

Studies on the Biology and Ultrastructure of *Nosema transitellae* sp. n. (Microsporidia: Nosematidae) in the Navel Orangeworm, *Paramyelois transitella* (Lepidoptera: Pyralidae)¹

WILLIAM R. KELLEN, DARLENE F. HOFFMANN, AND SUSAN S. COLLIER

Stored-Product Insects Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Fresno, California 93727

Received August 13, 1976

Nosema transitellae sp. n. was originally isolated from larvae of the navel orangeworm, *Paramyelois transitella*, reared on contaminated trash almonds. The life cycle of the pathogen includes the formation of quadrinucleate schizonts and sporonts that divide to form twin binucleate sporoblasts typical of the genus *Nosema*. The pathogen primarily invades fat and the silk and salivary glands. Larvae with heavy infections frequently develop melanized encapsulated fat lobes that are visible beneath the transparent cuticle. In laboratory tests, only the navel orangeworm and the red flour beetle, *Tribolium castaneum*, acquired lethal infections. The almond moth, *Cadra cautella*, the raisin moth, *Cadra figulilella*, the tobacco moth, *Ephestia elutella*, and the greater wax moth, *Galleria mellonella*, acquired benign infections. The sawtoothed grain beetle, *Oryzaephilus surinamensis*, was not susceptible. The ultrastructure of the spores of *N. transitellae* is similar to that of spores of *N. whitei* and *N. oryzaephili*.

INTRODUCTION

The navel orangeworm, *Paramyelois transitella*, has previously been reported to be susceptible to several species of microsporidia in laboratory tests. Kellen and Lindegren (1969, 1970, 1973) noted that the larvae were highly susceptible to *Nosema heterosporum*, *N. plodiae*, and *N. invadens* when they were reared on diets containing fresh spores. Also, Kellen and Lindegren (1970) reported that an undescribed species of *Nosema* and a *Pleistophora* had been isolated from diseased larvae that were reared in the laboratory on naturally contaminated trash almonds.

In 1975, we obtained several dried larval cadavers of the navel orangeworm that harbored oval to pyriform spores of a *Nosema* that appeared morphologically similar to *N. whitei* and *N. oryzaephili*. These two respective species were originally described from the red flour beetle,

Tribolium castaneum, and the sawtoothed grain beetle, *Oryzaephilus surinamensis*. Because a species of *Nosema* with spores of this type had not been reported previously from the navel orangeworm, a study was made to determine the life cycle, host range, and identity of the pathogen.

MATERIALS AND METHODS

Spores of *Nosema transitellae* sp. n. were first obtained from infected larvae of the navel orangeworm that had been reared on contaminated trash almonds in the laboratory. These in-hull nuts were collected in February 1975 from beneath 'Nonpareil' almond trees in an orchard near Ballico, Madera County, California. They were kept in 1-gal glass jars, and eggs from a laboratory culture of the navel orangeworm were added (26°C). Dried cadavers of mature larvae containing spores of *Nosema* were recovered after about 30 days. A laboratory culture of the pathogen was then established by rearing larvae on a bran diet containing 5×10^6 spores/g.

¹ Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

Stages in the life cycle of the pathogen were examined in smears of infected tissues fixed with methyl alcohol and stained with Giemsa's solution. Fresh spores were measured with an A. E. I. Vickers image-splitting eyepiece at 1000 \times .

For histological studies, tissues from infected larvae were fixed in Zenker's solution. Specimens were dehydrated in a graded series of alcohols and were embedded in paraffin. Sections were cut at 6 μm and were stained with Mallory's triple connective tissue stain. Also, samples of infected tissue were prepared for examination under an electron microscope by being fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. Specimens were embedded in Spurr's medium. Uncoated grids bearing sections stained with uranyl acetate and lead citrate were examined in a Siemens Elmiskop IA at 80 kV.

Bioassays were conducted to determine the comparative virulence of the *Nosema* in some common stored-product moths. Since dried spores frequently germinated when rehydrated, infected fresh larval tissues were used in the preparation of aqueous spore suspensions. Two milliliters of a known concentrated suspension of spores was added to 10 g of bran diet in 0.5-pt paper cartons to give 5×10^6 spores/g of diet. Two milliliters of water was added to the control cartons. Twenty-five neonate larvae were then placed in each carton and reared to the adult stage at 26°C. The percentage of mortality was based on the number of larvae that developed to emerge as adults. Tests were replicated three times. The following moths were tested: the almond moth, *Cadra cautella*; the raisin moth, *C. figulilella*; the tobacco moth, *Ephestia elutella*; and the greater wax moth, *Galleria mellonella*.

