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A *Nosema*-Type Microsporidian in *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

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Light and electron microscopy observations were conducted on a microsporidium (Protozoa: Microspora) detected in the Malpighian tubules of *Ectomyelois ceratoniae* from Tupungato, Mendoza Province, Argentina. The characteristics showed by the pathogen (monomorphic, diplokaryotic, apansporoblastic) allowed its placement in the genus *Nosema*. Unusually elongated sporoblasts appeared to be a particular attribute of the pathogen. Experimentally induced infections will be necessary to find out if other differential characters are present. The finding represents only the second report of microsporidia in argentine Lepidoptera.

KEY WORDS: *Nosema; Ectomyelois ceratoniae;* Lepidoptera; Pyralidae; microsporidium; walnut moths; Argentina.

INTRODUCTION

Natural populations of insects in the order Lepidoptera are particularly susceptible to microsporidiosis (Sprague, 1977). Records of microsporidian pathogens, mostly in the genera *Nosema* and *Vairimorpha,* infecting lepidopterous hosts, have been obtained worldwide. Some species, like *Nosema fumiferanae, Nosema pyrausta,* and *Vairimorpha necatrix,* have even received considerable attention as potential microbial control agents in those countries with advanced programs for the study of insect diseases (Brooks, 1988). However, such is not the case in Argentina, where research on Insect Pathology is in its infancy, and hence, in the initial, descriptive phase. In fact, only one reference (Jauch and Jauch, 1948) was found reporting microsporidian diseases in argentine Lepidoptera after an extensive bibliographical search.

The present paper deals with a microsporidium found in an economically important species of moth, *Ectomyelois ceratoniae,* that causes damage to walnuts in the province of Mendoza, Western Argentina.

MATERIALS AND METHODS

Infected insects were present in walnuts from Tupungato, Mendoza Province, Argentina. After dissection, different tissues and organs were examined for infection using fresh mounts with saline under phase contrast microscopy. Fresh mounts were also used for light microscopy observations and for measurements of spores with an ocular micrometer.

For transmission electron microscopy, small pieces of infected tissues were fixed in 2.5% (v/v) glutaraldehyde buffered with 0.1 M cacodylate buffer, *p*H 7.4. Postfixation was in 1% (w/v) OsO_4 followed by dehydration in an ethanol series. Spurr's resin was used for embedding. Silver sections were stained with uranyl acetate followed by lead citrate and photographed with a JEOL JEM CX electron microscope at 100 kV.

RESULTS

Infections were observed in larvae, pupae, and adults of *E. ceratoniae*. The overall prevalence of infection was 48% (n =

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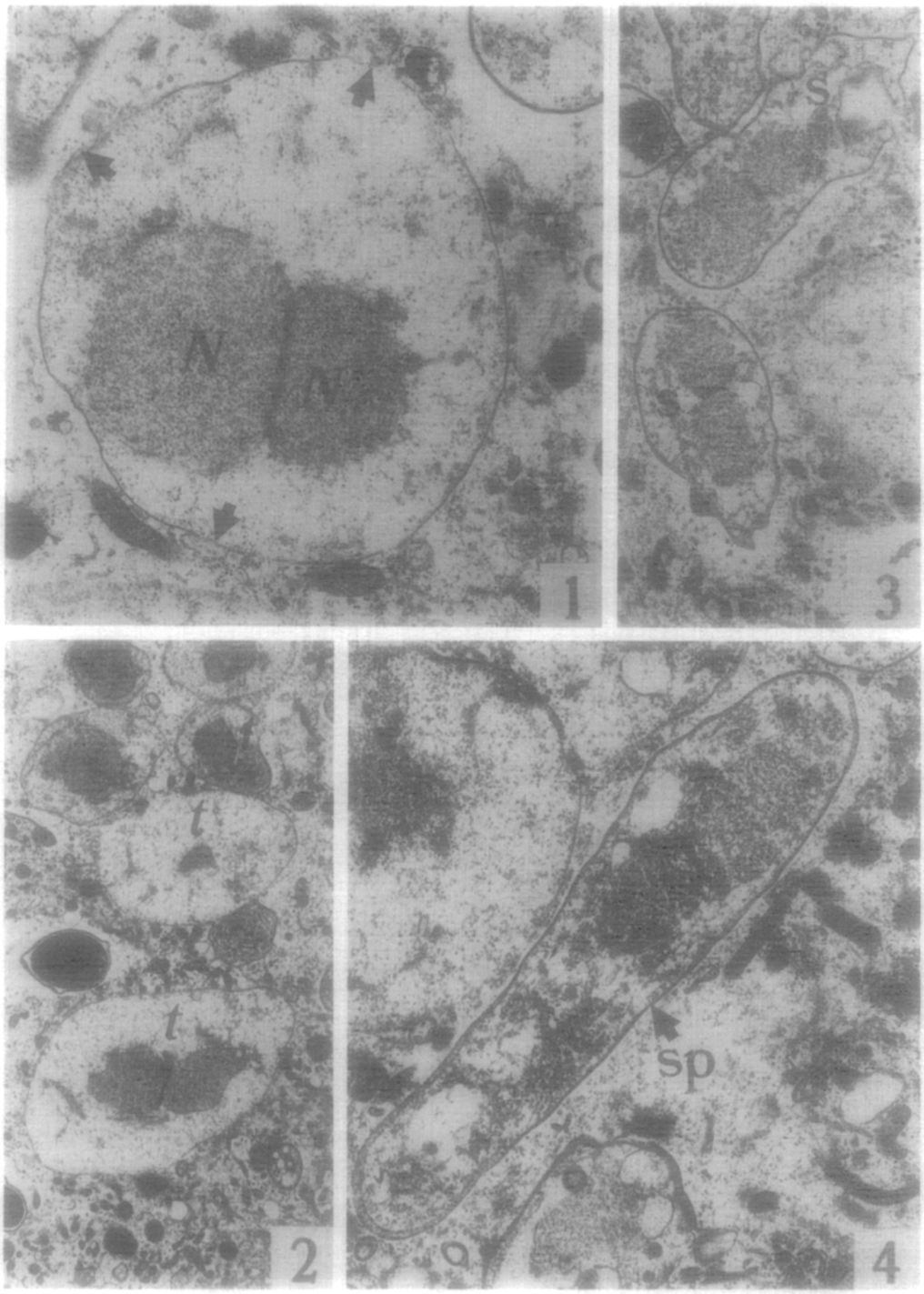
50). Although infected individuals sometimes appeared to be stunted and sluggish, no external sign or symptom of disease proved reliable enough to discriminate between infected and healthy insects. This made dissection a requirement in order to establish their condition. Infections were only found in Malpighian tubules despite extensive examination of other organs and tissues. Heavily infected Malpighian tubules were easily recognizable because they were pale in color and hypertrophied.

