ENTOMOPHAGA 38 (4), 1993, 551-564

INFLUENCE OF *NOSEMA BORDATI* (*MICROSPORIDA : NOSEMATIDAE*) ON THE BIOLOGICAL CYCLE OF *CHILO PARTELLUS* (*LEP : PYRALIDAE*), A STEM BORER OF CULTIVATED GRAMINAE

D. BORDAT (1), A. E. GOUDEGNON (2) & G. BOUIX (3)

(1) Entomology Laboratory, CIRAD/FLHOR, BP 5035, av. du Val de Montferrand, 34032 Montpellier Cedex 1, France
(2) Department of Zoology, FAST/UNB, 526 Cotonou, Benin
(3) Laboratory of Parasitology and Immunology, UM 11, place Eugène Bataillon, 34000 Montpellier, France

The biological cycle of *Chilo partellus* (Swinhoe) was described on artificial diet. From egg to adult, it lasted 32 to 49 days with an average of 36.6 days. About 2,000 larvae from the 2nd to the 5th instars were artificially infected by ingestion with doses of *Nosema bordati* Goudegnon, varying from 2×10^2 to 2×10^7 spores per ml. Only 72 survived (7.66 %) of these infected larvae. *N. bordati*, when present in the larvae, continued to multiply in the resulting pupae. The parasite affected the adults of this Pyralid reducing in a proportion of 5 the productivity of infected females and increasing the production of sterile eggs in the proportion of 8.

KEY-WORDS: rearing, infestation, artificial diet, mortality, immature stages, fecundity.

Chilo partellus (Swinhoe) is the most important pest on maize plant in Comoros archipelago (Bordat, 1982).

From 1973 to 1982, 500,000 adults of *Apanteles flavipes* (Cameron) were introduced to the island of Ngazidja (Comoros archipelago) to reduce the damage caused by this stem borer, without success (Bordat, 1982). In searching the causes of this failure, 5th instar larvae of *C. partellus* were collected from fields on the island of Ngazidja and dissected. They were found to be infected with a Microsporidian, later named *Nosema bordati* Goudegnon. It was then decided to study the influence of this pathogen on its host *C. partellus*. The host biological cycle was first studied. About 2,000 larvae from the 2nd to 5th instar of the Pyralid were infected with doses of Microsporidian varying from 2×10^2 to 2×10^7 spores per ml. The action of the pathogen was studied on these different biological instars of the host and its mode of transmission through the generations was also investigated.

MATERIALS AND METHODS

THE INSECT PEST

Described in 1884 by Swinhoe under the name *Crambus zonellus*, this species had been given several names up to the revision of the genus *Chilo* (Bleszynski, 1970). It was then

renamed *Chilo partellus* (Swinhoe). It belongs to the Order Lepidoptera, Family Pyralidae and Sub-family Crambinae.

According to the C.I.E. (1977), *C. partellus* is encountered in South Asia and East Africa and is in full expansion towards West Africa. In its area of geographic distribution, it attacks principally maize, but it is also found on rice, sorghum, millet and sugar cane (Rahman, 1945 ; Duerden, 1953). It is the major pest of maize in the Comoros archipelago (Bordat, 1983). The larvae used came from the island of Ngazidja. They were reared in the Entomology Laboratory of CIRAD/CA Montpellier.

Rearing was carried out at 25 ± 1 °C, 75 ± 5 % R.H. and a L : 12 D : 12 regime.

For egg-laying, adults were assembled in transparent polystyrene boxes, 290 × 280 × 100 mm, each containing 20 females and 30 males. The cover of the box had two openings. One, 150 mm square, was covered with gauze to ensure aeration ; the other, circular 20 mm diameter, was closed with a rubber stopper and served to introduce the Pyralid adults into the box. The adults were provided with drinking water by means of water-soaked pieces of synthetic sponge, which also served to maintain the required humidity. A band of corrugated paper served as egg-laying surface. The eggs laid in batches were collected each morning and placed for incubation in cylindrical boxes 105 mm diameter and 80 mm high. Incubation lasted 5 days. One day before hatching, the batches were cut and washed for 20 seconds in 20 % sodium hypochlorite solution to disinfect them, then rinsed in distilled water. They were then placed in groups of 20 to 30 eggs in boxes similar to those used for incubation, the bottom of which was covered with 5 to 10 mm of artificial diet. Larval development took place in these air-proof boxes. The last instar larvae (5^{th}), were kept for 10 days at 10 °C to prevent development of the diseases (Bordat *et al.*, 1984).

They were then placed in boxes similar to those used for egg-laying with pieces of artificial diet and rolls of corrugated paper for population.

The composition of the artificial diet is that described by Guennelon & Soria (1973), and modified by Bordat & Pichot (1978).

For the biological cycle study of *C. partellus,* 150 newly-hatched larvae were individually segregated in cyclindrical boxes 50 mm diameter and 15 mm deep containing a piece of artificial diet. Each larva was observed every 24 hours to determine the moulting date. The boxes and the diet pieces were changed every 48 hours until pupation and each pupa was observed up to emergence of the adults.

THE MICROSPORIDIA

With isolated oval spores devoid of a pansporoblastic membrane, *Nosema bordati* belongs to the Order Microsporidia, Family Nosematidae. It was described by Goudegnon in 1985 from 5^{th} instar larvae of *C. partellus* collected from fields in the island of Ngazidja.