Also, assays with larvae of the red flour beetle, *Tribolium castaneum*, and the sawtoothed grain beetle, *Oryzaephilus surinamensis*, were conducted. Infected dried larval cadavers of the navel orangeworm and a 10% mixture of dried yeast in

baking flour were ground with a mortar and pestle. The spore concentration was determined by suspending replicate 0.1-g samples of mixture in 5 ml of water and counting the spores with a Petroff Hausser cell counter. This stock mixture was then diluted with additional flour to produce a concentration of 4×10^6 spores/g. Twenty-five neonate larvae from a laboratory culture were then added to the flour mixture in 0.5-pt paper cartons. Control larvae were reared in flour mixture without spores.

RESULTS

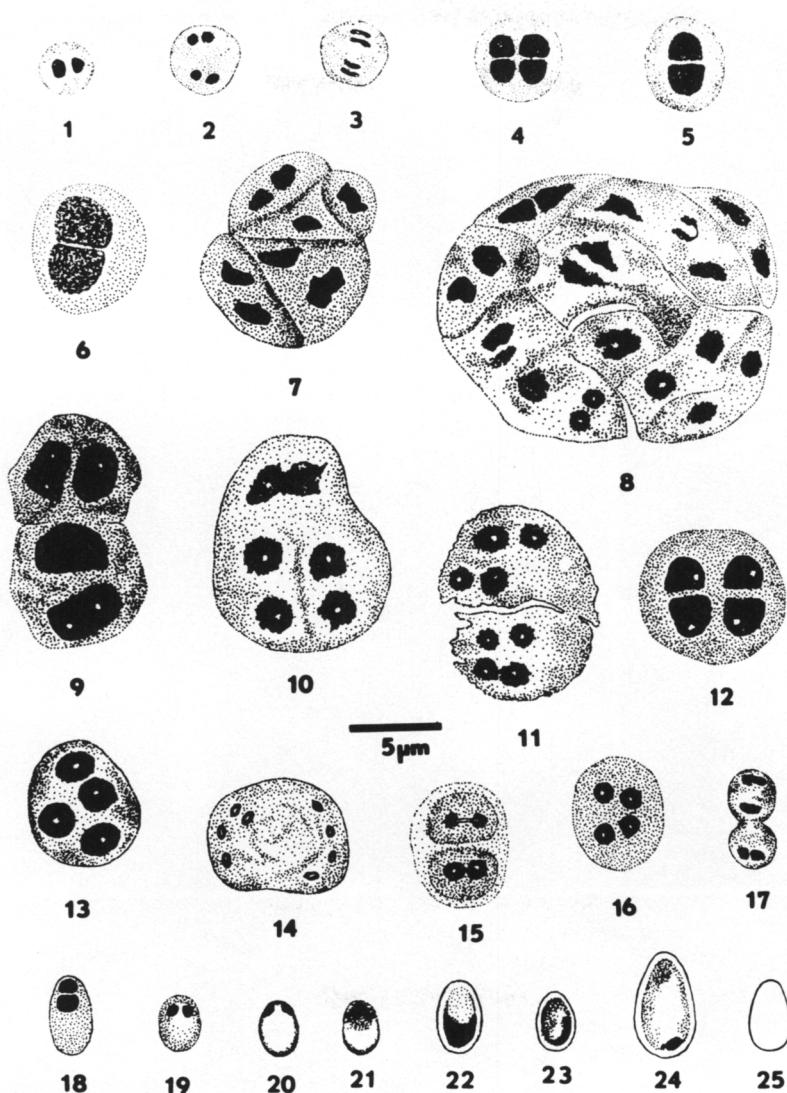
Life Cycle

Stages in the development of the *Nosema* in the navel orangeworm were observed in stained smears of larval fat tissue (Figs. 1-25). Many of the developmental forms were similar to those reported by Burges et al. (1971) for *N. oryzaephili* and by Milner (1972a) for *N. whitei*. Schizonts with four to eight nuclei were common in our smears made 7-10 days after initial infection; sporonts were abundant after approximately 15 days. Sporonts divided to form paired binucleate sporoblasts typical of the genus *Nosema*. Minimum generation time was approximately 6 days in larvae reared on bran diets containing 7×10^6 spores/g and maintained at 26°C.

