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A Microsporidian Pathogen of the Poroporo Stem Borer, *Sceliodes cordalis* (Dbld) (Lepidoptera:Pyralidae)

I. Description and Identification

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A microsporidian pathogen of the poroporo stem borer, *Sceliodes cordalis*, is described in terms of its developmental cycle, morphology, and host specificity. The characteristics of the pathogen, a *Nosema* species, were indistinguishable from 11 other published descriptions on the basis of both light and electron microscopic studies. Six out of seven other lepidopteran species became infected when dosed with spores.

KEY WORDS: Lepidoptera; Pyralidae; *Sceliodes cordalis;* Microsporidia; Nosematidae; *Nosema;* pathogen.

INTRODUCTION

The poroporo stem borer, *Sceliodes cordalis*, occurs in New Zealand and Australia where it has been recorded as a pest of eggplants, *Solanum melongena L.* (Davis, 1964) and pepino, *Solanum muricatum* Aiton (Martin, 1983). In New Zealand its natural host is the native shrub poroporo, *Solanum aviculare* Forst. f. and *Solanum laciniatum* Ait. which has been grown commercially for the extraction of the steroid precursor solasadine. In poroporo, larvae feed mainly within the berries but are also found in young shoots where they cause the tips to die back. Galbreath and Clearwater (1983) described pheromone trapping and Dhana (1984) described a nuclear polyhedrosis virus from larvae.

In this series of papers the effects of the microsporidian pathogen on *S. cordalis* are quantified as a basis for future work on the population dynamics of pest population. This paper describes the morphology, life cycle, and host specificity of the microsporidian and discusses its status as a possible new species within the genus *Nosema*. In the succeeding papers the interaction between the host and its parasite population is quantified in terms of the relationships between spore dose and host mortality (Mercer and Wigley, 1987a) and in surviving individuals, the effects of infection on reproductive success and the relationship between the intensity of these effects and parasite density (spore loads) (Mercer and Wigley, 1987b). The microsporidian is referred to from here as "NSC."

MATERIALS AND METHODS

A disease free, laboratory colony of *S. cordalis* was derived from three uninfected females which emerged from 77 prepupae collected at Pukekohe, South Auckland, New Zealand, on 9 October 1979. Eggs were routinely surface sterilized in 0.5% v/v sodium hypochlorite solution (10 min)

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and then rinsed in running tap water (10 min). Newly hatched larvae were transferred individually to a freshly cut flat surface at the apex of a surface sterilized poroporo fruit (0.25% v/v sodium hypochlorite, 20 min; running tap water, 20 min). Fruit, supported in ice-cube trays, were incubated at 25°C and 100% relative humidity. After 24 hr fruit were examined to determine whether larvae had successfully penetrated the cut surface and entered the fruit. Infested fruit were placed singly in plastic pots and incubated at 25°C in darkness. Pot lids were left partially open for ventilation. After 10–14 days cocoons were transferred to tubes and incubated at 25°C in darkness to prevent the onset of diapause. Females were weighed within 18 hr of emergence; each was paired with a freshly emerged male and placed in a pot lined with clear plastic film to act as an oviposition surface. Dates of emergence, oviposition, death, and egg hatch were recorded. Adults were frozen within 24 hr of death for later dissection and spore counts.

Spore suspensions were prepared from infected adults collected at Pukekohe, South Auckland, New Zealand. Adults were placed in an everted vinyl glove finger containing 1 ml of distilled water and pulverized to release the spores. After filtration through two layers of muslin the crude extract was centrifuged at 1500 rpm for 7 min. The supernatant was then discarded, the pellet resuspended, and the centrifugation repeated. The resuspended pellet gave a spore suspension suitable for per os infection of larvae. Spore concentrations were estimated both by a hemacytometer and a stained dried film technique (Wigley, 1980).

