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The influence of time of infection on transovarial transmission of Nosema pyrausta (Paillot) in Ostrinia nubilalis (Hübner), and impact of transovarially infected eggs on an egg parasitoid and a predator

Ahmad Said Sajap
Iowa State University

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The influence of time of infection on transovarial transmission of *Nosema pyrausta* (Paillot) in *Ostrinia nubilalis* (Hübner), and impact of transovarially infected eggs on an egg parasitoid and a predator

by

Ahmad Said Sajap

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INTRODUCTION

Microsporida (Microspora) have been shown to be promising biological control agents of insects. One of these is *Nosema pyrausta* (Paillot) (Nosematidae), an obligate intracellular parasite of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). *Nosema pyrausta* is host specific and perpetuates in natural European corn borer populations by vertical and horizontal transmission. All insect stages, egg, larva, pupa and adult are susceptible to infection.

The transmission of a microsporidium either in the egg (transovarial) or contamination on the surface of the egg of the host (transovum) is an advantage to the microsporidium. This process of vertical transmission essentially guarantees a continuous cycle of infection from one generation of the host to the next. The chances of transmission by the way of the egg however, may vary according to the intensity of a microsporidian infection in the maternal parent. Intensity of infection in the parent may also vary with the stage when the microsporidium is acquired and the quantity of microsporidium spores ingested. Quantitative information on such a relationship is greatly needed to understand the epizootiology of *N. pyrausta* in the European corn borer.

The presence of microsporidian parasites inside the eggs of the host however, may pose some problems to parasitoids and
predators which utilize the eggs for their development.

This dissertation reports data from the impact of N. pyrausta on biological parameters of the female host and the success of vertical transmission of the microspidium when different instars of the host are exposed to varying concentrations of microsporidian spores. Also, studies were made on an egg parasitoid, Trichogramma nubilale Ertle and Davis (Hymenoptera: Trichogrammatidae), and a predator Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) when N. pyrausta-infected eggs were either hosts or prey of these insects.

Ostrinia nubilalis (Hübner)

The European corn borer, O. nubilalis is an exotic pest in the United States. It was first collected in 1917 and since has spread to most of the corn growing areas and become one of the most important corn pests. The biology and ecology of the European corn borer are well documented by Brindley and Dicke (1963) and Brindley et al. (1975).

The European corn borer is a small yellowish to brownish moth. An adult female lays her eggs in masses on corn leaves. Eggs hatch in three to four days depending on the temperature. Young larvae feed slightly on the leaf surface, migrate to the whorl and subsequently into the corn stalk in which they pupate. The European corn borer has five instars which last
for about 25 to 30 days and a pupal stage that lasts for about 10 to 15 days. In Iowa, the European corn borer has two generations in a growing season and overwinters as a mature diapausing fifth instar.

The European corn borer has a range of natural enemies. A complex of native and indigenous parasitoids is part of the European corn borer ecology. There are six imported species known to have become permanently established (Baker et al., 1949). In Iowa, three of them, *Eriborus terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae), *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae) and *Lydella thompsoni* Hertig (Diptera: Tachinidae) are commonly found (Lewis, 1982). Among several native parasitoids, *Trichogramma minutum* Riley and *Aplomya caesar* (Aldrich) (Diptera: Tachinidae) have been reported to parasitize the European corn borer (Baker et al., 1949). Recently an egg parastoid, *T. nubilale* was discovered in Delaware (Ertle and Davis, 1975).

There are several insect predators of the European corn borer. The primary ones are predatory coccinellids, chrysopids, syrphids and an anthocorid (Sparks et al., 1966). The chrysopids are primarily represented by *C. carnea*.

Entomopathogens play a role in the regulation of corn borer populations. Two common entomogenous fungi, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Moniliaceae) and *Metarrhizium anisopliae* (Metchnikoff) Sorokin
(Deuteromycotina: Moniliaceae) are readily associated with the corn borer (Brooks and Raun, 1965). Among other entomopathogens, a microsporidium, *N. pyrausta* appears to be the most important debilitating factor in a European corn borer population (Zimmack and Brindley, 1957; Kramer, 1959a; Lewis et al., 1983).

*Nosema pyrausta* (Paillot)

**Historical background**

*Nosema pyrausta* is a microsporidium in the phylum Microspora. Morphologically, the spores are typically ovoidal or ovo-cylindrical, with the length and width varying between 4.2 and 2.1 um respectively (Kramer, 1959b). This microsporidium was first discovered in France by Paillot (1928) and was described as *Perezia pyraustae* Paillot. In 1961, this microsporidium was placed in the genus *Nosema* (Weiser, 1961). In the United States, *N. pyrausta* was first isolated by Steinhaus (1951) from European corn borer specimens collected at Ankeny, Iowa. This microsporidium was probably introduced from Europe along with the host insect.

**Host-range**

Like most microsporidia in the genus *Nosema*, *N. pyrausta* is host specific, and all stages of the host are susceptible to infection. This microsporidium is occasionally found
associated with parasitoids of the host (York, 1961). These parasitoids however, were infected only because they developed in an infected host.

**Life-cycle**

*Nosema pyrausta* has a life cycle typical of the order Microsporida. It is an obligate intracellular parasite unable to live outside the host cell except in the spore stage. In the European corn borer, infection begins with the ingestion of spores by the larva. Once inside the gut, spores germinate and initiate the infection cycle. According to Kramer (1959b) the typical development cycle occurs as follows: "The mature spore germinates with extrusion of the polar filament and the binucleate sporoplasm escapes from the spore. After penetrating suitable tissues of the host, the sporoplasm grows larger and becomes a schizont. At this point two paths of development are possible: (1) the binucleate schizont may continue to grow and develop into a tetranucleate sporoblast, which in turn produces two binucleate sporoblasts by binary fission, each of which develops directly into a spore; or (2) the binucleate schizont may increase in size and undergo one, two, or three nuclear divisions which produce four-, eight-, and 16-nucleate bodies. The four-, eight-, and 16-nucleate bodies may produce chain forms of binucleate daughter schizonts. The eight- and 16-nucleate bodies also may produce binucleate daughter schizonts by exogenous budding. The end
products of chain formation and budding are groups of binucleate schizonts which are distinguishable from the original schizont only in size. Since a gradation in size does exist among single binucleate schizonts, it is reasonable to assume that such forms simply grow and thus become equal to the original schizont."

From the midgut, infections spread to various tissues, particularly the Malpighian tubules, silk glands and reproductive tissues (Zimmack and Brindley, 1957; Kramer, 1959b). Other tissues, such as muscle and neural, and salivary glands can also be infected. These infections result in a debilitated insect and/or mortality. Infected larvae have higher mortalities and slower development rates (Zimmack et al., 1954; Kramer, 1959a; Siegel et al., 1986). While there might be no significant effect on the pupa, the emerging adults appeared to be adversely affected by infection with *N. pyrausta*. Adult longevity, oviposition, fecundity and fertility are greatly reduced (Windels et al., 1976).

*Nosema pyrausta* overwinters in infected diapausing European corn borer larvae and also in fecal material lodged in corn stalks. The longevity of the spore in its extracorporeal environment however, depends upon its ability to survive in the challenging interactions of temperature, moisture and solar radiation (Kramer, 1976).
Transmission of *N. pyrausta*

The subject of transmission of microsporidia is well documented (Canning, 1970; Tanada, 1976; Fine, 1984). In general, transmission of microsporidia occurs when spores are passed from one individual to another. Three routes of transmission may occur in an insect-microsporidia-transmission system. One route is referred to as vertical transmission, which has been defined by Fine (1984) as the direct transfer of infection from a parent to his or her progeny. Vertical transmission may involve the transfer of spores inside the egg (transovarial transmission) or on the external surface of the egg (transovum transmission). The second mode of transmission, horizontal, is the transmission of spores between individuals within or between generations but not directly from parent to offspring. The less common mode of transmission is by vectors such as parasites and parasitoids.

