

A Microsporidium, *Nosema pilicornis* sp. n., of the Purslane Sawfly, *Schizocerella pilicornis*¹

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A new microsporidian species, *Nosema pilicornis*, which infects the purslane sawfly, *Schizocerella pilicornis*, is described. This microsporidium infects most body tissues of the host. *N. pilicornis* was compared to other microsporidian species infecting Hymenoptera and to a group of similar microsporidia infecting Lepidoptera. *N. pilicornis* could be distinguished from all other microsporidian species on the basis of host range and ultrastructural characteristics of the spore. Spores were oval, containing 11 to 12 polar filament coils, and the polar filament had an angle of tilt of about 80°. *N. pilicornis* infected lepidopteran larvae, but only when heavy spore dosages were fed to early larval instars. *S. pilicornis* is a good but sporadic biological control agent of common purslane, *Portulaca oleracea*, a pernicious weed of vegetable, ornamental, and orchard crops. *N. pilicornis*, which is transovarially transmitted and causes high mortality in infected larvae, affects the performance of *S. pilicornis* as a biological control agent.

KEY WORDS: Microsporidium; *Nosema pilicornis* sp. n.; *Schizocerella pilicornis*; common purslane; biological control agent.

INTRODUCTION

Larvae of the purslane sawfly, *Schizocerella pilicornis*, feed on common purslane, *Portulaca oleracea*, a pernicious weed of vegetable, ornamental, and orchard crops. The purslane sawfly provides a good but sporadic biological control of common purslane.

In 1972 we observed that purslane sawfly larvae collected throughout the state of Illinois were infected with a microsporidium. Subsequent observations suggested that this microsporidium was a major factor in reducing purslane sawfly populations. This paper describes this microsporidium as a new species, *Nosema pilicornis* sp. n., and presents some observations on how it affects the purslane sawfly.

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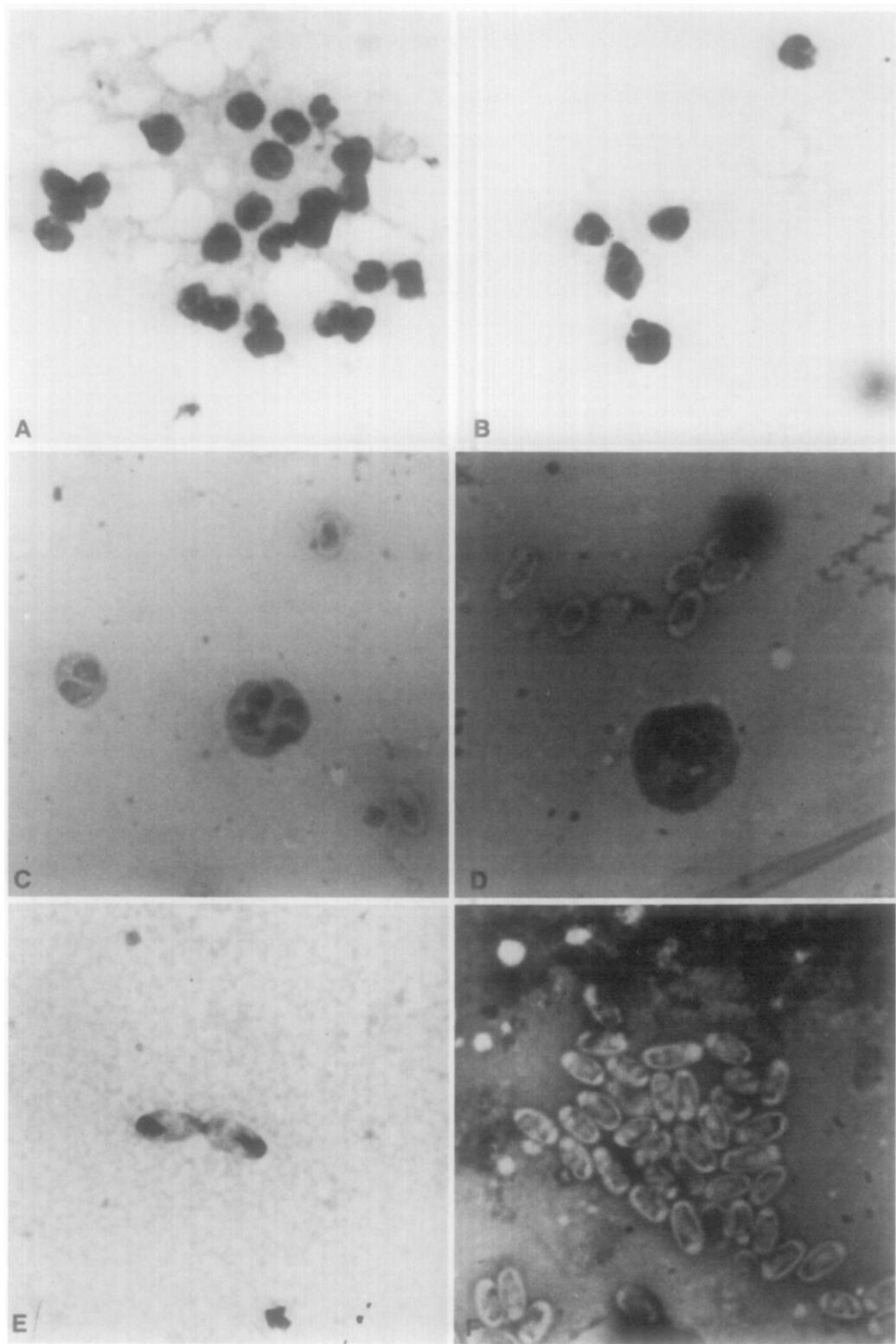
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MATERIALS AND METHODS