Larvae were infected by allowing them to feed individually on a freshly cut surface at the apex of a poroporo fruit onto which a known volume of spore suspension had been evenly applied. Larvae were enclosed over the moist, contaminated fruit surface within a length of clear plastic tubing attached to the fruit and sealed with pieces of plastic dough. Tubes were removed from the fruit 24 hr later, by which time larvae had burrowed inside, and the fruit placed in pots. Four hundred larvae from healthy adults were reared to third instar and 300 of these were fed with a suspension of 9.8×10^7 spores/ml. One hundred control larvae were fed with distilled water. Only larvae feeding within 12 hr were used. At 12-hr intervals for the first 96 hr (from the time of dosing) samples of three larvae were prepared for microscopic examination. After 96 hr all individuals had pupated and, from this time, three individuals were taken every 24 hr. Two smears were prepared from each sampled larva: one from the gut, other from the remaining tissues. Pupae and adults were not dissected but were broken open with fine forceps and repeatedly dabbed across a slide. Smears were fixed in absolute ethanol for 2 min, air dried, and then stained in 10% (v/v) Giemsa's stain (Gurr's Improved R66) in 0.02 M phosphate buffer (*p*H 6.9) for 45 min. Every 24 hr from the beginning of the experiment an additional sample of three individuals was removed and fixed in Bouin's fluid, embedded in 54°C melting point wax, sectioned at 5 μm, and stained in Giemsa's stain.

Each smear was examined at 250× and 900× (oil immersion) magnification. The morphology of stages and the time at which each stage first appeared were noted. Changes in the relative numbers of life stages were assessed by a series of sample counts from the stained smears. At each count, the number of each stage seen in a randomly chosen field of view at 900× magnification was recorded. Counting continued until more than 70 microsporidian cells were recorded or 30 min had elapsed.

Spores were measured in water mounts (Vavra and Maddox, 1976) with an ocular micrometer. Polar filaments were extruded by an alternate wetting and drying technique (Vavra and Maddox, 1976) and measured after staining, with an ocular micrometer.

To test host specificity larvae of seven species (Table 2) were fed on artificial diet (Singh, 1977) spread with a suspension of NSC spores. Smears or wet mounts of dead larvae and adults were examined for evidence of infection.

The developmental cycle of NSC in *Epiphyas postvittana* could not be distinguished from that seen in *S. cordalis* and *E. postvittana* was used in the electron microscopy study. Newly hatched larvae of *E. postvittana* were infected with NSC by feeding them individually on plugs of an artificial diet on to which 10 μl of spore suspension had been applied. After 10 days larvae were removed and the guts were dissected out in Karnovsky's fixative at room temperature. Ten minutes later, specimens were transferred to fresh fixative at 4°C for 1 hr. They were then washed twice in 0.12 M sodium cacodylate buffer, *p*H 7.4, and postfixed in 2.5% (w/v) osmium tetroxide in 0.1 M cacodylate buffer for 1 hr at 4°C. Two subsequent washes in 0.1 M sodium acetate were followed by en bloc staining in 0.25% (w/v) aqueous uranyl acetate for 1 hr at 4°C, and two more washes in 0.1 M sodium acetate. Specimens were then dehydrated in an alcohol series at room temperature and embedded in Spurr's resin. Sections were poststained in saturated uranyl acetate in 70% alcohol and Reynolds lead citrate.

RESULTS

Morphology and Development

The first stages were two binucleate meronts seen in a gut smear of *S. cordalis* made 48 hr after infection. By 60 hr, these binucleated meronts were numerous. Tetranucleate meronts first appeared at 72 hr and the first cleaving meronts were observed at 84 hr. Meronts were typically rounded in outline with closely apposed, deeply staining, diplokaryotic nuclei (Fig. 1). The cytoplasm stained a characteristic even blue color. Division of tetranucleate stages followed an elongation of the cell, a migration of the nuclei towards the poles, and constriction of the cytoplasm in the center (Figs. 2, 3).

Sporonts first appeared 72 hr after infection when two binucleate oval cells were observed. At 84 hr, both fusiform and oval binucleate sporonts were common (Fig. 4) and tetranucleate forms in various stages of cytoplasmic cleavage and nuclear division were seen (Figs. 5, 6). In some cases the cytoplasm was vacuolated and unevenly stained with irregular nuclei (Figs. 5, 6).