In the *N. pyrausta*-European corn borer system, vertical and horizontal transmissions readily occur. Vertical transmission of *N. pyrausta* in the European corn borer was first observed by Zimmack and Brindley (1957). They found that nurse cells, germarium and follicular epithelium of the ova were infected with the microsporidium. Kramer (1959a) showed that the egg surface may be contaminated by spores and the hatching larvae ingest these spores and become infected. Horizontal transmission is accomplished by larvae feeding on
spore-laden fecal material. The infection of midgut cells by microsporidia causes the midgut cells and spores to slough into the lumen and pass from the body to the fecal material. Lewis (1978) showed that migration by infected European corn borer larvae resulted in a wider spore dispersal through horizontal transmission and consequently a high incidence of N. pyrausta-infected larvae. Andreadis' (1986) showed that in the first generation of the European corn borer, horizontal transmission of N. pyrausta occurred mainly among larvae which inhabit the same corn plant. In the second generation, the European corn borer larvae actively disperse to other corn plants and this results in the spread of infection. Transmission by parasitoid vectors has also been reported. Siegel et al., (1986) demonstrated successful transmission of the spores from infected to uninfected European corn borer larvae by the parasitoid M. grandii.

*Trichogramma nubilale* Ertle and Davis

*Trichogramma nubilale* is an egg parasitoid of the European corn borer. This new species of *Trichogramma* was recently discovered in Delaware by Ertle and Davis (1975). *Trichogramma nubilale* is characterized by its dark brown to black body color, broad female genital capsule, and relatively long male genitalia (Ertle and Davis, 1975).
In the laboratory, *T. nubilale* requires about 9-10 days to complete its development. The adults live for about 9 days. A female can lay 60-75 eggs. More than one egg may be deposited in a single European corn borer egg (Ertle and Davis, 1975; Curl and Burbutis, 1977). In the field, *T. nubilale* overwinters in the pupal stage in the egg of its host (Burbutis et al., 1976; Curl and Burbutis, 1977). In Delaware, adult emergence occurs from late-March to late-April (Burbutis et al., 1976).

A recent survey conducted by Thorpe (1982) showed there were six species of *Trichogramma* found in corn agroecosystems in Maryland. These were *T. minutum* Riley, *T. exiguum* Pinto and Platner, *T. parkeri* Nagarkatti, *T. marylandense* Thorpe, and *T. retorridum* (Girault). However, no *T. nubilale* were collected. This may be due to either the absence of the parasitoid in the surveyed field or the failure of *H. zea* eggs, eggs used as the trap host, to attract a gravid *T. nubilale* female. Curl and Burbutis (1978) suggested that *T. nubilale* may not be host specific, but it may be selective for European corn borer eggs. Laboratory and field studies also indicated that *T. nubilale* demonstrated a high searching ability and host-preference (Curl and Burbutis, 1978; Need and Burbutis, 1979).

Results of recent research indicated that *T. nubilale* has the potential to be used as an effective and reliable
biological control agent of the European corn borer. The parasitoid could feasibly be mass produced and released (Burbutis and Goldstein, 1983). A high parasitization rate (85%) was reported from one of the field releases (Burbutis et al., 1977).

Field releases made by Kanour and Burbutis (1984) showed that a release of 300 females per day per 0.4 ha resulted in 40% parasitization of first generation European corn borer egg masses. Theoretically, they calculated a release of 2,400 and 12,000 females per day per hectare would be needed to achieve 80% parasitization of the first and the second generation European corn borer respectively.

In Europe, inundative releases of *Trichogramma* to control the European corn borer on corn have been carried out with considerable success. The species regularly used were *Trichogramma evanescens* Westwood (Hassan and Heil, 1980; Hassan, 1981) and *Trichogramma maidis* Pintureau and Voegele (Bigler, 1986). *Trichogramma nubilale* however, was not released. Results of laboratory research in Europe have shown that the performance of *T. nubilale* is not encouraging. The parasitoid had a low reproductive capacity and longevity in comparison with other *Trichogramma* (Russo and Voegelle, 1982).
Chrysoperla carnea (Stephens)

*Chrysoperla carnea* known as a common green lacewing, is a general predator of many small soft-bodied arthropods and their eggs. It is a cosmopolitan species occurring in various ecosystems. It is most common in man-altered ecosystems such as an agroecosystem or a cultural landscape. The taxonomic characters of the species have been thoroughly described by Killington (1936), Tauber (1974) and Garland (1985).

*Chrysoperla carnea* has a simple life-history. Eggs are laid on pedicels. At eclosion the larva ruptures the chorion with an egg burster. After a few hours clinging to the chorion, the larva crawls down the pedicel to the substrate. As in all chrysopids, the larva has three free-living larval instars. Tauber (1974) distinguishes the *C. carnea* larva from other species by its dorsal head markings with the anterior end more or less pointed towards the antennal sockets. This campodeiform larva is highly predaceous on aphids, psyllids, thrips and lepidopteran eggs and larvae (Hagen et al., 1976). The fully matured larva spins a spherical cocoon inside which it pupates. The pupa is a decticous type. The molting into an adult occurs through a short pharate adult phase. During the final adult emergence, meconium is discharged through the anus. This small black meconium is the waste matter accumulated in the midgut by the larva during its trophic activity. The alimentary canal runs the full length of the
body and is functionally closed between the midgut and hindgut (Gaumont, 1976). The adult, which is green in color may be distinguished from other species by its forewing venation, which usually possesses five inner series of gradate crossveins (Garland, 1985). It feeds primarily on honeydew and nectar. There are reports however, indicating *C. carnea* adults may also feed on corn pollen (Sheldon and Macleod, 1974). In a temperate region the adult diapaus in response to decreasing photoperiod (Tauber and Tauber, 1973).

Like many other chrysopids, *C. carnea* is a predator, important in the regulation of many insect populations. Its voracious larval feeding behavior has elicited interest in it as a biological control agent and it has consequently been reared in the laboratory. The mass rearing techniques have been described by a number of workers (Hagen and Tassan, 1970; Ridgway et al., 1970; Morrison and Ridgway, 1976; Morrison and King, 1977; Tulisalo, 1978). *Chrysoperla carnea* has been used as a biological control agent in greenhouses and in open fields. In greenhouses, releases of *C. carnea* larvae have been targeted to control pests such as aphids, tetranychid mites, whiteflies and thrips (Scopes, 1969; Hassan, 1977). In open fields *C. carnea* larvae have been used with considerable success to reduce the pest status of the grape mealybug in vineyards, bollworm and tobacco budworm in cotton (Doutt and Hagen, 1949; Ridgway and Jones, 1969) and aphids on vegetables
Applications of sucrose have been used in an attempt to increase numbers of insect predators including *C. carnea*, to suppress populations of the European corn borer (Schiefelbein and Chiang, 1966; Carlson and Chiang, 1973). The results of the experiments were not encouraging.

Like any other insects, *C. carnea* is also subject to attack by a wide range of natural enemies. These include entomophagous arthropods and entomopathogens. Many parasitoids have been recorded from all stages of *C. carnea* (Clancy, 1946; Principi, 1948; Alrouechdi et al., 1984). However, very limited information is available on the natural incidence of entomopathogens in chrysopids.

Entomopathogen-parasitoid-predator interactions

Parasitoids, predators and entomopathogens are the three important agents in biological control. In nature these agents may act independently or complementarily with each other. Thus interactions, which may be advantageous or detrimental, are likely to occur. The resultant effect of the interactions determines the compatibility of these organisms as pest control agents.

Studies on compatibility of parasitoids and predators with entomopathogens are still limited, but gaining more attention recently (Tamashiro, 1967; Brooks, 1973; Jaques and
Morris, 1981; Flexner et al., 1986). Examples of beneficial insects exposed to entomopathogens are listed by Flexner et al. (1986). Tamashiro (1967) categorized interactions between parasitoids and entomopathogens, from a point of view of pest control, as being detrimental, harmless and beneficial. The detrimental effects of a pathogen on a parasitoid occur when the parasitoids are susceptible to the entomopathogen, or the infected host becomes unattractive or unsuitable to the parasitoid for oviposition. The pathogen is said to be harmless and beneficial when the entomopathogen has no or little effect on the parasitoid and when the overall pest control is improved because of the interaction. The same interactions pertain to insect predators. Flexner et al. (1986) generalized the effect of entomopathogens on parasitoids and predators as follows: (1) in all entomopathogens, indirect mortality seems to be more prevalent than direct mortality (2) bacterial and protozoal entomopathogens cause higher direct mortality and (3) direct mortality caused by viruses has yet to be reported.