The original stock for laboratory experiments came from purslane sawfly larvae collected in Urbana, Illinois, in 1974. Purslane sawfly larvae were infected with the microsporidium by feeding them on purslane leaves dipped in a spore suspension containing 10^6 microsporidian spores per microliter. Observations on developmental stages were made by smearing infected tissue on a cover glass, drying for 30 min, fixing in absolute methanol for 10 min or over 1% osmium tetroxide vapors for 2 min, and staining in a 10% aqueous solution of Giemsa for 30 min. Infected larvae were also sectioned for histological observations, using methods described by Brooks (1970).

For transmission electron microscope studies, fresh infected tissues were prepared by the method of Hazard (1975) except that tissues were embedded in Spurr's (1969) low viscosity medium.

Fresh spores were immobilized with dilute agar by the method of Vavra (1964) and



measured under an oil immersion objective with a Cooke image-splitting eyepiece.

Cross-transmission studies were conducted on four species of lepidopterous larvae using the methods of Nordin and Maddox (1974).

Purslane sawfly larvae were collected from numerous locations throughout Illinois and examined for the presence of microsporidian infections. Infections were diagnosed by phase contrast microscopy.

RESULTS

The Giemsa-stained developmental stages and spores are shown in Figure 1. The earliest forms observed were binucleate schizonts (Fig. 1A). Tetranucleate schizonts were present but not as abundant as the binucleate forms (Fig. 1B). These tetranucleate schizonts apparently divide by binary fission to form binucleate schizonts. Larger schizonts with less compact nuclei and lighter staining cytoplasm were observed later in the developmental sequence (Fig. 1C, D). These forms contained either two, four, or eight nuclei with the binucleate and tetranucleate forms being much more abundant than the octonucleate forms. All schizonts, as well as the sporoblasts, appear to be diplokaryotic. We do not know what developmental path is followed by the octonucleate form. The tetranucleate sporont forms two sporoblasts (Fig. 1E). This disporoblastic development is common for the genus *Nosema* and is a characteristic of the type species *Nosema bombycis* (see Sprague, 1978). The spores (Fig. 1F) are formed singularly and are not enclosed in a developmental membrane. Spores are binucleate when stained by the method of Weiser (1976). Fresh, unstained spores are shown in Figure 2.