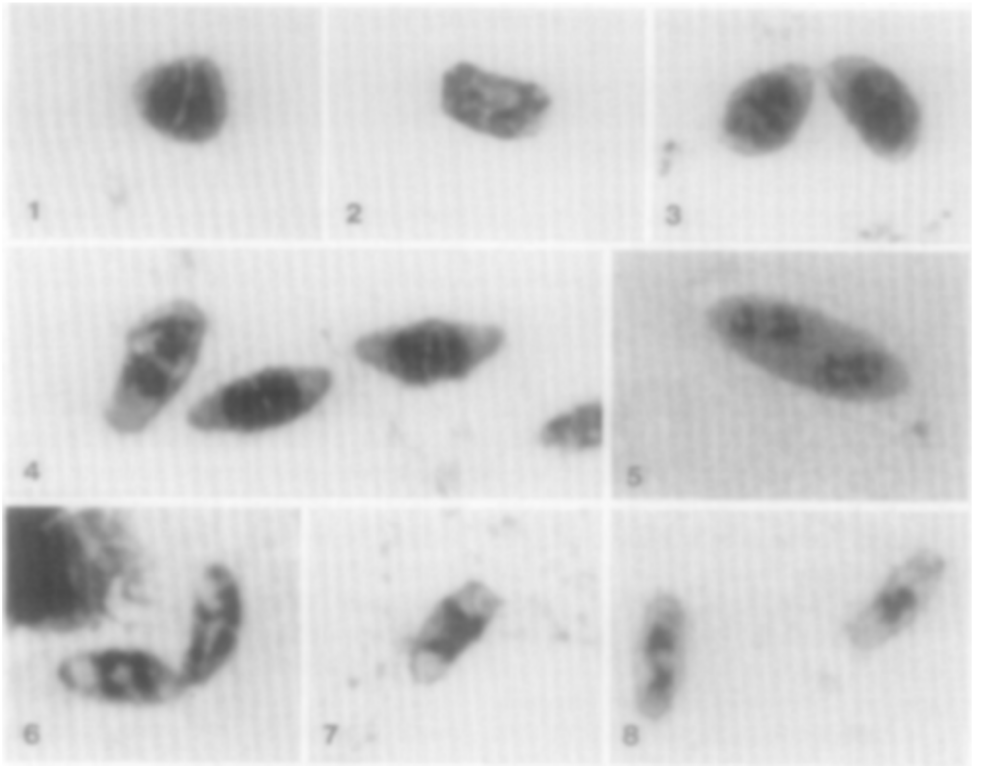
Sporoblasts and spores were first detected at 108 hr. Nuclei of sporoblasts were condensed and deeply staining. The cell outline varied from elongate and somewhat twisted to ovocylindrical. Sporoblasts ultimately developed into ovoid forms with condensed, closely apposed nuclei (Figs. 7, 8). The nuclei and cytoplasm of young spores stained deeply whereas mature spores only took up stain in a small terminal portion. Spores were ovoid to ovocylindrical and binucleate. No stages were found in control smears.

The proportions of each stage in relation to the total stages observed at each sample time are presented in Fig. 9. The proportions of meronts and sporonts initially peaked then gradually declined. Spores remained at a low level until 180 hr and then accumulated rapidly to comprise over half the stages seen.

The time taken for development from the spore phase to the spore phase of the next generation (Kramer, 1965) was 108 hr at 25°C. The interval elapsing before spores comprised 50% of the stages seen in a sample (Milner, 1972) was 180 hr (7.5 days).

Electron Microscopy

Meronts contained two or four nuclei in diplokaryotic arrangement (Figs. 10, 11). They were surrounded by simple unit membranes in direct contact with the host gut cell cytoplasm. The meront cytoplasm contained numerous ribosomes and the cysternae of rough endoplasmic reticulum.



FIGS. 1–8. Light micrographs of *Nosema* sp. (NSC) developmental stages from *Sceliodes cordalis*. Methanol fixed, Giemsa stained. ×4175. Fig. 1. Binucleate meront. Fig. 2. Early tetranucleate meront. Fig. 3. Cleaving tetranucleate meront. Fig. 4. Sporonts. Fig. 5. Non-vacuolated tetranucleate sporont. Fig. 6. Vacuolated cleaving tetranucleate sporonts. Fig. 7. Sporoblast. Fig. 8. Immature binucleate spores.

Sporont morphogenesis was characterised by the deposition of a layer of electron dense material in patches on the outer surface of the plasmalemma (Fig. 12). Host cell cytoplasm was detached in these areas of deposition and, as the patches of thickening progressively coalesced to form a continuous outer layer, the mature sporont came to lie in a vacuole in the host cell cytoplasm (Fig. 13). The differentiation of sporoblasts into spores was difficult to follow but was accompanied by the formation of parallel layers of rough endoplasmic reticulum, the morphogenesis of polar filaments and a further thickening of the outer wall (Fig. 14). Spores contained two nuclei in the diplokaryotic arrangement and a polar filament that was coiled between 7 and 14 times (Fig. 15).