**Microsporidium-parasitoid interactions**

One of the earliest microsporidium-parasitoid interactions was recognized in *Macrocentrus ancylivorus* Rohwer developing in a microsporidian-infected potato tuberworm *Phthorimaea operculella* (Zeller) (Lepidoptera: Pyralidae) (Allen and Brunson, 1945), in which 40-60% of the parasitoids
failed to emerge as adults (McCoy, 1947). Thompson (1958)
while studying a microsporidian disease of the spruce budworm,
*Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae)
caused by *Nosema fumiferanae* Thompson, observed a significant
parasitoid mortality among the *Apanteles fumiferanae* Viereck
(Hymenoptera: Braconidae) and *Glypta fumiferanae* Viereck
(Hymenoptera: Braconidae) larvae.

Throughout the years, many studies have revealed the
occurrence of microsporidium-parasitoid interactions in which
the parasitoids were detrimentally affected. Even though the
parasitoid might be detrimentally affected by developing in an
infected host, the parasitoid tissue might or might not be
directly invaded by the microsporidium. Thompson (1958)
showed histological evidence that the spores of *N. fumiferanae*
were concentrated in the midgut region of the *A. fumiferanae*
and *G. fumiferanae* larvae and their tissues were not infected.
A similar pattern of indirect infection has also been observed
*Pteromalus puparum* Linnaeus (Hymenoptera: Pteromalidae) in a
microsporidium-infected *Pieris rapae* Linnaeus (Lepidoptera:
Pieridae) (Laigo and Paschke, 1968), *Bonnetia comta* (Fällen)
(Diptera: Tachinidae) in *Vairimorpha* sp. (Microsporida:
Burenellidae)-infected *Agrotis ipsilon* (Hufnagel)
(Lepidoptera: Noctuidae) (Cossentine and Lewis, 1986). In
all cases, it was suggested that the detrimental effect on the
parasitoids was due to accumulation of nondigestible spores in
the parasitoid gut resulting in a fatal nutritional imbalance.

Direct invasion of microsporidia in various tissues of parasitoids developing in an infected host has been recognized from many microsporidium-parasitoid interactions. A few examples are: *Nosema destructor* Steinhaus and Hughes infecting *M. aenlivorus* (Allen and Brunson, 1945); *N. pyrausta* in *Chelonus annulipes* Wesmael (Hymenoptera: Braconidae), *M. grandii* and *L. thompsoni* (York, 1961); *Nosema mesnili* (Paillot) in *Pimpla instigator* (Fallén) (Hymenoptera: Ichneumonidae) (Hostounsky, 1970); *Nosema heliothidis* Lutz and Splendore and *Nosema campoletidis* Brooks and Cranford in *Campoletis soronensis* (Cameron) (Hymenoptera: Ichneumonidae) and *Cardiochiles nigricps* Viereck (Hymenoptera: Braconidae) (Brooks and Cranford, 1972); *Glugea gasti* McLaughlin (Microsporida: Glugeidae) in *Bracon mellitor* Say (Hymenoptera: Braconidae) (Bell and McGovern, 1975); *N. pyrausta* in *Trichogramma evanesens* Westwood (Huger, 1984); and *Nosema epilachnae* Brooks and *Nosema varivestis* Brooks in *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae) (Own and Brooks, 1986).

The susceptibility of a parasitoid to microsporidian invasion of its tissues varies with the stage of parasitoid development and the type of tissue. Infection was not seen in the larval *M. grandii* developing in *N. pyrausta*-infected European corn borer even though the midgut was filled with
spores. Heavy infection in cells of midgut epithelium, fat body, muscles, nerve and Malpighian tubules cells however, occurred in the pupa and the adult (Andreadis, 1980; Cossentine, 1985; Siegel et al., 1986). This pattern of systematic infection has also been observed with two Nosema spp. infecting P. faveolatus (Own and Brooks, 1986). Brooks and Cranford (1972) showed evidence that all stages of C. soronensis and C. nigriceps developing in Heliothis sp. infected with N. heliothidis and N. campoletidis were systematically infected with the microsporidia. The tissue infection in the larval stage, nevertheless was generally lighter than that in the pupa and the adult.

In general, regardless of the mode of infection, the infected parasitoids may exhibit one of several detrimental effects. Infected parasitoids usually experience a high larval and pupal mortality. The adults that do emerge are short lived and exhibit reduced fecundity.

**Microsporidium-predator interactions**

To date little is known about the interactions of entomopathogens and predatory insects. Most of the studies which have been carried out involved mainly bacteria. Very few studies have been done on interactions of viruses, fungi or protozoa with insect predators.

Like the parasitoids, predators may or may not be susceptible to microsporidia. A few insect predators which
have been reported to be susceptible to the microsporidia of their prey are; *Chrysopa californica* Coquillett to *Pleistophora californica* (Steinhaus and Hughes) (Microsporida: Pleistophoridae) (Finney, 1950), *Laricobius erichsonii* Rosenhauer (Coleoptera: Derodontidae) to *Nosema* sp. (Smirnoff and Eichhorn, 1970) and *Notonecta undulata* Say (Hemiptera: Notonectidae) to *Nosema algerae* Vavra and Undeen (Van Essen and Anthony, 1976). In general, insect predators are more resistant to microsporidia. The physical and physiological characteristics of a predator may prevent the invasion of microsporidia.
PART I: THE INFLUENCE OF TIME OF INFECTION ON TRANSOVARIAL TRANSMISSION OF NOSEMA PYRAUSTA (PAILLOT) IN OSTRINIA NUBILALIS (HÜBNER)
INTRODUCTION

The susceptibility of insects to microsporidia may vary with the instars in which the insects are exposed. The European corn borer can acquire an infection of *N. pyrausta* in all instars and/or in the egg. The effects however, are more severe with transovarially infected larvae (Siegel et al., 1986). Likewise Veber and Jasic (1961) working with *Nosema bombycis* Nægeli in *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) have shown that the fecundity of the moths was affected not only by the amount of spores ingested but also the instars in which they were exposed.

Transmission of microsporidia via eggs (transovarial or transovum) is known in many insects. Zimmack and Brindley (1957) showed that *N. pyrausta* infects the gonads of its host and internally contaminates the developing oocytes. Kramer (1959a), observed spores adhering to the surface of eggs. The details of time of infection (instar) and quantity of spores ingested that cause infection in the ovary and subsequent infection in the eggs however, are not well understood. Fine (1984) indicated that successful transmission of a pathogen from the parent to its progeny, is an important factor in the epizootiology of the pathogen. In this context, the present study was undertaken with the objectives to determine the influence of *per os* inoculation time and magnitude of inoculum on pupal weight, fecundity and longevity of female European
corn borer adults, and transovarial transmission of *M. pyrausta* in the European corn borer.
MATERIALS AND METHODS

Eggs from a *N. pyrausta*-free European corn borer colony were allowed to hatch and the larvae were reared on a meridic diet (Guthrie et al., 1985). On days 1, 4, 7, 10 and 13, representing first, second, third, fourth and fifth instars, the larvae were subjected to the following treatments.

**Larval infection:** *Nosema pyrausta* spores were obtained from homogenized tissues of European corn borer larvae. Four dilutions of *N. pyrausta* spore suspensions and a control (distilled water) were prepared in volumetric flasks. Two-tenth ml aliquots from each dilution were pipetted onto the surface of European corn borer diet in 15 ml plastic cups, such that a dosage of 0, 100, 200, 400 and 800 spores per mm² diet surface were obtained. The liquid was swirled and air-dried before the European corn borer larvae were introduced. In experiments with first, second and third instars, five larvae were placed in each cup, in experiments with fourth and fifth instars, three larvae were placed in each cup. The cups were sealed and kept in a walk-in incubator at 27°C and 70% R.H. After 48 hours, 50 larvae from each treatment were removed from the *N. pyrausta* treated diet and transferred individually into glass vials (18 x 65 mm) containing fresh diet. The vials were then sealed with cotton plugs. The vials were returned to the incubator for subsequent larval development and pupation. The pupae were removed from of the
vials, sexed and the female pupae were placed individually into 15 ml plastic cups for adult emergence. Pupal weights were recorded on the second day after pupation to avoid injury.

