleistophora* sp. infects midgut tissue). None of the assassin bugs became infected with microsporidia.

Previously, Franz *et. al.* (1955) and Smirnoff (1959) demonstrated the infectivity

TABLE 1. *Isolation of* Vairimorpha necatrix *and* Pleistophora *spores in excreta of* Zelus exsanguis *when fed microsporidan-infected hosts, and infectivity of excreted spores to saltmarsh caterpillars.*

Bug No.	Fed on infected host	1st Collection: Hours post feeding[a]	1st Collection: Spores/ mm²[b]	1st Collection: No. infected/ No. tested	2nd Collection: Hours post feeding[a]	2nd Collection: Spores/ mm²[b]	2nd Collection: No. infected/ No. tested
			Vairimorpha necatrix				
1	–	72	0	0/6	–	–	–
2	+	12	830	6/6	26	831	6/6
3	–	12	0	0/6	–	–	–
4	+	14	1283	6/6	36	1000	6/6
5	+	14	1153	6/6	36	1000	6/6
6	–	35	0	0/6	61	0	0/6
7	+	26	245	6/6	52	471	6/6
8	+	14	104	6/6	52	226	6/6
9	+	12	3210	6/6	26	1300	6/6
10	–	12	0	0/6	72	0	0/6
11	+	50	264	6/6	–	–	–
12	+	14	945	6/6	40	263	6/6
13	+	21	2640	5/6	35	0	0/6
			Pleistophora sp.				
14	+	48	9	0/6	72	0	0/6
15	+	24	396	2/6	72	1347	6/6
16	+	24	377	0/6	72	188	0/6
17	+	24	47	0/6	42	19	0/6
18	+	24	235	6/6	72	19	1/6
19	+	24	377	5/6	72	38	0/6
20	+	24	0	0/6	72	190	0/6

a-Hours postfeeding refers to the deposition of excreta after feeding on an infected host until transfer into a Petri dish (*i.e.* for bug No. 2, excrements deposited between 0-12 hr after feeding on a host were collected for 1st collection, and between 12-26 hr for 2nd collection).

b-Spores/ml can be computed by multiplying spores/mm² by 530 mm² (area of artificial diet surface) and then multiplying product by 10 ml.

TABLE 2. *Occurrence of* Vairimorpha necatrix *spores in urine and feces of* Zelus exsanguis *between 26 and 74 h after feeding on infected hosts* (=2nd collection from Table 1).

No. examined	No. with spores/	
Bug No.	Urine	Feces
1	—	—
2	2/3	1/1
3	—	—
4	1/1	4/5
5	1/7	2/2
6	—	—
7	2/2	0/0
8	0/1	1/1
9	2/3	2/2
10	—	—
11	—	—
12	0/5	1/1
13	0/2	0/1
Totals	8/24	11/13

of NPV after passage through the gut of predators with piercing-sucking mouthparts. I have demonstrated the survival of *V. necatrix* and *Pleistophora* spores after passage through the gut of the predatory bug, *Z. exsanguis*. The importance of predatory insects in the dissemination of pathogens from one locality to another has not been firmly established in natural insect populations. Further studies are needed in this area of insect ecology.

CONCLUSION

The assassin bug, *Zelus exsanguis* (Stål), after feeding on microsporidan-infected hosts, deposits excreta containing spores which are still infectious to test insects. Microsporidan spores were found in urine and feces; a high frequency was found in feces. None of the assassin bugs became infected with the microsporidia.

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