Histopathology

NSC produced no obvious internal or external macroscopic signs of infection. Nearly all larval and adult tissues were subject to infection. The most heavily infected tissues were the fat body, gut, and malpighian tubules. Infection also occurred in cuticular epithelium, muscle, brain, optical, and reproductive tissues. In the female, spores were observed in the oocyte nurse cells, immature oocytes, and in the yolk of mature oocytes. In the male, spores were seen in muscular tissue surrounding the ejaculatory duct, in the epithelial cells and body of the testis and among the stored spermatozoa.

Spore and Polar Filament Dimensions

The mean size of NSC spores collected

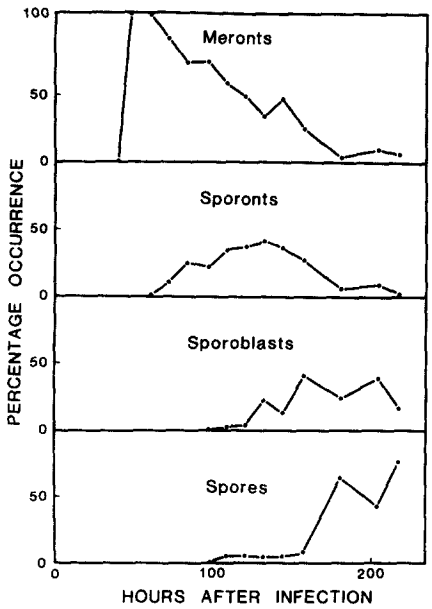


FIG. 9. Changes in the proportions of life stages of *Nosema* sp. (NSC) during development. Combined data from gut smears and "other tissues" smears. The proportions of each stage present at a particular time after the initial exposure to spores is given as a percentage of the total number of stages seen at that time.

at Pukekohe was 4.11 × 2.05 μm (range 2.93–5.26 × 1.51–2.73). An analysis of dimensions of spores from six adult *S. cordalis* is given in Table 1. Macrospores were occasionally seen and their stained appearance was similar to that of normal spores except that they were about twice as long (7.9 × 2.5 μm, fresh preparation).

Extrusion of polar filaments by mechanical pressure was unsuccessful, as was mixing a drop of spore suspension with final instar vomitus. The rehydration of an air-dried spore suspension was the most successful technique but even with this method few spores extruded their polar filaments and most of those which did were broken or only partially extruded. It therefore remains uncertain whether the maximum extruded length was recorded. Only three polar filaments appeared to be fully extruded and undamaged; their lengths were 79, 82, and 107 μm.