Longitudinal sections of spores, observed under a transmission electron microscope,

have features typical for the genus *Nosema* (Fig. 3). No membranes were seen around spores and spores were binucleate. Most species observed had 11 or 12 polar filament coils of uniform size lying in a single line along the posterior sides of the spores. The polar filament is located in the extreme posterior end of the spore.

Histological sections of heavily infected larvae show that most of the tissues in the body are infected. Midgut epithelium, Malpighian tubules, fat body, salivary glands, muscle tissue, and gonads were all infected. Although no attempt was made to determine time relationships and sequence of tissues infected, it was obvious that midgut epithelium was the first tissue to become infected. The infection then moved to the fat body and salivary glands and later to the other body tissues.

Heavily infected larvae appear lighter in color than healthy larvae, probably because of the infected fat body which develops enlarged lobes when heavily infected.

Fresh, unfixed spores are ovocylindrical (Fig. 2). Measurements of 50 spores gave a range of 3.9 to 5.2 $\mu\text{m} \times$ 1.8 to 3.0 μm and a mean of $4.7 \pm 0.4 \mu\text{m} \times 2.5 \pm 0.3 \mu\text{m}$.

When purslane sawfly larvae were fed spores during the last larval instar, they often pupated and developed into infected adults. Eggs laid by infected females were usually infected. The embryos of partially developed eggs, as well as newly hatched larvae, contained spores. The progeny of these infected females develop much more slowly than healthy larvae. Healthy purslane sawflies require an average of 130 hr at 23.9°C to develop through five larval instars from egg hatch to pupation (Gorske et al., 1977). Twenty larvae, from eggs laid by an infected female, were reared at 23.9°C. After 165 hr, they had only developed to the third larval stadium and all 20 died as third-stadium larvae.

FIG. 1. Stages of life cycle of *Nosema pilicornis*. $\times 1600$. (A) Early binucleate schizonts. (B) Early tetranucleate schizonts. (C) Late binucleate and tetranucleate schizonts. (D) Late octonucleate schizont. (E) Sporont forming two sporoblasts. (F) Mature spores.



FIG. 2. Fresh unfixed spores of *Nosema pilicornis*. Nomarski interference contrast; $\times 2700$.

All of the lepidopterous species to which purslane sawfly microsporidian spores were fed became infected. Larvae of the corn earworm, *Heliothis zea*, fall armyworm, *Spodoptera frugiperda*, yellow wollybear, *Diaecrisia virginica*, and *Autographa precationis* all developed positive infections. However, infections were always very light

and required a long time for spore development. Larvae could only be infected when spores were fed to first-instar larvae and even then only a small percentage of the larvae developed very light infections. Mid-gut epithelium and Malpighian tubules were infected in these lepidopteran larvae.