**Mating and oviposition:** Emerged female moths were paired with uninfected males. One pair was placed in an oviposition cage. The oviposition cage, measuring 60 mm by 90 mm, was made of copper mesh. A wax paper, serving as an oviposition site and a wet paper towel were placed on the top and the bottom of the cage respectively. These papers were secured by Petri dishes. The wax paper with egg masses was removed and replaced daily. The number of eggs laid was recorded. Ten pairs of adults were used in each treatment.

The egg masses were incubated for 3 days, (27°C, 70% RH) after which, the masses from each female were split into two samples. They were placed in 15 ml plastic cups and provided with a 10 mm wet cotton wick. One sample of egg masses was frozen and the other was allowed to hatch. The newly emerged larvae were also frozen. These frozen eggs and larvae were later examined for microsporidiosis.

**Determination of microsporidiosis in the eggs:** Egg mass were scraped from the wax paper and placed in 5 ml beakers containing a 1 ml solution of trypsin in a potassium buffer (pH 8). The beakers were placed in a water-bath at 36 - 38°C for 45 minutes. The trypsin digested the protein and
separated the egg masses into individual eggs. The trypsin and the eggs were poured into a filter funnel and were washed through four changes of distilled water and two changes of 70% ethyl alcohol. The trypsin treated eggs were again frozen until examined for microsporidiosis. The rate of microsporidian infection in the egg was determined by examining smears of 10 randomly selected eggs from each female within a treatment using 400x phase-contrast microscopy. This determination was made on eggs laid during the first seven days of oviposition.

**Determination of microsporidiosis in the larvae:** The rate of microsporidian infection in the larvae was determined from 10 randomly selected larvae from each female within a treatment. The larvae were examined for microsporidia using a method similar to that described for the egg.

**Histology:** Five larvae from each treatment were fixed in alcoholic Bouin's fluid at two day intervals after being exposed to *N. pyrausta*. These larvae were dehydrated in a graded series of ethyl alcohol, cleared in benzene and embedded in paraffin (Lewis et al., 1977). The embedded specimens were serially sectioned at 5 um and stained with Giemsa colophonium (Shortt and Copper, 1948). Two 2 day old female pupae and newly emerged adults, and egg masses from each treatment were also subjected to the same histological procedure except the pupae were double embedded with collodion
Photomicrographs were taken with a Leitz Lablux 20 microscope equipped with 5x4 Polaroid camera.

**Experimental design:** The experiment was designed in a randomized complete block with a split plot arrangement of treatments. The instars were the whole plots and spore concentrations the subplots. The experiment was replicated three times. Biological data; pupal weight, fecundity and longevity of female moths; microsporidian infection rates in egg and larvae were analyzed by analysis of variance and relationships between variables were determined using contrast coefficients.
RESULTS AND DISCUSSION

In this study, where larvae of different instars were exposed to varying concentrations of N. pyrausta spores, the results show that effects of the microsporidium on the host generally varied with the stadia in which they were exposed and with the amount of spores ingested. The larvae which were exposed in their first (1 day old) or second (4 days old) stadium failed to pupate or emerge as morphologically normal adults. This confirms the suggestion of Siegel et al. (1986) that larvae infected in their first and second stadium are more severely affected than larvae acquiring infection in the

Table 1. Effect of Nosema pyrausta (Paillot) on the pupal weight of Ostrinia nubilalis (Hübner) treated in different stadia

<table>
<thead>
<tr>
<th>Spores/mm² diet surface</th>
<th>Pupal wt (mg)ᵃ</th>
<th>Time of infection (Stadium)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Third</td>
</tr>
<tr>
<td>0</td>
<td>104.65</td>
<td>110.25</td>
</tr>
<tr>
<td>100</td>
<td>99.67</td>
<td>106.57</td>
</tr>
<tr>
<td>200</td>
<td>97.66</td>
<td>105.96</td>
</tr>
<tr>
<td>400</td>
<td>95.60</td>
<td>104.81</td>
</tr>
<tr>
<td>800</td>
<td>93.36</td>
<td>101.29</td>
</tr>
</tbody>
</table>

ᵃThe LSD value for comparing two means under the same spore concentration is 5.63 mg.
Table 2. Analysis of variance for pupal weight of *Ostrinia nubilalis* (Hübner) females exposed to varying concentrations of *Nosema pyrausta* (Paillot) in different stadia

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>51.25</td>
<td>25.63</td>
<td>0.46</td>
</tr>
<tr>
<td>Larva (stadium)</td>
<td>2</td>
<td>520.47</td>
<td>260.23</td>
<td>4.68</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>222.25</td>
<td>55.56</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>4</td>
<td>322.50</td>
<td>80.63</td>
<td>7.22**</td>
</tr>
<tr>
<td>Control vs others</td>
<td>(1)</td>
<td>238.59</td>
<td>238.59</td>
<td>21.38**</td>
</tr>
<tr>
<td>Residual</td>
<td>(3)</td>
<td>83.91</td>
<td>27.97</td>
<td>2.51</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>8</td>
<td>83.60</td>
<td>10.45</td>
<td>0.94</td>
</tr>
<tr>
<td>Error (b)</td>
<td>24</td>
<td>267.85</td>
<td>11.16</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant values at the 5 and 1% significance levels indicated by * and ** respectively.

late stadia. These two variables were omitted from further statistical analysis.

Analysis of data on pupal weights of the remaining instars show that the female pupae from larvae exposed to *N. pyrausta* (100 or 800 spores per mm² diet surface) in the third stadium had pupal weights significantly reduced by 4 to 10% (Tables 1 and 2). Female pupae from larvae treated in the fourth and fifth stadia appeared to be less affected. Presumably the time lapse between initial spore ingestion and
Table 3. Effect on *Nosema pyrausta* (Paillot) on the longevity of *Ostrinia nubilalis* (Hübner) females treated in different stadia

<table>
<thead>
<tr>
<th>Spores/mm² diet surface</th>
<th>Longevity (days)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of treatment (Stadium)</td>
</tr>
<tr>
<td></td>
<td>Third</td>
</tr>
<tr>
<td>0</td>
<td>12.34</td>
</tr>
<tr>
<td>100</td>
<td>10.21</td>
</tr>
<tr>
<td>200</td>
<td>8.61</td>
</tr>
<tr>
<td>400</td>
<td>7.95</td>
</tr>
<tr>
<td>800</td>
<td>8.37</td>
</tr>
</tbody>
</table>

ᵃThe LSD value for comparing two means under the same spore concentration is 1.40 days.

pupation was too short for the microsporidium to exert a significant detrimental effect on the pupating larvae. This is in contrast with work of Windels et al. (1976) where they found no significant difference between weights of infected and uninfected pupae. The insects used in their research however, were field collected and the times of infection were not known.

Although *N. pyrausta* infection had no significant effect on female pupal weights, when larvae were treated in the later instars the infection however, did affect adult longevity and fecundity. All females regardless of the stadium in which
Table 4. Analysis of variance for longevity of Ostrinia nubilalis (Hübner) females exposed to varying concentrations of Nosema pyrausta (Paillot) in different stadia

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>16.38</td>
<td>8.19</td>
<td>0.77</td>
</tr>
<tr>
<td>Larva (stadium)</td>
<td>2</td>
<td>18.41</td>
<td>9.20</td>
<td>1.50</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>30.36</td>
<td>7.64</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>4</td>
<td>86.39</td>
<td>21.59</td>
<td>31.35**</td>
</tr>
<tr>
<td>Control vs others</td>
<td>(1)</td>
<td>78.97</td>
<td>78.97</td>
<td>138.54**</td>
</tr>
<tr>
<td>Residual</td>
<td>(3)</td>
<td>7.42</td>
<td>2.47</td>
<td>3.58*</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>5.40</td>
<td>5.40</td>
<td>7.83**</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>(2)</td>
<td>2.02</td>
<td>1.01</td>
<td>1.46</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>8</td>
<td>6.89</td>
<td>0.86</td>
<td>1.25</td>
</tr>
<tr>
<td>Error (b)</td>
<td>24</td>
<td>16.53</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Significant values at the 5 and 1% significance levels indicated by * and ** respectively.