To extract the parasite spores, heavily infested 5^{th} instar larvae were crushed in a mortar. The mixture was homogenized in distilled water and filtered several times on cotton to eliminate fragments of host tissue. The filtrate was centrifuged at 2,000 r.p.m., and the pellet of sediment so formed, which contained the spores, was collected and the spore concentration determined using a Thoma blood-count cell. It was then stored in a refrigerator at 4 °C. Spore doses varying from 2×10^2 to 2×10^7 spores per ml of suspension were obtained by dilution of this pellet. They served for all the infections performed during this study. Tests were carried out on the 2^{nd} to 5^{th} instar larvae of *C. partellus.*

The artificial diet was finely chopped and the spore suspension was added in the proportion of 2 ml of water for 10 g of diet. This preparation was carefully mixed to ensure

an even distribution of spores, because the larvae of *C. partellus* feed in galleries. The larvae to be infected were fed on the contaminated diet for 48 hours. Then they were moved onto uncontamined diet which was replaced every 48 hours. The pupae obtained were observed up to emergence of the adults. Dead larvae and pupae which failed to give adults were counted and dissected to check the presence of the parasite. The adults obtained from infected larvae were used for a comparative fecundity study with healthy adults. Thirty groups, each of one female and two males, born from the 5th instar larvae and infected with a dose of 2×10^2 spores per ml of suspension, were constituted and placed in egg-laying boxes similar to those used for egg-incubation. Thirty other groups were formed with healthy adults as a control. The eggs batches were removed every 24 hours. The eggs per female were counted and observed up to hatching. Fertile and sterile eggs were counted. Larvae resulting from infected females were reared to pupation.

The host tissue infected by the parasite was subjected to histopathologic examination. Infected larvae and adults were fixed in Carnoy 2 liquid, cut and colored according to the azan technique of Heidenhain for photonic microscopy. Adult secretions were also examined under the microscope for the presence of pathogen spores. The egg chorion was examined for the presence of parasites using a scanning electron microscope.

TABLE 1

Length of the different larval instar periods of C. partellus, *and percentage of the different instars that pupated*

Larval instars	Length in days	
	Average	Extreme
1st	4	3-10
2nd	3.8	3-6
3rd	2.8	2-4
4th	3.4	2-4
5th	9.6	6-19
	5.6	3-14
6th	8.7	3-14
	3	2-6
7th	0	0
	4	2-8

RESULTS AND DISCUSSION

BIOLOGICAL CYCLE OF *C. PARTELLUS*

The eggs were whitish and flaccid because of their soft chorion. They were 1 mm long and 0,7 mm wide. They were laid by batches of one to 105 eggs with an average of 32 from the 153 counted batches. Bordat (1983) counted one to 208 eggs per batch with an average of 40. These eggs became progressively black as the embryos grew. Hatching occurred 5 days after laying (at 25 °C). Chatterji *et al.* (1968) reported 4 to 8 days, Reddy & Davies (1979) 6 days.

The first and second larval instar periods 3 to 10 days with respective averages of 4 and 3.8 days (table 1), 3rd and 4th instars were completed in 2 to 4 days with respective averages of 2.8 and 3.4 days. Two groups were distinguised among the 5th instar larvae : those that pupated at this instar finished it in 6 to 19 days (average 9.6 days), while those that moulted to the 6th completed in 3 to 14 days (average 5.6 days). An average of 52.5 % of the 150 larvae pupated at the 5th instar, 30 % at the 6th instar. The others, respectively 12.5 % and 5 %, reached the 7th and 8th instar but did not pupate. At 25 °C, the larval period lasted on an average of 23.6 and 29.3 days when pupation occurred at the 5th or 6th instar respectively. Chatterji *et al.* (1968) noted 17 days for the larval period when the larvae were fed on maize stems. Bordat (1983) counted 23.13 and 26.8 days for the larvae that pupated at the 5th and 6th instars respectively, when they were fed on artificial diet.

The pupae were ovoid and milky white when newly formed. They turned progressively brown. Male pupae weighed 58.52 mg on average, being lighter than female pupae at 75.68 mg. The pupal period lasted 8 days at 25 °C. From egg-laying to emergence of the adult, *C. partellus* completed its biological cycle in 36.6 to 42.2 days when pupation occurred at 5th or 6th instars respectively.

Influence of *N. bordati* on the biological cycle of *C. partellus*

The larvae fed on contamined diet moved up and stayed under the cover of the box after 24 hours of feeding, while the control larvae continued to feed. This stop in feeding could be due to the germination of the parasites spores on the gut of the larvae.

The infected larvae began to feed again 24 hours after their transfer onto uncontamined diet, but their feeding became progressively reduced during the course of the experiment. The larvae that survived stopped feeding after 40 days. They were smaller than the control larvae (plate 1, photo 1).