The purslane sawfly microsporidium is

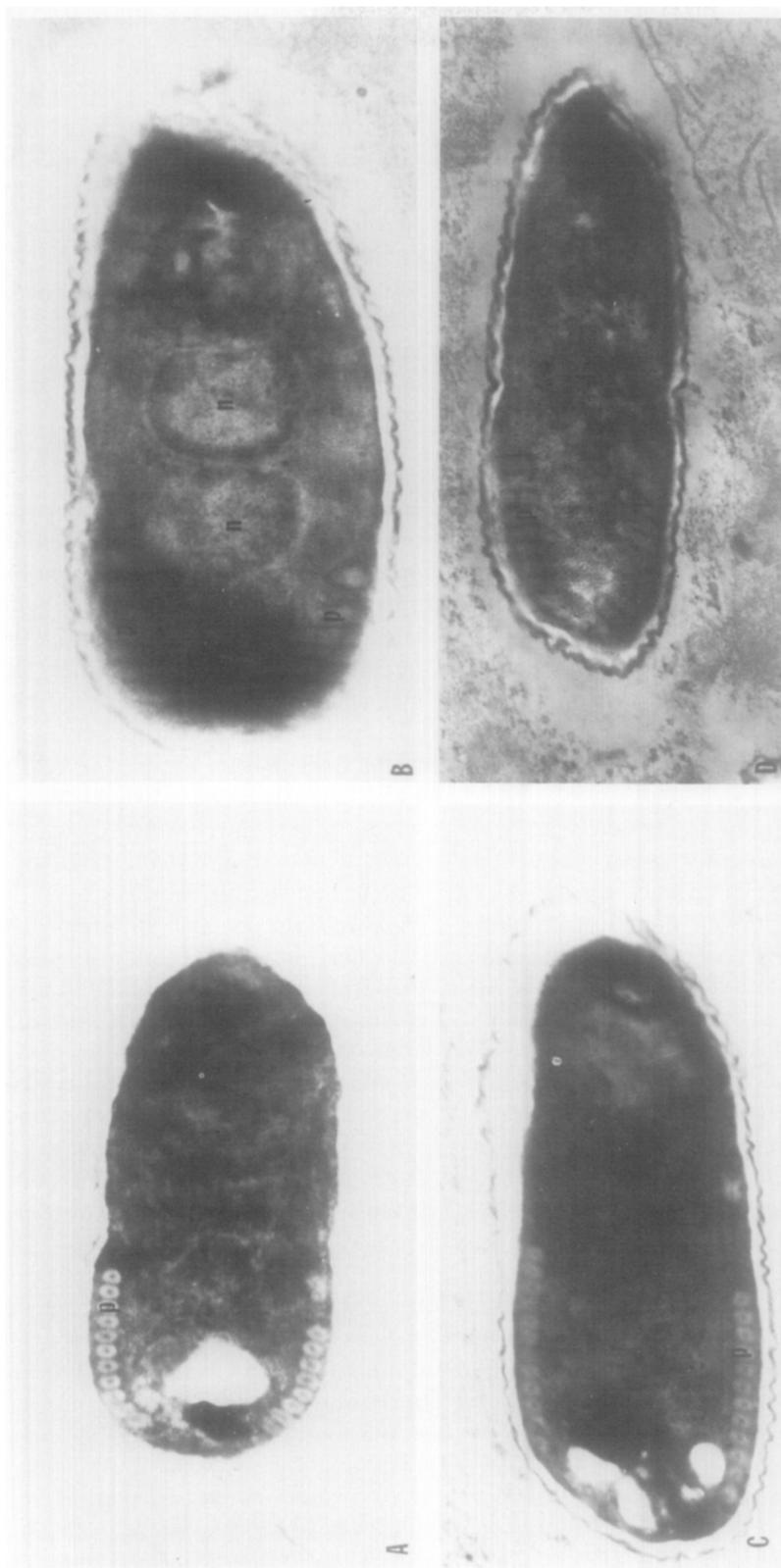


FIG. 3. Electron micrographs of thin longitudinal sections of *Nosema pilicornis* spores. $\times 18,000$. (A) Spore with 11 polar filament coils (P). (B) Spore showing the two nuclei (N) and posterior location of polar filament. (C) Spore with 12 polar filament coils. (D) Spore showing posterior location of polar filament.

widely distributed. It was recovered from purslane sawflies collected all over Illinois, from areas around Chicago in northern Illinois to Collinsville in the southern part of the state. A single collection from Stanford, Florida, also contained infected purslane sawfly larvae.

Three larval collections were made from a field near Urbana, Illinois, during 1975 (Table 1). While only three of the six generations occurring in Urbana were sampled, the incidence of infection appears to be increasing throughout the year. This same trend was observed in horseradish fields around Collinsville, Illinois. Early collections often contained high percentages of infected individuals. These observations were difficult to interpret, because the horseradish fields were often treated with insecticides for control of horseradish pests. This often eliminated purslane sawfly populations for varying periods of time.