Development of the parasite was in direct contact with the host cytoplasm. As it is normally the case while working with naturally infected insects, development was advanced, and hence, the earliest stages seen were those in late merogony or early sporogony. These transitional stages (Fig. 1), recognizable by the partially thickened plasmalemmas, had diplokaryotic nuclei and were mostly rounded in shape. Progressive elongation of these cells occurred during sporulation. This appeared to proceed always in the direction of the longer axis of the paired nuclei (Fig. 2). Sporonts (Fig. 3) had diplokaryotic nuclei and were easily recognized from transitional stages by their completely thickened cell membranes, elongated shapes, and vacuolated cytoplasms. Diplokaryotic sporoblasts (Fig. 4) were unusually elongated and appeared nonrefractile under phase contrast microscopy (Fig. 5). Fresh spores (Fig. 6) were ovocylindrical and measured 3.7 ± 0.01 by 1.3 ± 0.006 μm ($\bar{X}$ ± SE). Macrospores, sometimes almost twice the size of normal spores, were usually present. Longitudinal sections of mature spores (Fig. 7) revealed the diplokaryotic condition, a lamellar polaroplast, between 9 and 12 coils of the polar filament, and a wrinkled exospore.

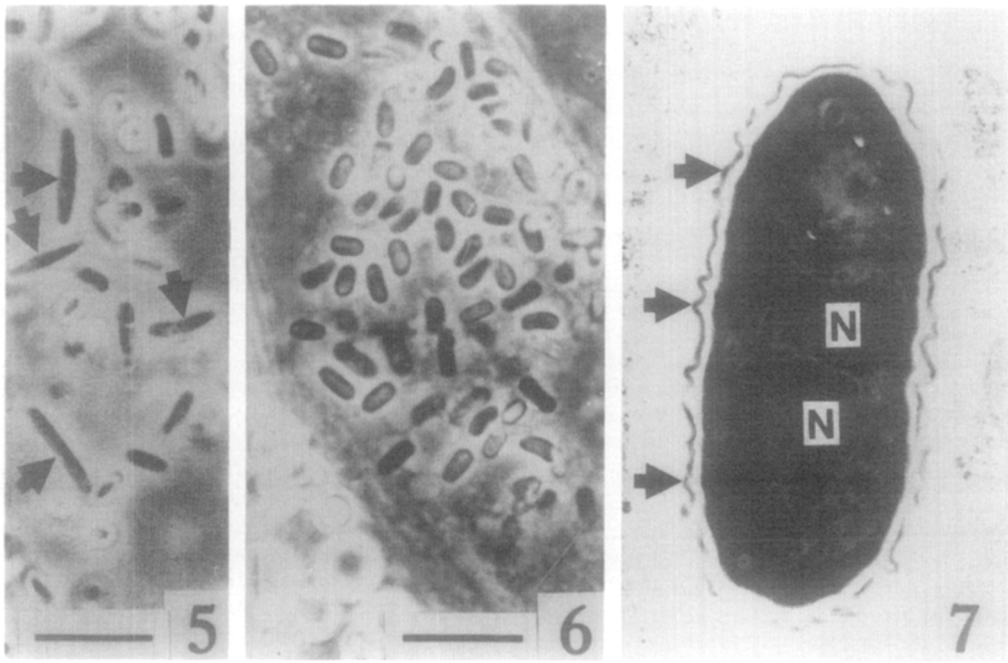
DISCUSSION

This study clearly demonstrated that the microsporidium in *E. ceratoniae* exhibits three of the four major characters normally used to characterize the genus *Nosema* (Sprague, 1978) as well as some others that have recently been mentioned for the genus (Larsson, 1988), specifically the type of polaroplast and the shape of spores. The pathogen in *E. ceratoniae* has only one sequence of sporogonic development (monomorphic), it develops in direct contact with the host cell cytoplasm (apansporoblastic), and the nuclei are paired, at least, during late merogony, sporogony, and sporogenesis (diplokaryotic). The fourth character, the formation of two sporoblasts from a sporoblast mother cell (disporous or disporoblastic), was not demonstrated but according to Vávra et al. (1981), such a condition should not be applied too rigidly because some particular exceptions occur. Furthermore, as has been pointed out by Brooks et al. (1985), many of the accepted species of the genus *Nosema* from different host groups have not been shown to possess all of the four characteristics.