Fresh spores measured 3.62 ± 0.26 $\times 2.38 \pm 0.15 \mu\text{m}$ ($n = 50$). Macrospores were common in our smears and measured $7.00 \pm 0.56 \times 3.23 \pm 0.12 \mu\text{m}$ ($n = 25$).

Pathogenicity

In larvae of the navel orangeworm, the pathogen invaded the silk and salivary glands and, to a lesser degree, the midgut, muscle, and nerve tissue. Furthermore, mature larvae with patent infections usually developed melanized encapsulated fat lobes that were evident beneath the transparent cuticle (Figs. 27, 31-34). In tissue sections, encapsulated lobes were ca. 0.2-0.3 mm in diameter. Many hypertrophied fat



FIGS. 1–25. Stages in the life cycle of *Nosema transitellaе* sp. n. in larvae of the navel orangeworm. Giemsa-stained. Figs. 1–8. First schizogony. Figs. 9–14. Second schizogony. Figs. 15, 16. Sporonts. Fig. 17. Formation of twin sporoblasts. Figs. 18–21. Sporoblasts. Figs. 22, 23. Mature microspores. Fig. 24. Macrospore. Fig. 25. Fresh microspore.

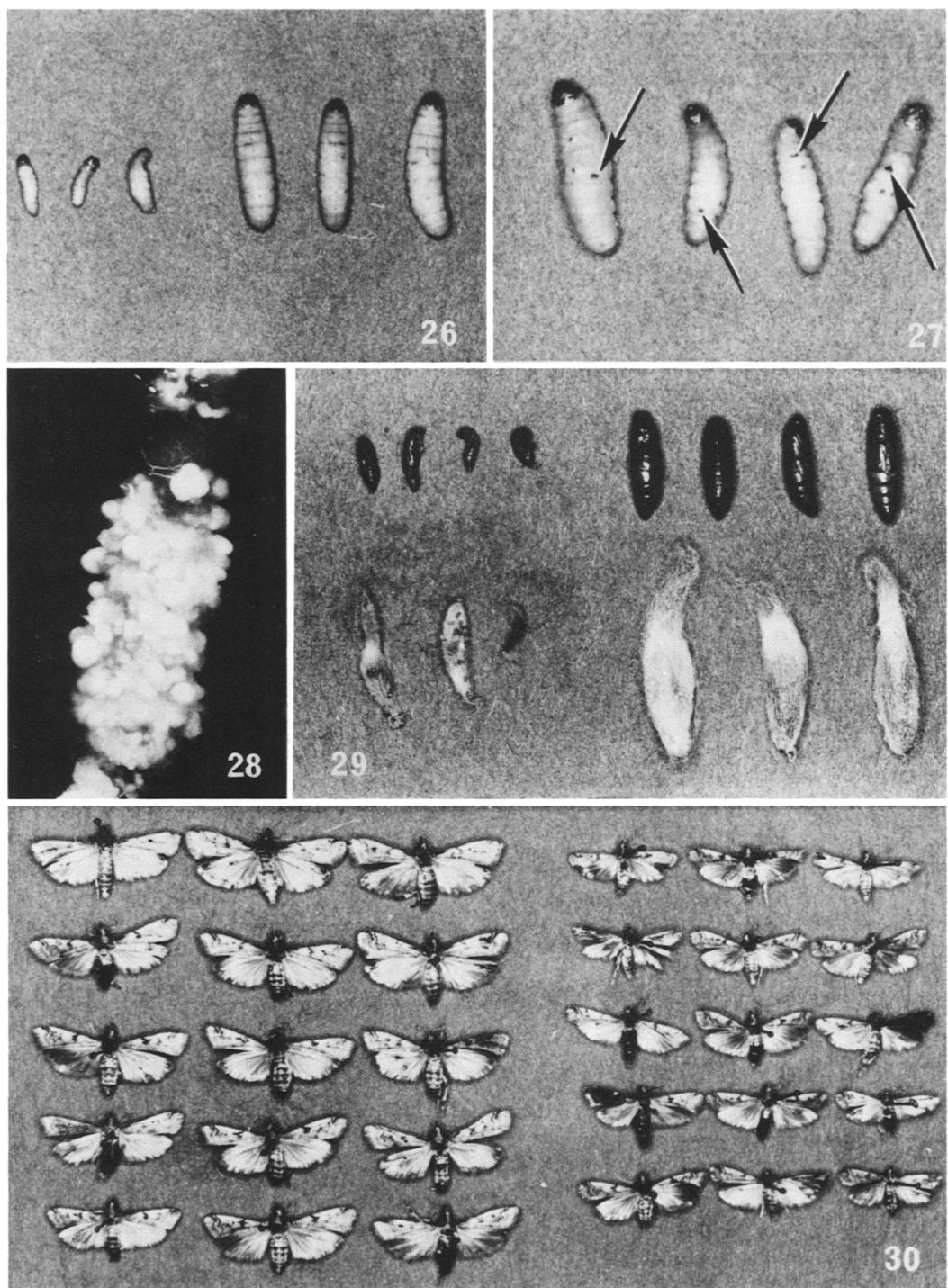
lobes filled with developing sporogonic forms occurred as clusters of grapes on the midgut (Fig. 28).