Infectivity to Other Lepidopteran Species

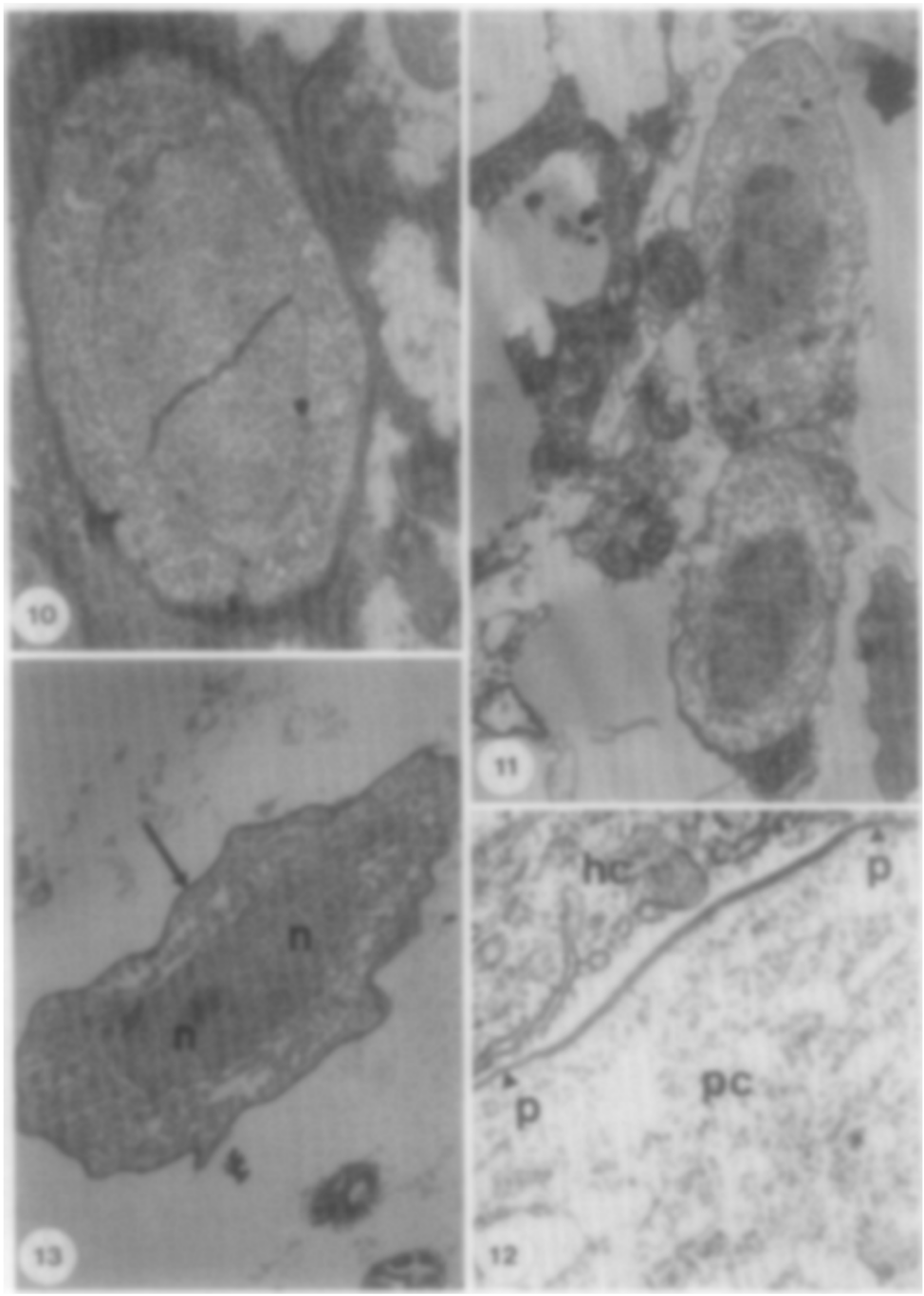
Six out of seven lepidopteran species fed NSC spores became infected (Table 2). NSC was infective but avirulent to *Galleria mellonella* (50% infected), but killed all infected larvae of *Agrotis ipsilon aneituma* (33% infected). Intermediate effects were noted for the other host species.

DISCUSSION

The results indicate that NSC should be assigned to the genus *Nosema* Naegeli, 1857, as it has the following characters in common with the type species *Nosema bombycis* Naegeli, 1857: monomorphic development, binucleate ovoid spores, a predominance of diplokaryotic nucleii in merogony and sporogony, two binucleate sporoblasts arising by binary fission from each sporont, and an absence of sporophorous vesicles.

NSC was compared with those species of *Nosema* considered valid by Thomson (1960) and Weiser (1961) and with those described since their reviews. The review papers by Nordin and Maddox (1974) and Wanatabe (1976) were useful in finding some descriptions. In many cases, the original description was not consulted but use made instead of the annotated list of species in Sprague (1977). Thirty-three descriptions of *Nosema* infecting lepidopteran species were found with sufficient data to make comparisons with NSC possible. Twenty-one of these were considered to be distinct from NSC because of differences in the development of vegetative stages or because of non-overlapping spore sizes or contrasting spore shapes. The wide host range of NSC precluded the use of host specificity as a descriptive character.

The remaining 12 species, listed in Table 3, cannot be distinguished from each other or from NSC on the basis of published descriptions. Direct comparisons of these species and NSC are needed before proper identification of NSC can be made.



FIGS. 10–13. Electron micrographs of *Nosema* sp. (NSC) developmental stages from the gut of *Epiphyas postvittana*. Fig. 10. Meront with diplokaryotic nuclei. ×20,000. Fig. 11. Dividing tetranucleate meront with cytoplasmic cleavage almost complete. ×8000. Fig. 12. Detail of thickening of the sporont wall. Detachment of host cell cytoplasm (hc) from parasite cytoplasm (pc) accompanies the deposition of a layer of electron dense material on the outer surface of the plasmolemma (p). Fig. 13. Binucleate sporont showing thickened sporont wall (arrow). n (nuclei). ×20,000.