Nosema is the largest microsporidian genus, containing approximately 200 species (Sprague, 1982). About 37.5% of these species were reported from Lepidoptera (Sprague, 1977), making this insect order the most common host group for the genus. With some remarkable exceptions, most of the *Nosema* species reported from Lepidoptera were poorly characterized or defined and too much emphasis placed upon the host specificity criterion. Many species have been created based almost solely on the isolation from a new host species instead of reporting differences at the microorganismal level, a procedure that later was considered unreliable by most authors. Following the former criterion, the microsporidium in *E. ceratoniae* would easily constitute a new species because no microsporidia have been reported for this host or other species in this genus. However, after a careful comparison with other microsporidia in Lepidoptera that are adequately defined (i.e., ultrastructural information available), only two differences at the organismal level were observed: sporoblasts were unusually elongated and spores



FIGS. 1–4. Transmission electron micrographs of stages in late merogony and sporogony of *Nosema* sp. in *Ectomyelois ceratoniae*. (1) Rounded stage in transition from merogony to sporogony showing nuclei (N) in diplokaryotic arrangement. Observe that most of the plasmalemma is thicker than a few stretches still showing a thin cell membrane (arrows) (×18,525). (2) Some transitional stages (t) showing gradual thickening of plasmalemmas and progressive body elongation along the longer axis of paired nuclei (×4750). (3) Elongated diplokaryotic sporonts (s) (×9405). (4) Diplokaryotic sporoblast (sp) (×16,340).



FIGS. 5–7. Sporoblasts and spores of *Nosema* sp. in *Ectomyelois ceratoniae* in fresh mounts (Figs. 5,6; bar: 10 μm) and transmission electron microscopy (Fig. 7). (5) No refringent, very elongated sporoblasts (arrows). (6) Ovocylindrical spores. (7) Mature spore showing diplokaryotic condition of nuclei (N) and wrinkled outline of the exospore (arrows) (×30,000).

were particularly small in width. These two distinctions are not considered valid enough for the creation of a new species at this time. Although sporoblasts in *E. ceratoniae* are remarkably elongated and could be used for identification as fresh mounts under phase contrast microscopy for practical needs, some degree of elongation of stages during late sporogony and sporogenesis is not unusual within the microsporidia. Besides, sporoblast morphology has seldom received much attention for taxonomic purposes because it is usually a highly variable stage undergoing intense reorganization. Spore size, once a primary character for the separation of species, is becoming less important, mainly because sporal phenotypic plasticity is an increasingly reported phenomenon for *Nosema* species (Walters, 1958; Armstrong et al., 1986; Mercer and Wigley, 1987; Hayasaka and Kawarabata, 1990). Also, the occurrence of strains within species is already well documented for the microsporidia (Kawarabata and Hayasaka, 1987).