Navel orangeworm larvae with chronic infections frequently were stunted and moribund, though they survived for several weeks (Fig. 26). However, patently infected larvae occasionally matured, spun cocoons, and developed into small deformed pupae (Fig. 29). Sublethally stunted larvae

survived to emerge as undersized adults (Fig. 30). The estimated LC₅₀ was 5 × 10⁵ spores/g at 26°C.

Ultrastructure

Electron micrographs of sporonts and spores are shown in Figures 35–38. Data from measurements of the number and angle of tilt of the coils of the polar filament are given in Table 1. The lowest angles of



FIGURES 26-30

anterior and posterior tilt were 35° and 43°, respectively.

Host Range

The four species of Lepidoptera tested, the raisin moth, almond moth, tobacco moth, and the greater wax moth, all survived to the adult stage without mortality. There were, however, small areas of infection in remnants of the silk glands in freshly emerged adults. Fat tissue and reproductive organs of the infected moths were normal.

Mortality of flour beetles was 80% after 30 days when they were reared on the contaminated flour diet. Sawtoothed grain beetles, however, survived to the adult stage without infection.

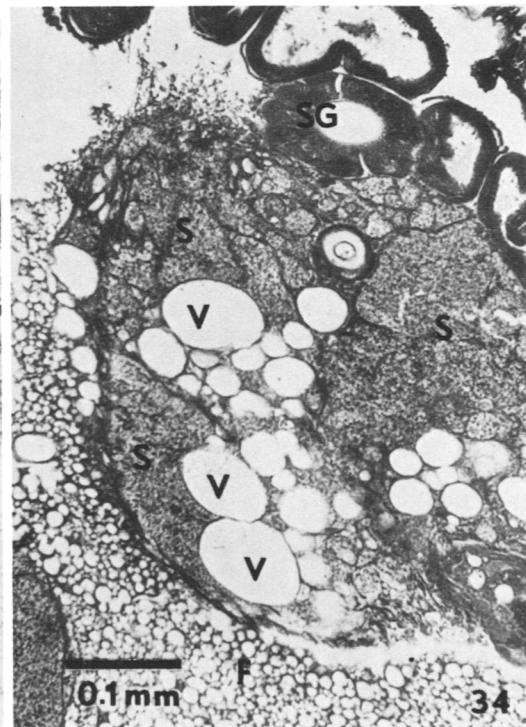
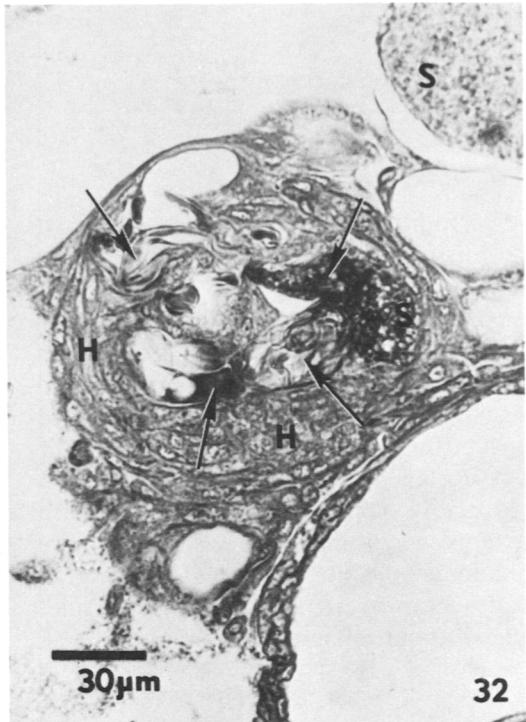
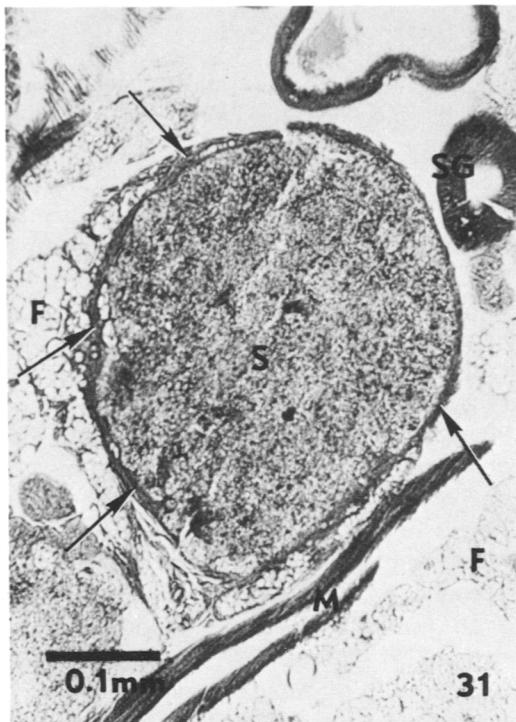
DISCUSSION