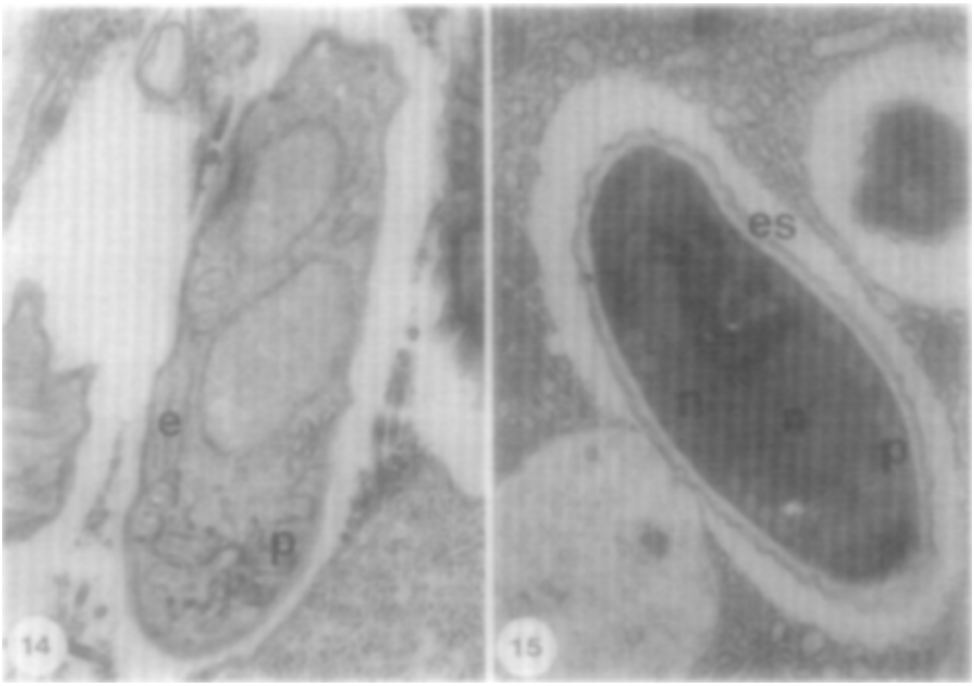


FIG. 14. Electron micrograph of a binucleate sporoblast exhibiting morphogenesis of the polar filament. p (polar filament). e (endoplasmic reticulum). ×20,000.

FIG. 15. Longitudinal secton through a mature spore showing nuclei (n), coiled polar filament (p), and rippled exospore (es). ×20,000.

TABLE 1

MEASUREMENTS (μm) OF *Nosema* SP. (NSC) SPORES FROM SIX *Sceliodes cordalis* MOTHS AT PUKEKOHE

Moth number	Length				Width			
	N	Mean ± SD[a] (95% confidence interval)		Range	*N*	Mean ± SD[a] (95% confidence interval)		Range
9	33	3.74 ± 0.58 (3.64–3.84)	A[b]	2.93–4.52	33	2.06 ± 0.16 (2.01–1.86)	B	1.75–2.36
11	33	3.93 ± 0.41 (3.86–4.00)	AB	3.01–4.73	33	1.81 ± 0.15 (1.76–1.86)	A	1.51–2.28
3	33	4.11 ± 0.38 (3.98–4.24)	B	3.42–4.97	33	2.06 ± 0.16 (2.01–2.11)	B	1.79–2.36
1	33	4.15 ± 0.36 (4.03–4.27)	BC	3.38–4.97	33	2.37 ± 0.15 (2.32–2.42)	C	2.04–2.73
19	33	4.42 ± 0.35 (4.36–4.48)	C	3.70–5.17	33	1.96 ± 0.16 (1.91–2.01)	B	1.63–2.32
7	33	4.46 ± 0.33 (4.35–4.57)	D	3.91–5.26	30	2.02 ± 0.21 (1.94–2.10)	B	1.67–2.49

[a] Standard deviation of the mean.

[b] Values without a letter in common differ significantly at $p < 0.05$ (least-significant difference, Studentized range).