Other well known *Nosema* in Pyralidae include: *Nosema transitellae* in the Navel Orangeworm, *Paramyelois transitella*, *Nosema* sp. in the Poroporo Stem Borer, *Sceliodes cordalis*, and *N. pyrausta* in the European Corn Borer, *Ostrinia nubilalis*. (Paillot, 1927; Kellen et al., 1977; Mercer and Wigley, 1987). The latter species has received considerable attention as a potential biological control agent (Maddox, 1986). *Nosema coliadis* in *Colias lesbia* is the only other microsporidium known to occur in an argentine lepidoptera (Pieridae) (Jauch and Jauch, 1948). Although a definitive comparisson between *Nosema* sp. in *E. ceratoniae* and *N. coliadis* would not be valid at present due to the lack of ultrastructural information on the latter, the probability that the two pathogens are conspecific is small if one considers that they do not share any feature in common, such

as host, geographical distribution, tissues affected, and spore size and shape.

The availability of experimentally transmitted infections upon establishment of a healthy colony of *E. ceratoniae* would provide further information on this pathogen that would in turn allow one to see if other useful differential characters are present.

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REFERENCES

ARMSTRONG, E., BASS, L., STAKER, K., AND HARRELL, L. 1986. A comparisson of the biology of a Nosema in *Drosophila melanogaster* to *Nosema kingi* in *Drosophila willinstoni*. *J. Invertebr. Pathol.*, **48**, 124–126.

BROOKS, W. M. 1988. Entomogenous Protozoa. *In* "Handbook of Natural Pesticides," Vol. V, "Microbial Insecticides" (C. M. Ignoffo, Ed.), CRC Press, Boca Raton, Florida.

BROOKS, W. M., HAZARD, E. I., AND BECNEL, J. J. 1985. Two new species of *Nosema* (Microsporida: Nosematidae) from the Mexican Bean Beetle *Epilachna varivestis* (Coleoptera: Coccinellidae). *J. Protozool.*, **32**, 525–535.

HAYASAKA, S., AND KAWARABATA, T. 1990. Cloning of a microsporidian, *Nosema bombycis* (Microsporida: Nosematidae) in insect cell cultures by a limiting dilution method. *J. Invertebr. Pathol.*, **55**, 35–40.

JAUCH, C. U., AND JAUCH, A. N. 1948. *Nosema coliadis*, nueva especie de microsporidio parásito de la isoca de los alfalfares (*Colias lesbia*). *An. Soc. Cient. Argent.*, **145**, 307–321.

KAWARABATA, T., AND HAYASAKA, S. 1987. An enzyme-linked immunoabsorbent assay to detect alkali-soluble spore surface antigens of strains of *Nosema bombycis* (Microspora: Nosematidae). *J. Invertebr. Pathol.*, **50**, 118–123.

KELLEN, W. R., HOFFMANN, D. F., AND COLLIER, S. S. 1977. Studies on the biology and ultrastructure of *Nosema transitellae* sp. n. (Microsporidia: Nosematidae) in the Navel Orangeworm, *Paramyelois transitella* (Lepidoptera: Pyralidae). *J. Invertebr. Pathol.*, **29**, 289–296.

LARSSON, J. I. R. 1988. Identification of Microsporidian Genera (Protozoa: Microspora)—a guide with comments on the taxonomy. *Arch. Protistenkd.*, **136**, 1–37.

MADDOX, J. V. 1986. Possibilities for manipulating epizootics caused by Protozoa: A representative case-history of *Nosema pyrausta*. *In* "Fundamental and Applied Aspects of Invertebrate Pathology" (R. A. Samson, J. M. Vlak, and D. Peters, Eds.), Pousen and Looijen, Wageningen, The Netherlands.

MERCER, C. F., AND WIGLEY, P. J. 1987. A microsporidian pathogen of the Poroporo Stem Borer, *Sceliodes cordalis* (Dbld) (Lepidoptera: Pyralidae). *J. Invertebr. Pathol.*, **49**, 93–101.

PAILLOT, A. 1927. Sur deux protozoaires nouveaux parasites des chenilles de *Pyrausta nubilalis* Hb. *C. R. Acad. Sci.*, **185**, 673–675.

SPRAGUE, V. 1977. Annotated list of species of microsporidia. *In* "Comparative Pathobiology. Systematics of the Microsporidia" (L. A. Bulla and T. C. Cheng, Eds.), Plenum, New York.

SPRAGUE, V. 1978. Characterization and composition of the genus *Nosema*. *Misc. Publ. Entomol. Soc. Am.*, **11**, 5–16.

SPRAGUE, V. 1982. Microspora. *In* "Synopsis and Classification of Living Organisms" (S. P. Parker, Ed.), McGraw-Hill, New York.

VÁVRA, J., CANNING, E. U., BARKER, R. J., AND DESPORTES, I. 1981. Characters of Microsporidian Genera. *Parasitology*, **82**, 131–142.

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.