TABLE 2

Cumulative percentages of dead 2nd instar larvae of C. partellus *according to spore quantities of* N. bordati

Number of days after infestation	Cumulative percentages of dead larvae according to spore quantities						
	Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
4	8	0	5.71	12.85	14.28	15.27	18.92
8	8	18.57	22.85	30	17.14	16.67	18.92
12	13.7	18.57	28.57	30	17.14	34.72	21.62
16	13.7	28.57	37.14	30	22.85	36.11	29.72
20	13.7	30	37.14	31.42	27.14	44.44	35.13
24	15.68	32.86	41.42	37.14	27.14	44.44	39.19
28	15.68	37.14	41.42	40	30	47.22	41.89
32	17.64	42.85	47.14	51.43	42.86	55.55	47.29
36	17.64	45.71	51.42	54.28	45.71	59.72	78.38
40	—	57.14	70	90	72.86	73.6	91.89
44	—	72.85	82.88	95.71	85.71	86.11	95.94
48	—	88.57	97.14	100	98.57	94.44	98.65
52	—	95.71	100	—	100	97.22	100
56	—	98.57	—	—	—	100	—
60	—	100	—	—	—	—	—

INFLUENCE OF *N. BORDATI* ON 2nd INSTAR LARVAE OF *C. PARTELLUS*

We observed a rapid increase in larval mortality for all spore doses used from the 4th day after the contamination date (table 2). This first increase was lower than that which occurred after 30 days. There was no significant difference between the action of all spores doses tested.

This was probably due to multiplication of the Microsporidian, which invaded all host tissues (plate 1, photo 3, and 4 ; plate 2, photo 1 to 3) and depleted their food reserves. This could explain the small size of infested larvae (plate 1, photo 1).

This is, however, not the case for all the Microsporidia since *Plistophora coecorum* Chapman & Kellen infected only gastric caeca of *Culiseta inornatus* Newstead (Chapman & Kellen, 1967), while *P. crangoni* Breed & Olson, preferred the skeletal muscles of its hosts (Breed & Olson, 1977), and *Nosema blissi* Liu & Mac Ewen, limited itself to Malpighian tubes of *Blissus leuropterus hirtus* Montandon (Liu & Mac Ewen, 1977).

N. bordati prolonged the larval period of *C. partellus* infected at the 2nd instar to near 60 days while the normal larval life lasted 23.6 to 29.2 days. This could be due to juvenile substances (Maurand & Bouix, 1969).

N. bordati was more virulent when it infected a secondary host. It destroyed 100 % of 5th caterpillars of *Sesamia nonagrioïdes botanephaga* Tams & Bowden infected with a dose of 1.43×10^3 spores/ml in 9 days and 4th stage caterpillars of *Helicoverpa armigera* (Hübner) in 16 days (Goudegnon, 1985). This phenomenon was also observed with *N. manierae*, pathogen of *Chilo zacconius* Bleszynski, which killed 91.9 % of 4th instar larvae of *H. armigera* in 9 days (Toguebaye, 1981).

The virulence of *N. bordati* varies from primary hosts to secondary hosts.

TABLE 3

Cumulative percentages of dead 3rd instar larvae of C. partellus *according to spore quantities of* N. bordati

Number of days after infestation	Cumulative percentages of dead larvae according to spore quantities						
	Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
4	1.75	3.12	10	11.86	—	22.58	
8	3.5	1.75	4.64	10	13.55	3.17	22.58
12	—	1.75	4.64	10	13.55	4.76	22.58
16	—	1.75	4.64	15	13.55	4.76	24.19
20	—	1.75	7.81	16.67	15.25	6.34	25.8
24	—	5.26	12.5	20	20.34	17.46	25.8
28	—	15.79	15.62	30	28.81	26.98	32.26
32	—	19.29	20.31	33.33	37.29	31.74	37.09
36	—	28.07	37.5	41.67	45.76	41.27	48.38
40	—	29.82	42.18	55	54.23	53.96	59.67
44	—	36.84	51.56	58.33	67.8	71.43	66.13
48	—	47.36	56.25	63.33	83.05	76.19	79.03
52	—	52.63	65.62	70	86.44	84.13	88.7
56	—	54.38	65.62	75	91.52	92.06	95.16
60	—	57.89	68.75	88.33	96.61	96.82	100
64	—	61.4	—	—	—	100	—