they acquired an infection, had their adult longevity reduced by at least 2 days (Tables 3 and 4). These infected females also laid significantly fewer eggs than the uninfected females (Tables 5 and 6). Previous studies by Zimmack and Brindley (1957), Windels et al. (1976) and Siegel et al. (1986) have shown that infected females lay about 30 to 50% fewer eggs than uninfected females. In this study, it is shown that not
Table 5. Effect of *Nosema pyrausta* (Paillot) on the fecundity of *Ostrinia nubilalis* (Hübner) treated in different stadia

<table>
<thead>
<tr>
<th>Spores/mm² diet surface</th>
<th>Number of eggs per female¹</th>
<th>Time of treatment (Stadium)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Third</td>
</tr>
<tr>
<td>0</td>
<td>450.26</td>
<td>444.30</td>
</tr>
<tr>
<td>100</td>
<td>160.83</td>
<td>217.31</td>
</tr>
<tr>
<td>200</td>
<td>117.31</td>
<td>217.68</td>
</tr>
<tr>
<td>400</td>
<td>105.24</td>
<td>151.56</td>
</tr>
<tr>
<td>800</td>
<td>94.39</td>
<td>146.79</td>
</tr>
</tbody>
</table>

¹The LSD value for comparing two means under the same spore concentration is 60.58 eggs per female.

only the total number of eggs laid by an infected female was significantly reduced but also the reduction evidently depended on the initial concentration of *N. pyrausta* spores that the larva ingested. The mean total of eggs laid by the infected females decreased by as much as 48% with an increase of spore concentration to which the larvae were exposed.

Adults from larvae treated with 100 spores per mm² diet surface during the third, fourth and fifth stadia had a reduction of the mean number of eggs by 64, 51 and 48% respectively.

Similarly the adults from larvae treated with 800 spores per mm² diet surface had a reduction of the mean number by 79,
Table 6. Analysis of variance for fecundity of *Ostrinia nubilalis* (Hübner) treated with varying concentrations of *Nosema pyrausta* (Paillot) in different stadia

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>2819.53</td>
<td>1409.76</td>
<td>0.09</td>
</tr>
<tr>
<td>Larva (stadium)</td>
<td>2</td>
<td>26077.07</td>
<td>13038.53</td>
<td>0.80</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>64932.40</td>
<td>16233.10</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>4</td>
<td>570871.07</td>
<td>142717.77</td>
<td>110.46**</td>
</tr>
<tr>
<td>Control vs others</td>
<td>(1)</td>
<td>549555.72</td>
<td>549555.72</td>
<td>425.35**</td>
</tr>
<tr>
<td>Residual</td>
<td>(3)</td>
<td>21315.35</td>
<td>7105.12</td>
<td>5.49**</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>21053.75</td>
<td>21053.75</td>
<td>16.30**</td>
</tr>
<tr>
<td>Lack of fits</td>
<td>(2)</td>
<td>261.60</td>
<td>130.80</td>
<td>0.10</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>8</td>
<td>18505.23</td>
<td>2313.15</td>
<td>1.79</td>
</tr>
<tr>
<td>Error (b)</td>
<td>24</td>
<td>31008.19</td>
<td>1292.01</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant values at the 5 and 1% significance levels indicated by * and ** respectively.

67 and 63% respectively. The stadium in which the larvae were treated with *N. pyrausta* did not significantly influence the number of eggs laid by the adults even though the adults treated in their third stadium consistently laid fewer eggs than those adults treated in their fourth and fifth stadia.

The daily egg production data from the first seven days of the oviposition period, show that the oviposition rate
decreased significantly over days with the uninfected adults laying significantly more eggs than the infected adults (Table 7). On the average the uninfected adults laid 68 eggs on the first day, while the infected adults previously treated with 100 and 800 spores per mm² diet surface during the larval stage laid a mean number of 45 and 34 eggs, a reduction of 34 to 50% respectively (Fig. 1). On the seventh day, the number of eggs laid by the uninfected adults dropped by 49% compared to day 1 while the number laid by the infected adults dropped by more than 75%. A similar trend in the daily egg production by adults from larvae treated at different stadia was observed because the time (stadia) during which the larvae were exposed to the spores and the interactions were not significant. Figures 2, 3 and 4 illustrate these trends.

Transovarial transmission: The prevalence of transovrially infected eggs was affected by the concentration of spores initially ingested and the day of oviposition (Table 8). The effect of the time (stadia) at which the larvae were exposed had no significant effect on transovarial transmission, even though eggs from adults treated as larvae in the third stadium invariably laid a higher percentage of infected eggs. These adults laid a total of 73% infected eggs over 7 days as compared to about 58% from adults treated in the fourth and fifth stadia. Similarly, Kramer (1959b) found that 54% of the eggs laid by infected adults were infected
Table 7. Analysis of variance for mean daily egg production for 7 days by *Ostrinia nubilalis* (Hübner) treated with varying concentrations of *Nosema pyrausta* (Paillot)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>741.58</td>
<td>370.79</td>
<td>0.22</td>
</tr>
<tr>
<td>Larva (Stadium)</td>
<td>2</td>
<td>3245.64</td>
<td>1622.82</td>
<td>0.94</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>6871.14</td>
<td>1717.79</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>4</td>
<td>53259.46</td>
<td>13314.87</td>
<td>78.44**</td>
</tr>
<tr>
<td>Control vs others</td>
<td>(1)</td>
<td>50711.98</td>
<td>50711.98</td>
<td>298.75**</td>
</tr>
<tr>
<td>Residual</td>
<td>(3)</td>
<td>2547.48</td>
<td>849.16</td>
<td>5.00*</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>2507.27</td>
<td>2507.27</td>
<td>14.77**</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>(2)</td>
<td>40.21</td>
<td>40.21</td>
<td>0.00.24</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>8</td>
<td>1459.91</td>
<td>182.48</td>
<td>1.08</td>
</tr>
<tr>
<td>Error (b)</td>
<td>24</td>
<td>4073.94</td>
<td>169.75</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>6</td>
<td>41072.18</td>
<td>6845.36</td>
<td>98.80**</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>40105.98</td>
<td>40105.98</td>
<td>555.45**</td>
</tr>
<tr>
<td>Quadratic</td>
<td>(1)</td>
<td>719.62</td>
<td>719.62</td>
<td>9.97**</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>(4)</td>
<td>246.58</td>
<td>61.65</td>
<td>0.85</td>
</tr>
<tr>
<td>Day x Larva</td>
<td>12</td>
<td>1031.14</td>
<td>85.93</td>
<td>1.19</td>
</tr>
<tr>
<td>Day x Spore</td>
<td>24</td>
<td>1524.15</td>
<td>63.51</td>
<td>0.88</td>
</tr>
<tr>
<td>Day x Spore x Larva</td>
<td>48</td>
<td>2195.72</td>
<td>45.74</td>
<td>0.63</td>
</tr>
<tr>
<td>Error (c)</td>
<td>180</td>
<td>12996.98</td>
<td>72.21</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant values at the 5 and 1% significance levels indicated by * and ** respectively.
Fig. 1. Mean total egg production by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) during the larval stage

*N. pyrausta* (spores/mm² diet surface)

* = 0
+ = 100
♦ = 200
△ = 400
x = 800
MEAN TOTAL EGG PRODUCTION

DAY OF OVIPosition
Fig. 2. Mean daily egg production by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the third stadium

*N. pyrausta* (spores/mm² diet surface)

- * = 0
- + = 100
- ♦ = 200
- △ = 400
- x = 800
Fig. 3. Mean daily egg production by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the fourth stadium

*N. pyrausta* (spores/mm² diet surface)

- $* = 0$
- $+ = 100$
- $\cdot = 200$
- $\Delta = 400$
- $\times = 800$
MEAN DAILY EGG PRODUCTION

DAY OF OVULATION

0 10 20 30 40 50 60 70 80

1 2 3 4 5 6 7
Fig. 4. Mean daily egg production by Ostrinia nubilalis (Hübner) adults treated with varying concentrations of Nosema pyrausta (Paillot) in the fifth stadium

N. pyrausta (spores/mm² diet surface)

• = 0
+ = 100
♦ = 200
△ = 400
x = 800
MEAN DAILY EGG PRODUCTION

DAY OF OVIPOSITION

0  10  20  30  40  50  60  70  80

0  1  2  3  4  5  6  7
with *N. pyrausta*. Generally, increased spore dosage resulted in a higher percentage of transovarially infected eggs. The overall rate of infection increased linearly by 15% when the spore dosage was increased by a factor of eight.