Although the *Nosema* from the navel orangeworm is relatively distinct in spore size and tissue specificity, we initially sought to determine whether it was conspecific with either *N. whitei* or *N. oryzaephili*. *N. whitei* is a common pathogen of flour beetles and occurs frequently as a contaminant in laboratory cultures. Its host range among Lepidoptera is unknown. Milner (1972a, b, c) presented a comprehensive report of the biology and ultrastructure of *N. whitei* in the red flour beetle, noting that fresh spores averaged $4.6 \times 2.9 \mu\text{m}$ and that schizonts with more than two nuclei comprised only 1% of the population; moreover, he concluded that the sporonts formed only a single sporoblast. These characteristics are somewhat

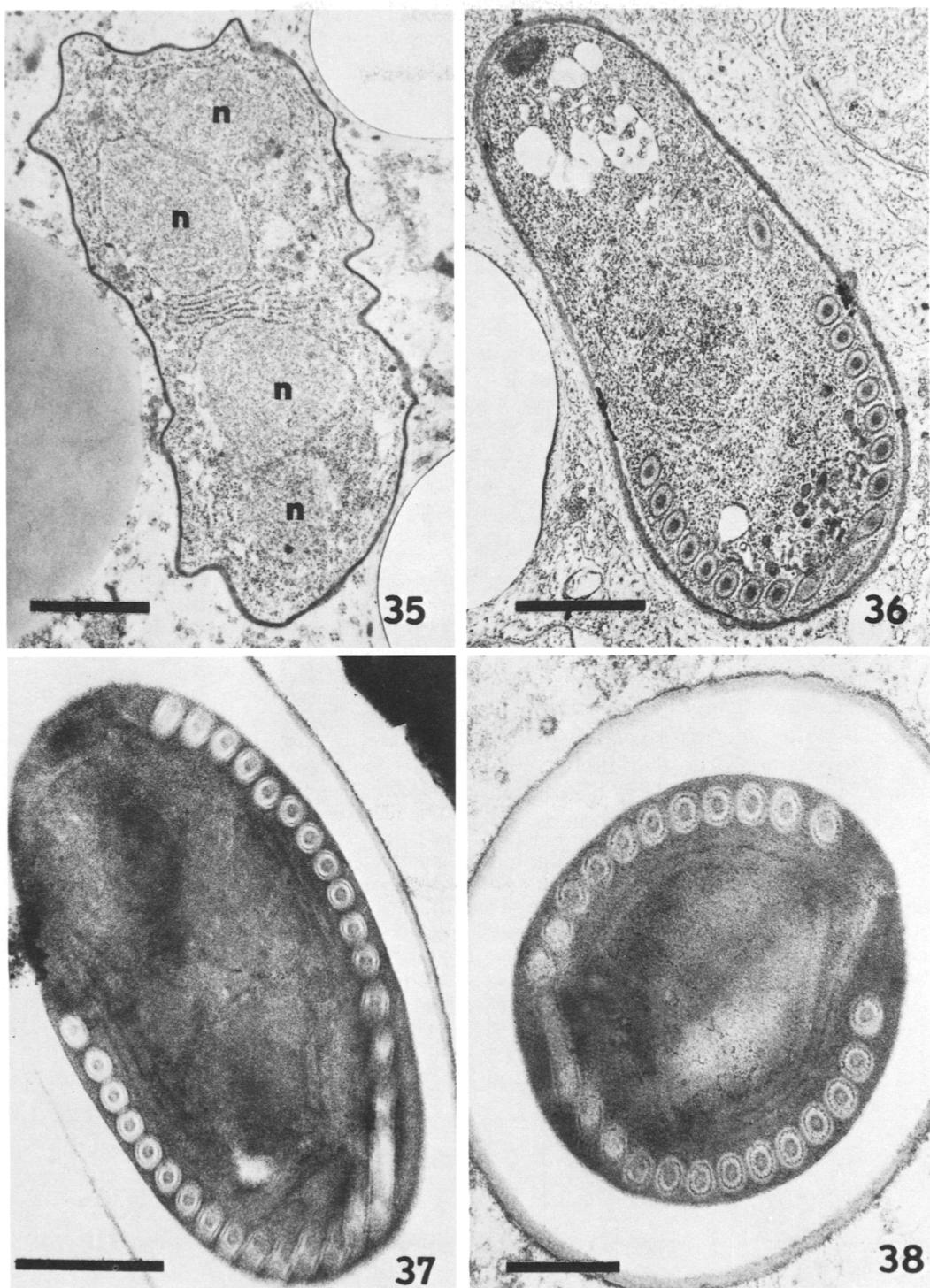
different from those we noted in the *Nosema* from the navel orangeworm, but, nonetheless, we questioned the identity of our species because our tests showed that red flour beetles were highly susceptible. Subsequently, however, we obtained a culture of *N. whitei* for cross-transmission tests. Navel orangeworm larvae proved to be only slightly susceptible to infection by *N. whitei*, and invasion was limited to small areas in fat tissue and silk glands. Macrospores were absent. The differences in life cycle and susceptibility finally led us to conclude that the pathogen from the navel orangeworm is distinct from *N. whitei*.

Burges et al. (1971, 1974) described a species of *Nosema* morphologically similar to *N. whitei* from the sawtoothed grain beetle. This pathogen, *N. oryzaephili*, was considered to be a separate species largely because of its low virulence in the red flour beetle. They reported, however, that five of sixteen species of beetles and three of five species of moths were susceptible in laboratory