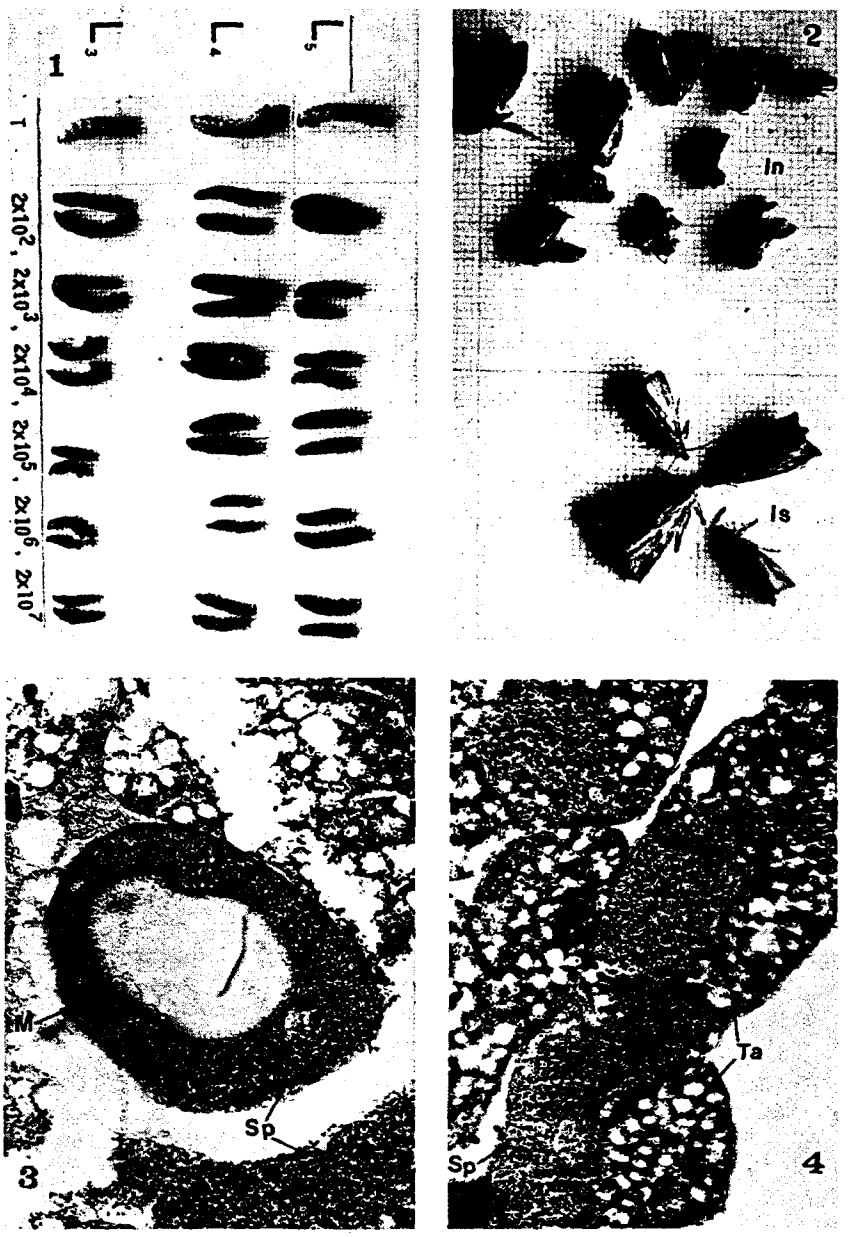

Plate 1. Effects of *N. bordati* on the larvae and adults of *C. partellus*.
Photo 1 : Influence on the size of different instar larvae infected with different spore doses of the pathogen (× 1).
Photo 2 : Deformed adults obtained from larvae infected with spores (× 1.4).
Photo 3 : Infected Malpighian tube (× 420).
Photo 4 : Infected fat tissue (× 420).
In : *Nosema* infected individuals ; Is : healthy individuals ; L3, L4 and L5 : 3rd, 4th and 5th instar larvae ; Sp : spores ; T : control ; Ta : Fat tissue ; tM : Malpighian tube ; 2 × 10^2 to 2 × 10^7 : spore doses.

INFLUENCE OF *N. BORDATI* ON 3rd INSTAR LARVAE OF *C. PARTELLUS*

The larval mortality began slowly and increased rapidly from the 20th day (table 3). It was related to the spore dose used to infect these larvae. There was no significant difference between the effects 2×10^2 and 2×10^3 spores per ml. The effect was also similar to those of 2×10^5 to 2×10^7 spores per ml. As seen in 2nd instar larvae, Malpighian tubes and fat tissue (plate 2, photo 3 and 4), muscles (plate 2, photo 1), gut walls (plate 2, photo 2 and 3), salivary glands etc. were invaded by the parasite.

Pupation occurred much later in the control group (70 days compared to 36.6 days). The number was inversely proportional to the spore doses applied (table 4). Their weight was lower than that of the control group. The average weights were 48.05, 40.60 and 40.50 mg respectively for larvae infected with 2×10^2, 2×10^3 and 2×10^4 spores per ml, while that of the control pupae was 80.20 mg (table 5). Decrease in larval feeding and depletion of the tissue food reserves by the parasite can explain this loss of weight by the infected pupae.

TABLE 4

Mortality induced by different spore doses of N. bordati *in the populations of immature instars of* C. partellus *infected at the 3rd larval instar*

Spore doses		Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
Number of larvae used		57	57	64	60	59	63	62
Dead larval	number	2	35	44	53	57	63	62
	percentage	3.5	61.4	68.75	88.3	96.61	100	100
Dead pupae	number	14	16	16	7	2	—	—
	percentage	28.08	89.48	93.75	100	100	—	—

TABLE 5

Influence of N. bordati *on the weight of pupae resulting from infected larvae of* C. partellus

Spore doses / instar	Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
3rd	80.2	48.05	40.6	40.5	—	—	—
4th	65.72	72.45	57.7	53.96	41.6	28.55	25.81
5th	82.91	67.9	62.73	66.22	47.44	35.31	39.5

All pupae obtained from these 3rd instar larvae infected with 2×10^4 and 2×10^5 spores/ml failed to become adults (table 4). The adults developing from larvae infected with the dose of 2×10^2 and 2×10^3 were abnormal and unable to fly (plate 1, photo 2), they did not mate and laid no eggs. The spore doses ranging from 2×10^4 to 2×10^7 were lethal for the immature instars of the borer when they were infested at the 3rd larval instar.

INFLUENCE OF *N. BORDATI* ON 4th INSTAR LARVAE OF *C. PARTELLUS*

In this test, it was observed that the number of pupae obtained was large despite the infection of the larvae (table 6 and 7). In fact, at doses of 2×10^2 and 2×10^3, the LT 50 was not attained and for doses between 2×10^4 to 2×10^7, it occurred within 35 to 48 days.