DISCUSSION

The genus *Nosema* is characterized by disporoblastic sporogony giving rise to isolated oval spores in contact with host-cell cytoplasm and having binucleated sporoblasts and spores. *N. pilicornis* possesses all these characteristics and, therefore, belongs to the genus *Nosema*.

Several microsporidia have been described from insects in the order Hymenoptera. Some parasitic Hymenoptera are infected by the microsporidia of their hosts and several such relationships have been reported (Blunck, 1952; Brooks, 1973; Hostounský, 1970; Steinhäus and Hughes, 1949; York, 1961). These are specific cases which involve the interaction of host, insect parasite, and microsporidium. Since they are obviously not comparable to *N. pilicornis*, they will not be considered further.

Nosema campoletidis Brooks and Cranford and *Nosema cardiochiles* Brooks and Cranford were described by Brooks and Cranford (1972) as pathogens of the hy-

menopterous parasites, *Campoletis sonorensis* and *Cardiochiles nigroceps*. *N. campoletidis* also infected hymenopterous hyperparasites of *Campoletis sonorensis*, but neither of these microsporidia infected larval *Heliothis zea*, the lepidopterous host of the hymenopterous parasites. Both *N. campoletidis* and *N. cardiochiles* exhibit monosporoblastic spore development and have elongate sporoblasts and spores (Brooks and Cranford, 1972). *N. pilicornis* is disporoblastic and has oval sporoblasts and spores.

Nosema apis Zander is a well-known parasite of the honey bee, *Apis mellifera*. Fantham and Porter (1913) reported that other bees as well as representatives of other insect orders were susceptible to *N. apis*. These reports, however, have never been substantiated, and Kramer (1964) was unable to infect insects other than Hymenoptera with *N. apis*. *N. apis* is primarily a parasite of the alimentary tract of adult bees. Larval bees are not infected by *N. apis*. This is in contrast to *N. pilicornis* which infects most body tissues of all developmental stages of the purslane sawfly. The ultrastructural features of *N. apis* and *N. pilicornis* are also different; spores of *N. apis* have 18 polar filament coils (Wildfuhr and Fritzsch, 1969) while *N. pilicornis* spores have 11 to 12 polar filament coils.

Allen and Buren (1974) observed what appeared to be a *Nosema* infection in the ant, *Solenopsis invicta*, collected in Brazil. They gave no details about the infection but it is very unlikely that this *Nosema* is conspecific with *N. pilicornis*.

Thelohania pristiphorae Smirnoff infects several different sawfly (Tenthredinidae) species (Smirnoff, 1966). This species is easily distinguished from *N. pilicornis* on the basis of generic differences.

We found no reports of either naturally occurring or experimentally induced *Nosema* infections in sawflies (Tenthredinidae). Those microsporidia discussed above, which have been described from other Hy-

menoptera, can be readily distinguished from *N. pilicornis*.

Since *N. pilicornis* will infect lepidopterous larvae, we must consider the possibility that it is conspecific to some of the microsporidia infecting Lepidoptera. *N. pilicornis* resembles a group of *Nosema* species from Lepidoptera such as *N. trichoplusiae* Tanabe and Tamashiro and *N. bombycis* which Nordin and Maddox (1974) considered indistinguishable on the basis of their original descriptions. The spore size, tissues infected, and the developmental stages observed for *N. pilicornis* do not significantly distinguish it from this group of *Nosema* species from Lepidoptera.

N. pilicornis does differ from this group of similar microsporidia in Lepidoptera in the position of the polar filament, the angle of tilt of the polar filament, and the limited infection produced in lepidopteran larvae. The location of the polar filament in *N. pilicornis* is at the extreme posterior end of the spore (Fig. 3), while those *Nosema* which we have observed from Lepidoptera are located more toward the center of the spore (Maddox, unpubl.). We have examined the spore ultrastructure of *Nosema* from the following Lepidoptera: *Nosema* sp. from *Ostrinia nubilalis*, *Nosema* sp. from *Loxagrotis albicosta*, *Nosema* sp. from *Hyphantria cunea*, *N. trichoplusiae* Tanabe and Tamashiro from *Trichoplusia ni*, and *N. invadens* Kellen and Lindegren from *Plodia interpunctella*. All of these *Nosema* spp. have an angle of tilt of the polar filament, determined by the method of Burges et al. (1974), of 40 to 50°. The polar filament of *N. pilicornis* has an angle of tilt of about 80°.