tests. Susceptible moths were: the almond moth, the tobacco moth, and the Mediterranean flour moth, *Anagasta kuehniella*. Although *N. oryzaephili* has a broad host range, we were unable to transmit the pathogen to larvae of the navel orangeworm. Moreover, as indicated, we were also unable to infect sawtoothed grain beetles with our navel orangeworm *Nosema*. Therefore, we concluded that our *Nosema* represents a new species that is morphologically and ultrastructurally similar to *N. whitei* and *N. oryzaephili*. We propose the name *N. transitellae* sp. n. for this species.

Figs. 26–30. Effects of infection by *Nosema transitellae* sp. n. developmental stages of the navel orangeworm. Fig. 26. Stunted larvae after 30 days of infection and healthy mature larvae of same age. Fig. 27. Chronically infected larvae showing melanized encapsulated fat lobes visible through cuticle (arrows). Fig. 28. Hypertrophied fat lobes harboring spores of *N. transitellae* adjacent to larval midgut. Fig. 29. Stunted and healthy pupae (top) and cocoons of diseased and healthy larvae (bottom) compared. Fig. 30. Stunted adults with sublethal infections of *N. transitellae* and normal adults (at left).



Figs. 31-34. Histological sections through fat lobes of larvae of the navel orangeworm infected with *Nosema transitella* sp. n. Mallory connective tissue stained. Fig. 31. Fat lobe with accumulation of spores showing early stage of hemocytic encapsulation (arrows). Fig. 32. Encapsulated fat lobe with areas of dense melanization (arrows). Fig. 33. Infected lobe showing intrusion by hemocytes (arrows) into area of fat tissue filled with spores. Fig. 34. Invaded fat lobe with much vacuolization throughout tissue occupied by spores. F, fat; G, gut wall; H, hemocytes; M, muscle; S, spores; SG, salivary gland; V, vacuole.