Whereas pupation lasted about 20 days in the case of healthy larvae of *C. partellus*, this period was prolonged to 54 days at a dose of 2×10^2 and to 70 days for these infected with a dose of 2×10^5. This prolongation was comparable to that observed in the case of infected larvae of the 2nd and 3rd instars. The average weight of infected pupae was 65.72 mg for control pupae and respectively 62.45, 57.70, 53.96, 41.60, 28.55 and 25.81 mg for pupae obtained from larvae infected with doses ranging from 2×10^2 to 2×10^7 spores/ml (table 5).

TABLE 6

Cumulative percentages of dead 4th instar larvae of C. partellus *according to spore quantities of* N. bordati

Number of days after infestation	Cumulative percentages of dead larvae according to spore quantities						
	Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
4	11.25	6.25	12.79	8.75	10.22	5.75	7.5
8	16.25	7.5	13.95	10	14.77	6.89	17.5
12	17.5	8.75	15.16	10	15.9	11.49	25
16	17.5	16.25	22.09	17.5	18.18	18.39	27.5
20	17.5	18.75	22.09	25	23.86	24.13	30
24	18.75	22.5	23.25	31.25	30.68	27.58	40
28	—	22.5	23.25	33.75	32.95	31.03	40
33	—	25	25.58	36.25	39.77	33.33	42.5
36	—	26.25	25.58	45	45.45	43.67	52.5
40	—	32.5	25.58	45	50	68.96	60
44	—	—	29.07	47.5	63.63	68.96	81.25
48	—	—	32.55	51.25	68.18	80.45	90
52	—	—	32.55	52.5	72.72	81.6	92.5
56	—	—	32.55	52.5	72.72	85.05	92.5
60	—	—	33.72	56.25	72.72	85.05	92.5

This progressive decrease in weight of the infected pupae was the effect of the low feeding of the infected larvae and the exhaustion of the host tissue food reserves by the Microsporidian which invaded all organs of its host (plate 1, photo 3 and 4; plate 2, photo 1 to 3).

The dose of 2×10^2 killed 67.5 % of immature instars of *C. partellus* (table 7), when infected at the 4th instar larval. This percentage increased rapidly with spore doses of 2×10^3 and 2×10^4, killing respectively 79 and 85 % of immature instars which is not significant difference. The effects of doses of 2×10^5, 2×10^6 and 2×10^7 (96.6, 100 and 100 % respectively), were also not significantly different.

INFLUENCE OF *N. BORDATI* ON 5th INSTAR LARVAE OF *C. PARTELLUS*

The dose effect was equally clear in relation to the mortality of infected 5th instar larvae (table 8). The percentage of mortality was not significantly different from that of the

TABLE 7

Mortality induced by different spore doses of N. bordati *on 4th instar larvae of* C. partellus *and in pupae resulting from them*

Spore doses		Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
Number of larvae used		80	80	86	80	88	87	80
Dead larvae	Number	16	26	30	45	64	74	74
	percentage	20	32.5	35	56.3	73	85.1	92.5
Number of pupae obtained		64	54	56	35	24	13	6
Dead pupa	Number	4	28	38	23	21	13	6
	percentage	6.3	51.9	67.9	65.7	87.5	100	100
Number of adults obtained		60	26	18	12	3	—	—
Total percentage mortality		25 a	67.5 b	79 c	58 c	96.6 d	100 d	100 d

$a \neq b \neq c \neq d$ at 5 % (Test of Duncan).

control at the dose of 2×10^2, being 12.7 % and 18.3 % respectively (table 9). There was a small increase at the dose of 2×10^3 (25.3 %) then a big increase at doses of 2×10^4 and 2×10^5, 44.4 and 58.7 % respectively (table 9). The percentage of mortality was very important at doses of 2×10^6 and 2×10^7, being 85 % an 80 % respectively (table 9).

TABLE 8

Cumulative percentages of dead 5th instar larvae of C. partellus *according to spore quantities of* N. bordati

Number of days after infestation	Cumulative percentages of dead larvae according to spore quantities						
	Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
4	2.94	8.53	4.82	11.11	7.5	2.5	16.25
8	7.84	9.76	7.23	12.34	10	2.5	27.5
12	8.82	13.41	7.231	12.34	10	2.5	27.5
16	11.76	13.41	9.64	13.38	11.25	5	33.75
20	11.76	13.41	13.25	13.38	11.25	6.25	33.75
24	11.76	13.41	13.25	13.38	11.25	7.5	38.75
28	12.74	13.41	16.87	18.52	18.75	11.25	42.5
32	—	13.41	16.87	20.99	23.75	15	48.75
36	—	14.63	18.07	28.39	31.25	33.7563.75	
40	—	18.29	34.56	48.75	5.0	72.5	
44	—	—	20.48	34.56	53.75	56.25	72.5
48	—	—	22.89	41.97	55	68.75	75
52	—	—	25.3	44.44	58.75	81.25	80
56	—	—	25.3	44.44	—	85	80
60	—	—	25.3	44.44	—	85	80

There were significant differences between the percentages of pupal mortality induced by the doses of 2×10^3 to 2×10^7. These were respectively 74.2 %, 66.6 %, 87.9 %, 75 % and 68.8 % for the above mentioned doses (table 9). At the lowest dose of 2×10^2, 56.7 % of the pupae did not give adults (table 9). This diversity in the percentages of pupae mortality could be due to the number of spores ingested by the larvae when fed on contaminated diet and the multiplication of the Microsporidian in the pupae.