TABLE 2
INFECTION OF OTHER LEPIDOPTERAN SPECIES WITH *Nosema* SP. (NSC)

Insect	Percentage infected	Sample size
Noctuidae		
Agrotis ipsilon aneituma (Walker)	33	42
Chrysodeixis eriosoma (Doubleday)	71	17
Helicoverpa armigera conferta (Walker)	73	15
Mythimna separata (Walker)	0	28
Tortricidae		
Ctenopseustis obliquana (Walker)	83	36
Epiphyas postvittana (Walker)	25	32
Galleriidae		
Galleria mellonella (L.)	50	36

An interesting aspect of this study was the difference in the mean dimensions of spores between individual moths. Several factors have been shown to influence the size of microsporidian spores. Spore size may be altered by host age, the tissues in which the spores develop, the treatment of the tissue after removal from the host, and the medium in which the spores are measured (Blunck, 1954; Walters, 1958). Malone and Wigley (1981) showed that the spore size of *Nosema carpocapsae* differed between localities and from a single locality after four passages through laboratory reared codling moth. Studies with NSC show that spore dimensions can vary significantly even between individual moths and show the importance of taking a large sample of host individuals for reporting spore size. We support the conclusion of Nordin and Maddox (1974) that spore size is an important characteristic for describing microsporidian species but urge that the number of sources of host individuals and full statistics be reported for spore measurements.

Permanent slides have been deposited in the New Zealand National Protozoan Type Collection at Entomology Division, Department of Scientific and Industrial Research, Mount Albert Research Centre, Auckland, New Zealand.

TABLE 3
Nosema SPECIES FROM LEPIDOPTERA WHICH ARE INDISTINGUISHABLE FROM *Nosema* SP. (NSC)

Name and author	Reference
N. aporivora Veber, 1957	Veber, 1957
N. bombycis Naegeli, 1857	Ishihara, 1969
N. cactoblastis Fantham, 1939	Fantham, 1939
N. cactorum Fantham, 1939	Fantham, 1939
N. destructor Steinhaus and Hughes, 1949	Steinhaus and Hughes, 1949
N. disstriae (Thomson, 1959)	Thomson, 1959
N. heliothidis Lutz and Splendore, 1904	Kramer, 1959
N. lotmarae Weiser, 1961, emend.	Weiser, 1961
N. manierae Toguebaye and Bouix, 1983	Toguebaye and Bouix, 1983
N. phryganidiae Lipa and Martignoni, 1960	Lipa and Martignoni, 1960
N. sp. (NGL) Watanabe, 1976	Watanabe, 1976
N. sphingidis Brooks, 1970	Brooks, 1970

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REFERENCES

BLUNCK, H. 1954. Mikrosopridien bei *Pieris brassicae* L. ihren Parasiten and Hyperparasiten. *Z. Agnew. Entomol.* 36, 316–333.

BROOKS, W. M. 1970. *Nosema sphingidis* sp.n., a microsporidian parasite of the tobacco hornworm, *Manduca sexta*. *J. Invertebr. Pathol.* 16, 390–399.

DAVIS, J. J. 1964. The egg fruit caterpillar. *Qld. Agric. J.*, **90**, 76–78.

DHANA, S. 1984. A nuclear polyhedrosis virus of the poroporo stem borer. *New Zealand Entomol.*, **8**, 67–68.

GALBREATH, R. A., AND CLEARWATER, J. R. 1983. Pheromone monitoring of *Sceliodes cordalis*, a pest of pepino. Proceedings, *New Zealand Weed Pest Control Conf.*, **36**, 128–130.

FANTHAM, H. B. 1939. *Nosema cactoblastis*, sp. n., and *Nosema cactorum*, sp. n., microsporidian parasites of speices of *Cactoblastis* (Lepidoptera) destructive to prickly pear. *Proc. Zool. Soc. Lond.*, **108**, 689–705 + Pl. 1.

ISHIHARA, R. 1969. The life cycle of *Nosema coliadis* as revealed in tissue culture cells of *Bombyx mori*. *J. Invertebr. Pathol.*, **14**, 316–320.

KRAMER, J. P. 1959. On *Nosema heliothidis* Lutz and Splendor, a microsporidian parasite of *Heliothidis zea* (Boddie) and *Heliothis virescens* (Fabricius) (Lepidoptera, Phalaenidae). *J. Insect Pathol.*, **1**, 297–303.

KRAMER, J. P. 1965. Generation time of the microsporidian *Octosporea muscaedomesticae* Flu in adult *Phormia regina* (Meigen) (Diptera, Calliphoridae). *Z. Parasitenkd*, **25**, 309–313.