The *Nosema* spp. considered by Nordin and Maddox (1974) produce active infections in lepidopteran larvae and could infect these larvae as late larval instars. When fed to lepidopteran larvae, *N. pilicornis* produced only very light infections and only when fed to early larval instars.

We believe the differences in polar filament location and angle of tilt and differ-

TABLE 1

INFECTION OF PURSLANE SAWFLY, *Schizocerella pilicornis* BY *Nosema pilicornis*. CHAMPAIGN, ILLINOIS, 1975

Date	Number of larvae examined	Number of infected larvae	Percentage infected	Generation
6/9	—	—	—	1
7/22	30	3	10	3
8/15	20	9	45	5
8/27	28	13	46	6

ences in infectivity to lepidopteran larvae convincingly distinguish *N. pilicornis* from similar *Nosema* species in Lepidoptera.

In our opinion, *N. pilicornis* is sufficiently different from all other microsporidia to justify its description as a new species. We propose the name *Nosema pilicornis* in keeping with the specific name of its host *Schizocerella pilicornis*.

In some years at some locations the purslane sawfly is a very effective biological control agent of purslane, an important weed in vegetable crops, but control is not consistent during the early growing season from year to year. This is probably caused in part by pesticide treatments which are sometimes applied to vegetable crops for pest control (Gorske et al., 1976). Purslane sawfly populations also appear to fluctuate because of *N. pilicornis* infections. Those areas with a high incidence of *N. pilicornis* infections in the fall have very low purslane sawfly populations in the spring (Gorske, 1975). This is not surprising, since the progeny of infected sawfly females usually dies as larvae. If the purslane sawfly is to be an effective biological control agent of purslane, it must control purslane early in the season, when it is most serious as a competitive weed. *N. pilicornis* appears to affect the early-season buildup of purslane sawfly populations. Moreover, if the purslane sawfly is introduced into new areas for biological control of purslane, only healthy individuals must be introduced.

The purslane sawfly has great potential

as a biological control agent of purslane, but more research, including additional studies on *N. pilicornis* is needed.

SYSTEMATICS

Host and tissues infected. All stages of the purslane sawfly, *Schizocerella pilicornis*. Most body tissues were infected. Several lepidopteran larvae can be infected experimentally, if spores are fed to first-instar larvae. Infections in Lepidoptera were light and only midgut epithelium and midgut tissues were infected.

Morphology. Spore formation is disporoblastic. Mean spore size \pm SE of fresh unfixed spores was $4.7 \pm 0.4 \mu\text{m} \times 2.5 \pm 0.3 \mu\text{m}$ (range $3.9-5.2 \mu\text{m} \times 1.8-3.0 \mu\text{m}$, $n = 50$). Spores had 11 to 12 polar filament coils and the polar filament was located at the extreme posterior end of the spore. The angle of tilt was about 80° .

Type location. Champaign, Illinois.

Type slides. Slides will be deposited with the Illinois Natural History Survey and Dr. Klaus Reutzler, International Protozoan Type Slide Collection, Smithsonian Institution, Washington, D. C.

Derivation of name. *Nosema pilicornis*. The specific name from the host *Schizocerella pilicornis*.

Differentiating characters. On the basis of host range, limited susceptibility of lepidopteran larvae, number of polar filament coils, location of the polar filament in the spore, and angle of tilt of the polar filament, *N. pilicornis* can be distinguished from all other *Nosema* species described from Hymenoptera and Lepidoptera.

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