TABLE 9

Mortality induced by different spore doses of N. bordati *on 5th instar larvae of* C. partellus *and in pupae resulting from them*

Spore doses		Control	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	2×10^7
Number of larvae used		102	82	83	81	80	80	80
Dead larvae	Number	13	15	21	36	47	68	64
	percentage	12.7	18.3	25.3	44.4	58.7	85	80
Number of pupae obtained		89	67	62	45	33	12	16
Dead pupae	Number	14	38	46	27	29	9	11
	percentage	15.7	56.7	74.2	66.7	87.9	75	68.8
Number of adults obtained		75	29	16	18	4	3	5
Total percentage mortality		26.5 a	64.6 b	80.7 c	77.8 c	95 d	93.7 d	d

a ≠ b ≠ c ≠ d at 5 % (Test of Duncan).

As in the case of the 4th instar, the 5th instar infected with a dose of 2×10^5 gave almost no adults ; but when they occurred these adults were unable to fly and laid no eggs (plate 1, photo 2). Only adults derived from the larvae infected with 2×10^2 spores per ml laid eggs. However, 64.6 % of the immature instars infected at this dose died before they reached adult instar.

From the above data, we could conclude that the Microsporidian continued to multiply in the pupae of *C. partellus*. Contrary to *Pliestophora oncoperae* which infected *Oncopera alboguttata* (Walker) without affecting its life cycle (Milner & Lutton, 1980), *N. bordati* provoked high mortality in the population of the immature instars of *C. partellus* irrespective of the dose of spores used. The percentage mortality decreases according to the age at which the larvae were infected. The lower instars, 2nd and 3rd, were more sensitive to parasite infection. The 4th and 5th instars were more resistant to light infection and could give productive adults. It would therefore be more beneficial to infect the youngest instars should *N. bordati* be used to control the populations of *C. partellus*.

INFLUENCE OF *N. BORDATI* ON THE FERTILITY AND PRODUCTIVITY OF ADULT FEMALES OF *C. PARTELLUS*

The control adults laid in total 13,645 eggs with an average of 455 eggs per female (table 10). Infected females produced only 2,829 eggs with an average of 94 eggs/female. *N. bordati* reduced by a factor of 5 the productivity of the infected females.

TABLE 10

Influence of N. bordati *on the productivity of infected adults of* C. partellus

Adults	Number	Average egg number per female	Percentage of sterile eggs
Control	30	455	1.74
Infected	30	94	60.61

A total of 93.36 % of the control eggs hatched (table 10). A total of 60.6 of the eggs laid by infected females were sterile. In all, 1,141 eggs from the infected females had developed into embryos, but only 154 hatched, or 5.44 % of all eggs laid by infected females. Larvae from infected females died before the 3rd instar. Therefore, *N. bordati* affected significantly the productivity of females and fertility of eggs of *C. partellus*. Other Microsporidians produce similar effects on their hosts.

Veber & Jasic (1961) noted that *N. bombycis* Nägeli reduced the fecundity of *Bombyx mori* L. and *Hyphantria cunea* (Drury) when the 4th or 5 th instar larvae were lightly infected with the pathogen. Etienne (1975) observed that microsporidial diseases reduced mating, the number of laid eggs and egg fertility of *Chilo sacchariphagus* (Böjer). High larval and pupal mortalities occurred in populations of *Ostrinia nubitalis* (Hübner) naturally infected by *Perezia pyraustae* Paillot (Frye & Colson, 1974).

TRANSMISSION OF *N. BORDATI* THROUGH ITS HOST GENERATIONS

Eggs laid by infected females showed the presence of the pathogen spores on the chorion (plate 2, photo 5). Newly-hatched larvae infected themselves by ingestion of spores when they emerged. Stained sections of infected female genital organs revealed the pathogen spores in the ovules (plate 2, photo 4). Crushed embryos of eggs laid by these females contained the parasite spores. They newly-hatched larvae could be infected by the ovarian route.

Infected adult secretions contained spores which could contaminate the egg chorion during mating and egg-laying. Transovarian transmission of Microsporidae was frequent. Some generae, parasites of Mosquitos, are transmitted solely by this means (Kellen & Lipa, 1960 ; Kellen *et al.*, 1965 ; Chapman *et al.*, 1970). *Nosema* sp. is transmitted through the generations of *Autheraea assamensis* Helfer (Talukdar, 1980) in the same manner.

CONCLUSION

In the case of *Chilo partellus* (Swinhoe) only lighty-infected larvae could survive infection with *Nosema bordati* Goudegnon. Those heavily infected died before the adult state.

About 2,000 2nd to 5th instar larvae of *C. partellus* were artificially infected by ingestion with doses of *N. bordati*, varying from 2×10^2 to 2×10^7 spores/ml. Only 72 survived, that is 7.66 %. This high mortality was due to the multiplication of the pathogen in all instars of the host.

N. bordati prolonged its host larval period. Pupation occurred at 54 to 70 days against 23 to 20 days for healthy individuals.