FIGS. 35-38. Electron micrographs of stages of *Nosema transitellae* sp. n. in the navel orangeworm. Fig. 35. Division of sporont to form twin binucleate sporoblasts (n, nucleus). Fig. 36. Young spore showing early formation of the polar filament. Figs. 37, 38. Longitudinal sections of mature spores showing two aspects of the angle of tilt of the coiled polar filament. (Figs. 35, 36: bar = 1.0 μm ; Figs. 37, 38: bar = 0.5 μm).

TABLE I

NUMBER OF COILS AND ANGLE OF TILT OF THE POLAR FILAMENT IN MEDIAL LONGITUDINAL SECTIONS OF SPORES OF *NOSEMA TRANSITELLAE* SP. N. FROM LARVAE OF THE NAVEL ORANGEWORM,
PARAMYEOLOIS TRANSITELLA

Spore No.	Number of coils	Angle of tilt (degrees)	
		Anterior	Posterior
1	12	68	68
2	13	68	65
3	13	53	—
4	12	50	46
5	11	75	68
6	13	50	—
7	11	52	—
8	12	35	—
9	15	50	43
10	12	43	—
Average	12.4	54.5	58.0

In Weiser's (1961) key to Microsporidia, *N. transitella* may be included in the couplet that keys to *N. perezoides*. Spore shape distinguishes these species, *N. transitella* being slightly more pyriform.

The holotype and paratype slides of *N. transitella* are deposited in the collection of the National Museum of Natural History of the Smithsonian Institution.

ACKNOWLEDGMENTS

The authors wish to thank D. K. Hunter of the ARS, U. S. Department of Agriculture, for providing us with the original isolate of *N. transitella* in larvae of the navel orangeworm. We also thank H. D. Burges of the Glasshouse Crops Research

Institute, England, and J. V. Maddox of the Illinois Natural History Survey for samples of *N. oryzaephili* and *N. whitei*.

REFERENCES

- BURGES, H. D., CANNING, E. U., AND HURST, J. A. 1971. Morphology, development, and pathogenicity of *Nosema oryzaephili* n. sp. in *Oryzaephilus surinamensis* and its host range among granivorous insects. *J. Invertebr. Pathol.*, **17**, 419-432.
- BURGES, H. D., CANNING, E. U., AND HULLS, I. K. 1974. Ultrastructure of *Nosema oryzaephili* and the taxonomic value of the polar filament. *J. Invertebr. Pathol.*, **23**, 135-139.
- KELLEN, W. R., AND LINDEGREN, J. E. 1969. Host-pathogen relationships of two previously undescribed Microsporidia from the Indian meal moth, *Plodia interpunctella* (Hübner), (Lepidoptera:Phycitidae). *J. Invertebr. Pathol.*, **14**, 328-335.
- KELLEN, W. R., AND LINDEGREN, J. E. 1970. Previously unreported pathogens from the navel orangeworm, *Paramyelois transitella*, in California. *J. Invertebr. Pathol.*, **16**, 342-345.
- KELLEN, W. R., AND LINDEGREN, J. E. 1973. *Nosema invadens* sp. n. (Microsporida:Nosematidae), a pathogen causing inflammatory response in Lepidoptera. *J. Invertebr. Pathol.*, **21**, 293-300.
- MILNER, R. J. 1972a. *Nosema whitei*, a microsporidan pathogen of some species of *Tribolium*. I. Morphology, life cycle, and generation time. *J. Invertebr. Pathol.*, **19**, 231-238.
- MILNER, R. J. 1972b. *Nosema whitei*, a microsporidan pathogen of some species of *Tribolium*. II. Ultrastructure. *J. Invertebr. Pathol.*, **19**, 239-247.
- MILNER, R. J. 1972c. *Nosema whitei*, a microsporidan pathogen of some species of *Tribolium*. III. Effect on *T. castaneum*. *J. Invertebr. Pathol.*, **19**, 248-255.
- WEISER, J. 1961. Die Mikrosporidien als Parasiten der Insekten. *Monogr. Angew. Entomol.*, No. 17, 1-149.