The prevalence of transovarial transmission was also significantly affected by the day on which the eggs were laid. The percentage of infected eggs increased with each successive day of oviposition (Fig. 5). All adults regardless of the stadium in which the larvae were treated with 100, 200, 400 and 800 spores per mm² diet surface laid 29, 33, 50 and 42% infected eggs on the first day respectively. On the seventh day, the percentage infected eggs increased to 75, 87, 87 and 86%, resulting in a linear increase of 37 to 54%. This supports the suggestion by Siegel et al. (1986) that the rate of transovarial infection may depend on the day of oviposition period on which the eggs were laid.

The analysis also indicates that there were no significant differences between all interacting effects. This was evident from the pattern in the incidence of infection in eggs laid by all infected adults. Figures 6, 7 and 8 illustrate the pattern of infection in eggs laid by adults from larvae treated with varying dosages of *N. pyrausta* in their third, fourth and fifth stadia. All adults developing from larvae treated with 100 spores per mm² diet surface laid significantly fewer infected eggs compared to the control.
Table 8. Analysis of variance for rate of transovarially infected eggs laid over 7 days by *Ostrinia nubilalis* (Hübner) treated with varying concentrations of *Nosema pyrausta* (Paillot)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>3045.49</td>
<td>1522.75</td>
<td>1.29</td>
</tr>
<tr>
<td>Larva</td>
<td>2</td>
<td>12220.87</td>
<td>6110.43</td>
<td>5.16</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>4736.99</td>
<td>1184.25</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>3</td>
<td>8632.22</td>
<td>2877.41</td>
<td>10.51**</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>7605.81</td>
<td>7605.81</td>
<td>27.77**</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>(2)</td>
<td>1026.72</td>
<td>513.36</td>
<td>0.10</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>6</td>
<td>296.79</td>
<td>3.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Error (b)</td>
<td>18</td>
<td>4929.19</td>
<td>273.84</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>6</td>
<td>55264.20</td>
<td>9210.70</td>
<td>117.61**</td>
</tr>
<tr>
<td>Linear</td>
<td>(1)</td>
<td>53538.28</td>
<td>53538.28</td>
<td>683.63**</td>
</tr>
<tr>
<td>Quadratic</td>
<td>(1)</td>
<td>1037.05</td>
<td>1037.05</td>
<td>13.24**</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>(4)</td>
<td>688.87</td>
<td>172.22</td>
<td>2.20</td>
</tr>
<tr>
<td>Day x Larva</td>
<td>12</td>
<td>1468.24</td>
<td>122.35</td>
<td>1.56</td>
</tr>
<tr>
<td>Day x Spore</td>
<td>18</td>
<td>1698.16</td>
<td>94.34</td>
<td>1.20</td>
</tr>
<tr>
<td>Day x Spore x Larva</td>
<td>36</td>
<td>2340.77</td>
<td>65.02</td>
<td>0.83</td>
</tr>
<tr>
<td>Error (c)</td>
<td>144</td>
<td>11277.38</td>
<td>78.32</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Significant values of the 5 and 1% significance levels indicated by * and ** respectively.
Fig. 5. Percent daily infection in eggs laid by Ostrinia nubilalis (Hübner) adults treated with varying concentrations of Nosema pyrausta (Paillot) in the larval stage.

N. pyrausta (spores/mm² diet surface)

- = 100
+ = 200
♦ = 400
△ = 800
Fig. 6. Percent daily infection in eggs laid by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the third stadium

*N. pyrausta* (spores/mm² diet surface)

- = 100
+ = 200
♦ = 400
▲ = 800
Fig. 7. Percent daily infection in eggs laid by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the fourth stadium.

*N. pyrausta* (spores/mm² diet surface)

-  = 100
+  = 200
♦  = 400
△  = 800
Fig. 8. Percent daily infection in eggs laid by *Ostrinia nubilalis* (Hübner) adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the fifth stadium

*N. pyrausta* (spores/mm² diet surface)

- = 100

+ = 200

♦ = 400

△ = 800
throughout the seven days of oviposition. At this concentration, the percentages of infection on the first day of oviposition were 42, 24 and 22% when the adults from larvae were treated in the third, fourth and fifth stadia respectively. On day seven, these percentages increased to 85, 66 and 74%. This is a significant increase of ca. 43 to 52%. The increase in the percentage of infection in eggs from adults treated as larvae with the higher dosages was also within this range.

**Vertical (transovarial plus transovum) transmission:** The data on the prevalence of vertical transmission were collected from neonates emerging from eggs laid during the first three days of oviposition. These data are similar to those collected from infected eggs in the previous study. The analysis shows that the percentage of infection in these neonates was dependent on the spore dosage to which the larvae were exposed and the day of oviposition by the adults. The effect of the stadia in which the larvae were exposed was not significantly different (Table 9). The average percentages of infection for the three day period, with treatments at 100, 200, 400 and 800 spores per mm² diet surface were 71, 77, 83 and 81% respectively.

The analysis also shows that the percent of infection increased linearly with the day of oviposition. This linear response however, differed significantly with different stadia
Table 9. Analysis of variance for rate of vertically infected eggs laid over 3 days by *Ostrinia nubilalis* (Hübner) treated with varying concentrations of *Nosema pyrausta* (Paillot)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F values&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>2953.45</td>
<td>1476.73</td>
<td>0.92</td>
</tr>
<tr>
<td>Larva</td>
<td>2</td>
<td>10176.86</td>
<td>5088.43</td>
<td>3.18</td>
</tr>
<tr>
<td>Error (a)</td>
<td>4</td>
<td>6405.53</td>
<td>1601.38</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>3</td>
<td>2554.42</td>
<td>851.47</td>
<td></td>
</tr>
<tr>
<td>Linear (1)</td>
<td></td>
<td>1929.85</td>
<td>1929.85</td>
<td>8.62**</td>
</tr>
<tr>
<td>Lack of fit (2)</td>
<td></td>
<td>624.57</td>
<td>312.29</td>
<td>1.39</td>
</tr>
<tr>
<td>Spore x Larva</td>
<td>6</td>
<td>634.35</td>
<td>105.73</td>
<td>0.47</td>
</tr>
<tr>
<td>Error (b)</td>
<td>18</td>
<td>4028.56</td>
<td>223.81</td>
<td></td>
</tr>
<tr>
<td>Day (1)</td>
<td>2</td>
<td>7405.51</td>
<td>3702.75</td>
<td>36.25**</td>
</tr>
<tr>
<td>Linear (1)</td>
<td></td>
<td>7384.57</td>
<td>7384.57</td>
<td>72.30**</td>
</tr>
<tr>
<td>Lack of fit (1)</td>
<td></td>
<td>20.94</td>
<td>20.94</td>
<td>0.21</td>
</tr>
<tr>
<td>Day x Larva</td>
<td>4</td>
<td>1687.08</td>
<td>421.77</td>
<td>4.13**</td>
</tr>
<tr>
<td>Day&lt;sub&gt;1&lt;/sub&gt; x Larva (2)</td>
<td></td>
<td>1352.94</td>
<td>676.47</td>
<td>6.62**</td>
</tr>
<tr>
<td>Day&lt;sub&gt;1&lt;/sub&gt; (5th vs oth.) (1)</td>
<td></td>
<td>1349.16</td>
<td>1349.16</td>
<td>13.21**</td>
</tr>
<tr>
<td>Day&lt;sub&gt;1&lt;/sub&gt; (4th vs 3rd) (1)</td>
<td></td>
<td>3.78</td>
<td>3.78</td>
<td>0.04</td>
</tr>
<tr>
<td>Day&lt;sub&gt;2&lt;/sub&gt; x Larva (2)</td>
<td></td>
<td>334.14</td>
<td>169.07</td>
<td>1.63</td>
</tr>
<tr>
<td>Day x Spore</td>
<td>6</td>
<td>133.60</td>
<td>22.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Day x Spore x Larva</td>
<td>12</td>
<td>424.66</td>
<td>35.39</td>
<td>0.35</td>
</tr>
<tr>
<td>Error (c)</td>
<td>48</td>
<td>4902.55</td>
<td>102.14</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant values at the 5 and 1% significance levels indicated by * and ** respectively.
treated. Further analysis shows that the linear increase in the percentage of neonates infected from eggs laid by adults treated with *N. pyrausta* in the fifth stadium was significantly different from those in the third and fourth stadia (Fig. 9). The infection rate increased by 33% from day one to day three in neonates from adults treated as larvae in the fifth stadium compared to 14% increase in neonates from adults treated as larvae in the third and the fourth stadia.