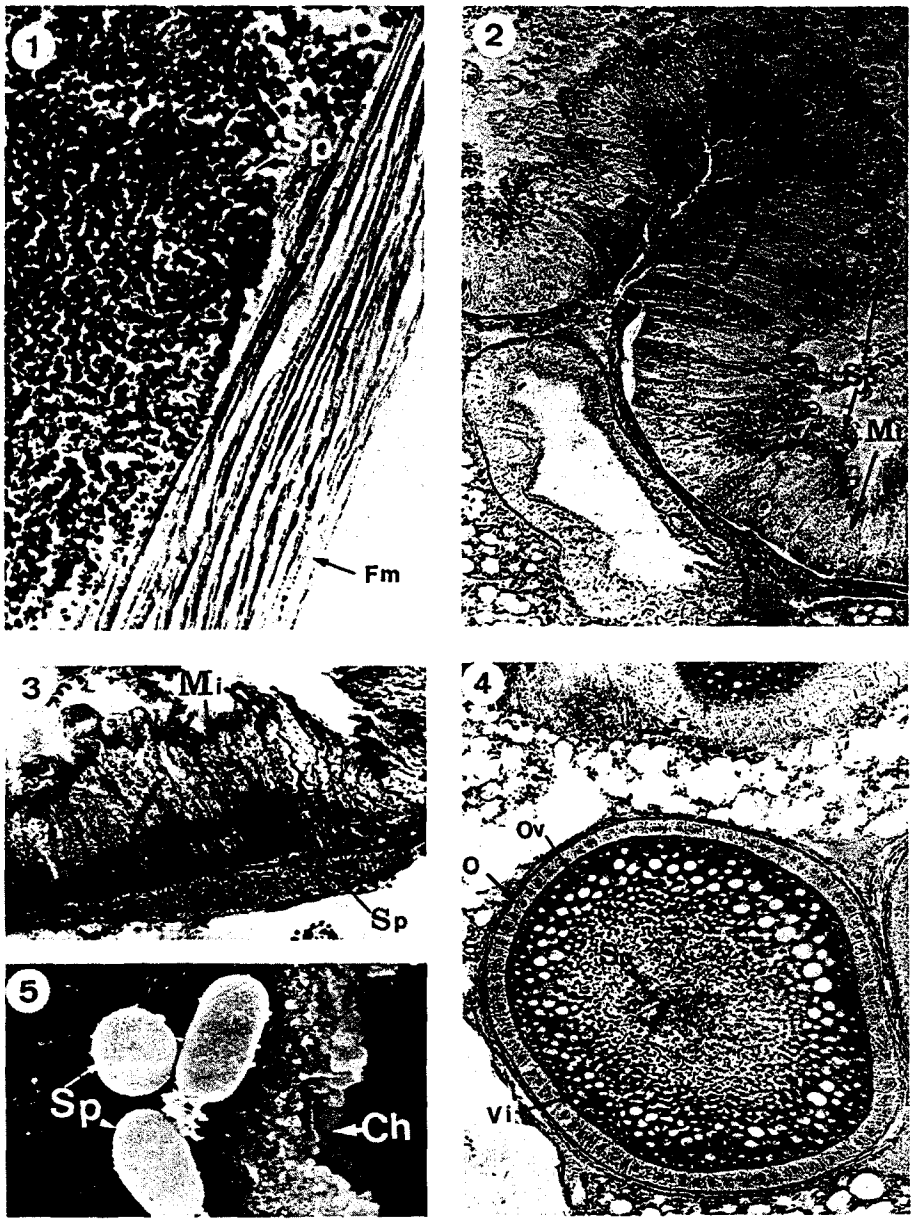

Plate 2. Histopathologic study of *C. partellus* infected with spores of *N. bordati*.
Photo 1 : Infected muscles (× 560).
Photo 2 : Infection of the intestinal mucosa (× 360).
Photo 3 : Infection of the gut wall (× 560).
Photo 4 : Spores in an ovocyte (× 420).
Photo 5 : Spores scanned on the egg envelope (× 8,600).
Ch : egg envelope ; Fm : muscular fibres ; Mi : intestinal mucosa ; O : ovariole ; Ov : ovocyte ; Sp : spores ; Vi : Vitellus.

The Microsporidian equally affected the adults of this Pyralid, reducing by a factor of 5 the productivity of females and increasing the production of sterile eggs by a factor of 8. However, the reproductive ability of females, on average more than 450 eggs per healthy female, allowed the transmission of *N. bordati* from generation to generation, despite the high percentage of sterile eggs laid (60 %).

RÉSUMÉ

Influence de *Nosema bordati (Microsporidia : Nosematidae)* sur le cycle biologique de *Chilo partellus (Lep. : Pyralidae)*

Une infestation expérimentale des chenilles de différents stades de *Chilo partellus* (Swinhoe) par *Nosema bordati* Goudegnon a été menée en laboratoire. Le parasite est très pathogène pour son hôte dont il peut réduire sensiblement les populations dans la nature.

Received : 29 October 1992 ; Accepted : 7 February 1994.

REFERENCES

Bleszynski, S. — 1970. A revision of the world species of *Chilo* Zincken (Lepidoptera, Pyralidae). — *Bull. British Mus. Nat. His. Entomol.*, 25, 4, 99-195.

Bordat, D. et Pichot, M. — 1978. *Chilo zacconius* Blesz. Technique d'élevage sur milieu artificiel et observation sur sa biologie en laboratoire. — *Agron. Trop.*, 33, 4, 337-343.

Bordat, D. — 1982. Introduction à l'île de Ngazidja (Grande Comore) d'*A. flavipes* (Hym., Braconidae) pour lutter contre le foreur du maïs *Chilo partellus* (Swinhoe). — *Rapport de mission, IRAT/Montpellier,* 28 p.