LIPA, J. J., AND MARTIGNONI, M. E. 1960. *Nosema phrysanidia* n. sp., a microsporidian parasite of *Phrysanidia californica* Packard. *J. Insect Pathol.*, **2**, 396–410.

MARTIN, N. A., 1983. Identifying pepino pests. *Hort. News*, **5**, 18–19.

MALONE, L. A., AND WIGLEY, P. J. 1981. The morphology and development of *Nosema carpocapsae*, a microsporidian pathogen of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) in New Zealand. *J. Invertebr. Pathol.*, **38**, 315–329.

MERCER, C. F., AND WIGLEY, P. J. 1987a. A microsporidian pathogen of *Sceliodes cordalis*. II. Mortality and infectivity. *J. Invertebr. Pathol.*, **49**, 102–107.

MERCER, C. F., AND WIGLEY, P. J. 1987b. A microsporidian pathogen of *Sceliodes cordalis*. III. Effects on natality. *J. Invertebr. Pathol.*, **49**, 108–115.

MILNER, R. J. 1972. *Nosema whitei*, a microsporidian pathogen of some species of *Tribolium*. I. Morphology, life cycle, and generation time. *J. Invertebr. Pathol.*, **19**, 231–238.

NORDIN, G. L., AND MADDOX, J. V. 1974. Microsporida of the fall web-worm, *Hyphantria cunea*. I. Identification, distribution, and comparison of *Nosema* sp. with similar *Nosema* spp. from other Lepidoptera. *J. Invertebr. Pathol.*, **24**, 1–13.

SINGH, P. 1977. "Artificial Diets for Insects, Mites and Spikers," 594 pp. IFI/Plenum Data Co., New York.

SPRAGUE, V. 1977. Classification and Phylogeny of the Microsporidia, *In* "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, eds.), Vol. 2, pp. 1–30. Plenum, New York/London.

STEINHAUS, E. A., AND HUGHES, K. M. 1949. Two newly described species of microsporidia from the potato tuberworm, *Gnorimoschema operculella* (Zeller) (Lepidoptera, Gelechiidae). *J. Parasitol.*, **35**, 67–75.

THOMSON, H. M. 1959. A microsporidian parasite of the forest tent caterpillar, *Malacosoma disstria* Hbn. *Canad. J. Zool.*, **37**, 217–221.

THOMSON, H. M. 1960. A list and brief description of the microsporidia infecting insects. *J. Insect Pathol.*, **2**, 346–385.

TOGUEBAYE, B. S., AND BOUIX, G. 1983. *Nosema manierae* sp.n., a microsporidian parasite of *Chilo zacconius* Blezenski 1970 (Lepidoptera: Pyralidae), the natural host, and *Heliothis armigera* (Hubner 1808) (Lepidoptera: Noctuidae), experimental host: developmental cycle and ultrastructural. *Z. Parasitenkd.*, **69**, 191–205.

VAVRA, J., AND MADDOX, J. V. 1976. Methods in Microsporidiology. *In* "Comparative Pathobiology" (L. A. Bulla and T. C. Cheng, eds.), Vol. 1, pp. 281–319. Plenum, New York/London.

VEBER, J. 1957. *Nosema aporivora* n.sp., dalsi parasit belaska ovocneno (*Aproia crataegi* L.). *Vestn. Cesk. Spol. Zool.*, **21**, 187–188.

WALTERS, V. A. 1958. Structure, hatching, and size variation of the spores in a species of *Nosema* (Microsporidia) found in *Hyalophora cecropia* (Lepidoptera). *Parasitology*, **48**, 113–130.

WATANABE, H. 1976. A *Nosema* species of the Egyptian cotton leafworm, *Spodoptera litura* (Lepidoptera): Its morphology, development, host range and taxonomy. *J. Invertebr. Pathol.*, **28**, 321–328.

WEISER, J. 1961. Die mikrosporidien als parasiten der insekten. *Monogr. Angew. Entomol.*, **17**, 149 pp.

WIGLEY, P. J. 1980. Counting micro-organisms. *In* "Microbial Control of Insect Pests" (J. M. Kalmakoff and J. F. Longworth, eds.), pp. 29–35. D.S.I.R. Bull. 228, Govt Printer, Wellington, N.Z.