Even though the stadium in which the larvae were exposed to *N. pyrausta* had no significant effect, the adults treated as larvae in the fifth stadium invariably produced fewer infected larvae. Treatment with 100 spores per mm² diet surface in the third, fourth and fifth stadia produced subsequent infections in neonates of 70, 76 and 42% in day one respectively. These rates increased to 85, 94 and 74% by day three. When the corresponding larvae were treated with 800 spores per mm² diet surface, the infection of neonates on day one was 73, 82 and 53% and on day three was 94, 94 and 88% (Figs. 10, 11 and 12).

European corn borer larvae commonly consume the chorion at eclosion and thereby increase the intensity and incidence of infection. This study shows that the pattern of larval infection in neonates from the first three days of oviposition was 18% higher than the infection in the eggs from the same period (Table 10). The larval behavior coupled with the fact
Fig. 9. Percent daily infection in *Ostrinia nubilalis* (Hübner) larvae hatched from eggs laid by adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the third, fourth and fifth stadia.

Stadium

- = Third
+ = Fourth
♦ = Fifth
DAY OF OVIPSOTION

% INFECTION

1  2  3
Fig. 10. Percent daily infection in Ostrinia nubilalis (Hübner) larvae hatched from eggs laid by adults treated with varying concentrations of Nosema pyrausta (Paillot) in the third stadium

N. pyrausta (spores/mm² diet surface)

• = 100
+ = 200
♦ = 400
△ = 800
Fig. 11. Percent daily infection in *Ostrinia nubilalis* (Hübner) larvae hatched from eggs laid by adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the fourth stadium

*N. pyrausta* (spores/mm² diet surface)

- • = 100
- •+ = 200
- •† = 400
- •△ = 800
Fig. 12. Percent daily infection in *Ostrinia nubilalis* (Hübner) larvae hatched from eggs laid by adults treated with varying concentrations of *Nosema pyrausta* (Paillot) in the fifth stadium

*N. pyrausta* (spores/mm² diet surface)

- • = 100
- • = 200
- • = 400
- △ = 800
Table 10. Percentage of infection by *Nosema pyrausta* (Paillot) in eggs and larvae produced by infected *Ostrinia nubilalis* (Hübner) adults treated at different instars on day one and three of oviposition

<table>
<thead>
<tr>
<th>Spores/mm² surface diet</th>
<th>Percent infection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1ᵃ</td>
<td>Day 3ᵇ</td>
</tr>
<tr>
<td></td>
<td>Egg (%)</td>
<td>Larva (%)</td>
</tr>
<tr>
<td>100</td>
<td>29.45</td>
<td>62.73</td>
</tr>
<tr>
<td>200</td>
<td>33.85</td>
<td>66.42</td>
</tr>
<tr>
<td>400</td>
<td>41.52</td>
<td>74.81</td>
</tr>
<tr>
<td>800</td>
<td>45.59</td>
<td>69.81</td>
</tr>
</tbody>
</table>

ᵃThe LSD value for comparing means under the same spore concentration for day 1 is 13.23%.
ᵇThe LSD value for comparing means under the same spore concentration for day 3 is 10.75%.

that microsporidia multiply rapidly after embryogenesis of the host may be in part, the reasons for the relatively higher rate of neonate infection.

Even though the incidence of infection in the neonate was relatively higher, the pattern of infection between these two stages was similar. Indications are that the incidence of infection in the progeny is directly related to an increase in the intensity of infection in the female parent. Furthermore, an infection in the parent depends on the initial number of spores ingested. As the microsporidia continuously multiply,
more reproductive tissues become infected. Consequently, the chances of transovarial and transovum transmission of \textit{N. pyrausta} are greatly increased. It is also apparent, although not significantly different, that the increase in the incidence of infection, by transovarial and/or transovum means was relatively lower in the adults developing from larvae treated in an early stadium than in adults developing from larvae treated in a later stadium. It is probable that at the onset of oviposition, the intensity of infection in the adults developing from larvae treated in an early stadium had almost reached its maximum, including infection in reproductive tissues thus no additional tissues were available to be infected. Adults developing from larvae treated in later stadia, had fewer tissues infected and thus there was a greater potential for additional infection and multiplication of \textit{N. pyrausta}. Histological observations presented in the succeeding section support this premise.

\textbf{Histology:} All adults, regardless of the stadia in which the corresponding larvae were exposed to \textit{N. pyrausta} spores, acquired infection in their reproductive tissues. Infections were evident in the reproductive tissues in all larvae seven days after exposure to \textit{N. pyrausta} spores. Larvae exposed during the first, second and third stadia acquired an infection in their ovaries while they were still in the larval stage whereas there was no infection in insects exposed during
the fourth and fifth stadia until the pupal stage. These observations are very significant in reference to the work by Zimmack (1956) in which he found no infection in the ovaries of immature European corn borers, even though tissues of the Malpighian tubules, silk glands and muscles were heavily infected with this microsporidium. He assumed that infection of the reproductive tissues occur during the late pupal stage.

First instars exposed to *N. pyrausta* had an infection initially in the outer epithelial cells (Fig. 13). The ovary consists of a mass of undifferentiated germ cells enclosed in the ovarian sac through the early third stadium of development. The infection remained in the epithelial cells until the insects reached the fourth and fifth stadia. At this time of the insects development, the ovary had differentiated into four distinct ovarioles, each surrounded by layers of epithelial cells. At this time the epithelial, stroma and germ cells were also infected in those larvae that had a more intense infection of *N. pyrausta* (Figs. 14 and 15). This massive destroying of germ cells by the accumulating microsporidia directly affects the ovarian development and subsequent oogenesis.

The process of histolysis and histogenesis occurring during the pupal stage of the host, apparently did not hinder further infection of the reproductive tissues by the microsporidium. *Nosema pyrausta* spores were found in the
Fig. 13. *Nosema pyrausta* (Paillot) spores infecting an ovary of a third instar *Ostrinia nubilalis* (Hübner) (400X)

Os = ovarian sac; Ov = ovariole; sp = spore
Fig. 14. *Nosema pyrausta* (Paillot) spores infecting stroma of a fifth instar *Ostrinia nubilalis* (Hübner) (400X)

El = epithelial layer; Ov = ovariole; sp = spore; St = stroma

Fig. 15. *Nosema pyrausta* (Paillot) spores infecting germ cells, stroma and epithelial cells of a fifth instar *Ostrinia nubilalis* (Hübner) (400X)

El = epithelial layer; Ov = ovariole; sp = spore
epithelial cells, stroma and germ cells (Figs. 16 and 17). Larvae exposed to *N. pyrausta* during the fifth stadium however, had a less intense or no infection at all. By this stage, the ovary had grown to a considerable length and had developed to an extent that follicles were observed in the lower region of the ovarioles.

In an adult the ovary, which is morphologically different from that in the immature stage, had grown to a considerable size and developed into strings of follicles. In this polytrophic type of ovary, each follicle consists of an oocyte and a number of trophocytes. This complex is interconnected by ring canals (Berry, 1985). At the promixal end of the ovariole, there is an epithelial plug which evidently constrains the follicles within the ovarioles prior to oviposition (Fig. 18).