Bordat, D. — 1983. Mise au point de l'élevage de masse d'*Apanteles chilonis* Matsumura, 1912 et d'*Apanteles flavipes* (Cameron, 1881) (Hyménoptère Braconidae) sur trois Lépidoptères Pyralidae foreurs de graminées *Chilo zacconius* Bleszynski, 1970 ; *Chilo partellus* (Swinhoe, 1884) et *Diatraea saccharalis* (Fabricius, 1794) dans un objectif de lutte biologique. — *Thèse de Doctorat d'Université, USTL, Montpellier,* 184 p.

Bordat, D., Coquard, J. & Renand, M. — 1984. Quelques moyens de lutte pour enrayer les nosémoses de trois foreurs des graminées, élevés en laboratoire sur milieu nutritif artificiel. — *Agron. Trop.*, 39, 3, 275-285.

Breed, G. M. & Olsen, R. E. — 1977. Biology of the Microsporidian parasite *Pleistophora crangoni* n. sp. in three species of Crangonid and Shrimps. — *J. Invert. Pathol.*, 30 : 387-405.

Chapman, H. C. & Kellen, W. R. — 1967. *Plistophora coecorum* sp. n., a Microsporidian of *Culiseta inornata* (Diptera, Culicidae) from Louisiana. — *J. Inv. Pathol.*, 9, 500-502.

Chapman, H. C., Clark, T. B. & Peterson, J. J. — 1970. Protozoans, Nematodes and viruses of anophelines. — *Entomol. Soc. Amer.*, 7, 134-139.

Chatterji, S. M., Siddiqui, K. H., Panwar, V. P. S., Sharma, G. C. & Young, W. R. — 1968. Rearing of maize stem borer *C. zonellus* on artificial diet. — *Indian J. Entomol.*, 30, 8-12.

Commonwealth Institute of Entomology, London. — 1977. *Distribution maps of insect pest.* — Serie 1, Map n° 184.

Duerden, J. C. — 1953. Stemborers of cereals crops at Kongwe Tanganika. — *East Afr. Agric. J.*, 19, 105-119.

Etienne, J. — 1975. Lutte biologique aux Comores. — *IRAT/Comores, Rapport de mission,* 19-25.

Frye, R. D. & Colson, L. C. — 1974. Fecundity and survival in populations of the European corn borer infected with *Perezia pyraustae.* — *J. Inv. Pathol.*, 24, 378-379.

Goudegnon, A. E. — 1985. Le foreur ponctué des graminées, *Chilo partellus* (Swinhoe, 1884) (Lepidoptera : Pyralidae) et ses parasites, *Apanteles flavipes* (Cameron, 1891) (Hymenoptera : Braconidae) et *Nosema bordati* n. sp. (Microsporida : Nosematidae), cycle de développement et inter-relations. — *Thèse de 3e cycle, USTL, Montpellier,* 230 p.

Guennelon, G. & Soria, F. — 1973. Mise au point au laboratoire d'un élevage permanent de la pyrale du riz *Chilo suppressalis* sur milieu artificiel. — *Ann. Zool. écol. anim.,* 5, 547-558.

Kellen, W. R. & Lipa, J. J. — 1960. *Thelohania californica* n. sp., a Microsporidian parasite of *Culex tarsalis* Coquillett. — *J. Insect Pathol.,* 2, 161-166.

Kellen, W. R., Chapman, H. C., Clark, T. B. & Lindegren, J. E. — 1965. Host parasite relationships of some *Thelohania* (Microsporidia, Nosematidae) from Mosquitoes. — *J. Inv. Pathol.,* 7, 161-166.

Liu, H. S. & Mac Ewen, F. L. — 1977. *Nosema blissi* n. sp. (Microsporida, Nosematidae) a pathogen of the Chink Bug *Blissus leucopterus hirtus* (Hemiptera, Lygaeidae). — *J. Inv. Pathol.,* 29, 141-146.

Maurand, J. & Bouix, G. — 1969. Mise en évidence d'un phénomène secrétoire dans le cycle de *Thelohania fibrata* (Strickland, 1913) Microsporidie parasite des larves de *Simulium.* — *C. R. Acad. Sci.,* 269, 2216-2218.

Milner, R. J. & Lutton, G. G. — 1980. Interaction between *Oncopera alboguttata* (Lepid. Hepialidae) and its Microsporidian Pathogen *Pleistophora oncoperae* (Protozoa, Microsporida). — *J. Inv. Pathol.,* 36, 198-202.

Rahman, K. A. — 1945. Biology and control of maize and jawar borer *C. zonellus.* — *Indian J. Agr. Sci.,* 14, 303-307.

Reddy, K. V. S. & Davies, J. C. — 1979. A new medium for mass rearing of sorghum borer *C. partellus* (Swinhoe), Lep. Pyralidae, and its use in resistance screening. — *Indian J. Plant. Prot.,* 6, 48-55.

Talukdar, J. N. — 1980. Prevalence of transovarian infection of Microsporidian parasite infesting Muga silkworm : *Antheraea assamensis.* — *J. Inv. Pathol.,* 36, 273-280.

Toguebaye, B. S. — 1981. Etude de l'infestation expérimentale d'*Heliothis armigera* (Hübner, 1808) (Lepidoptera, Noctuidae) par *Nosema manierae* n. sp. Microsporidie parasite de *Chilo zacconius* Bleszynski, 1970 (Lepidoptera, Pyralidae). — *Thèse de Doctorat de 3e cycle, USTL, Montpellier,* 261 p.

Veber, J. & Jasic, J. — 1961. Microsporidia as a factor in reducing the fecundity in insects. — *J. Insect pathol.,* 3, 103-111.