All tissues within the germarium and the vitellarium of the ovariole are susceptible to *N. pyrausta* infections. Figure 19 shows *N. pyrausta* infection within the germ cells and epithelial cells in the germarium. In the vitellarium, where oocytes and trophocytes are enveloped forming follicles, *N. pyrausta* spores were observed (Fig. 20). As the follicle matures the oocyte has a greater cytoplasmic volume than does the trophocyte. At this stage of development *N. pyrausta* spores were detected in both the oocyte and the trophocytes (Fig. 21). Presumably, the microsporidium had moved with the
Fig. 16. *Nosema pyrausta* (Paillot) spores infecting epithelial cells and stroma of an *Ostrinia nubilalis* (Hübner) pupa (400X)

El = epithelial layers; Ov = ovariole; sp = spore; St = stroma

Fig. 17. *Nosema pyrausta* (Paillot) spores infecting germ cells of an *Ostrinia nubilalis* (Hübner) pupa (400X)

El = epithelial layers; Ov = ovariole; sp = spore; St = stroma
Fig. 18. Follicles within ovarioles of an *Ostrinia nubilalis* (Hübner) adult (250X)

Ep = epithelial plug; Oc = oocyte; Tc = trophocyte

Fig. 19. *Nosema pyrausta* (Paillot) spores infecting germ cells and epithelial cells in germarium of an *Ostrinia nubilalis* (Hübner) adult (450X)

El = epithelial layers; Ov = ovariole; sp = spore; St = stroma
Fig. 20. *Nosema pyrausta* (Paillot) spores infecting trophocytes of an *Ostrinia nubilalis* (Hübner) adult (400X)

Fe = follicular epithelium; Oc = oocyte; sp = spore; Tc = trophocyte

Fig. 21. *Nosema pyrausta* (Paillot) spores infecting oocyte and trophocytes of *Ostrinia nubilalis* (Hübner) adult (400X)

Fe = follicular epithelium; Oc = oocyte; sp = spore; Tc = trophocyte
nutrients from the trophocytes to the oocyte through the intercytoplasmic connections. Zimmack (1956) indicated that the trophocytes were the chief site of infection from which *N. pyrausta* spores spread to the oocyte. It has been indicated that *(Nosema)=Vairimorpha plodiae* Kellen and Lindegren invasion in oocytes of the Indian-meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) was preceded by multiplication in the associated trophocytes (Kellen and Lindegren, 1973). In this study, it was not determined whether or not *N. pyrausta* multiplied in the trophocytes prior to movement to the oocyte.

As the follicles mature, the trophocytes cease further growth and are compressed into a narrow disc by the oocyte. By this stage, the follicular epithelium has completely enveloped the oocyte and the potential of a transovarial transmission of *N. pyrausta* has been determined. Figure 22 shows *N. pyrausta* spores trapped in the oocyte and the degenerating trophocytes with the remaining microsporidia left outside the oocyte. Within the oocyte, the microsporidia are concentrated in the central ooplasm and are seldom found in the cortical ooplasm (Figs. 23 and 24). This phenomenon agrees with the observations of Zimmack (1956), Brooks (1968) and Kellen and Lindegren (1973) studying the European corn borer, the corn earworm, *Heliothis zea* (Bodie) (Lepidoptera: Noctuidae) and the Indian-meal moth respectively. Two days
Fig. 22. *Nosema pyrausta* (Paillot) spores in oocyte and in degenerating trophocytes (400X)

Fe = follicular epithelium; Oc = oocyte; sp = spore; Tc = trophocyte
Figs. 23 and 24. *Nosema pyrausta* (Paillot) spores concentrated in central ooplasm

Fe = follicular epithelium; Oc = oocyte; sp = spore

Fig. 23: 250X
Fig. 24: 400X
after the egg was laid, the microsporidia were no longer confined to the central region of the yolk. Figure 25 shows a two day old egg with an embryo developing within N. pyrausta-infected yolk.

The aggregation of microsporidia in the central ooplasm might be important in the host-microsporidium relationship. This localization, as indicated by Stempell (1909) studying N. bombycis in B. mori prevents the microsporidia from interfering with the formation of embryonic organs during embryogenesis. Kellen and Lindegren (1973) suggested that microsporidia in the yolk remain dormant until susceptible organs developed during embryogenesis.

Accessory organs of the reproductive system were also infected with N. pyrausta however, infection was not uniform. Figure 26 shows N. pyrausta infections occurred randomly in epithelial cells of the common oviduct. It is probable that these infected tissues serve as potential sources of microsporidia to contaminate the egg and allow for transovum transmission. The surface of the chorion might be contaminated with the microsporidium as the egg escapes from the follicular epithelium and moves posteriorly through ruptured epithelial plugs into the oviduct and out through the vagina. Kramer (1959a) observed N. pyrausta spores lodged in the foveae of the chorion of the European corn borer egg.
Fig. 25. **Nosema pyrausta** (Paillot) spores in yolk of an **Ostrinia nubilalis** (Hübner) egg (1000X)

Em = embryo; sp = spore; Yk = yolk

Fig. 26. **Nosema pyrausta** (Paillot) spores in epithelial cells of the reproductive tract of **Ostrinia nubilalis** (Hübner) adult (400X)

sp = spore
In summary, the results of this study corroborate the previous works of Zimmack and Brindley (1957), Kramer (1959a), Windels et al. (1976) and Siegel et al. (1986), that an infection of *N. pyrausta* in early instars impacts directly causing abnormal pupation, emergence and mortality. An infection is most severe when acquired in the first or second instar; many such larvae died prior to adult emergence and those that did emerge did not oviposit. An infection of later instars results in a reduction in pupal weight, fecundity and longevity. These effects appear to be dependent on the dosage of spores initially ingested by the corresponding larvae. The rate of *N. pyrausta* infection in the progeny of infected adults also varied with the dosage of spores initially ingested during the stadium in which the larvae were exposed. The data also support observations made by Andreadis (1984), where he found higher levels of infection occurred when the European corn borer population was low.
PART II: THE IMPACT OF NOSEMA PYRAUSTA (PAILLOT) ON AN EGG PARASITOID, TRICHOGRAMMA NUBILALE ERTLE AND DAVIS
INTRODUCTION

*Trichogramma nubilale* is an egg parasitoid of the European corn borer, *O. nubilalis*. A considerable amount of research has been conducted on its biology and host relationships since its discovery and subsequent description by Ertle and Davis (1975). *Trichogramma nubilale* is well adapted to the European corn borer (Burbutis et al., 1977), showing a high preference for European corn borer eggs (Curl and Burbutis, 1978). A mass release of *T. nubilale* against the European corn borer has been made with considerable success (Kanour and Burbutis, 1984). Long term successes in suppressing the European corn borer with *T. nubilale* in a corn agroecosystem however, are questionable. It is known that a large segment of the natural populations of European corn borer are infected with a debilitating microsporidian pathogen, *N. pyrausta* (Kramer, 1959c; Peairs and Lilly, 1974; Hill and Gary, 1979; Andreadis, 1986). This microsporidium has also been found associated with or infecting several parasitoids associated with the European corn borer, i.e., *C. annulipes* (York, 1961) and *M. grandii* (Andreadis, 1980; Siegel et al., 1986; Cossentine and Lewis, 1987) *T. evanescens* (Huger, 1984) and *L. thompsoni* (Cossentine, 1985). It is speculated that this microsporidium is a factor in the disappearance of *L. thompsoni* (Hill et al., 1978) and the decline of *M. grandii* (Andreadis, 1980; 1982). Heretofore the
interactions between T. nubilale and N. pyrausta were not known. This study was conducted to assess the susceptibility of T. nubilale developing in N. pyrausta-infected European corn borer eggs and its impact on fecundity of the emerging adults.
MATERIALS AND METHODS

*Trichogramma nubilale* used in this study were obtained from Dr. Paul P. Burbutis, Department of Entomology and Applied Ecology, University of Delaware, Newark. These parasitoids were reared continuously on European corn borer egg masses. The colonies were kept in plastic containers (5 cm x 12 cm) with the open ends covered with perforated polyethylene lids. Honey was streaked on the inside as a food source for the adult parasitoids. The rearing and experiments were conducted in a cam-operated environmental chamber (25 ± 2°C, 70 ± 5% RH and 16L : 8D).

**Impact of *N. pyrausta*-infected eggs on the development of *T. nubilale***: Five 0-24 hr old *N. pyrausta*-infected and-uninfected European corn borer egg masses (ca. 20 eggs per mass) were placed separately in an oviposition unit. A unit consisted of a clear 30-ml plastic cup with a hole on the side plugged with cotton and covered with a disc of no. 1 filter paper secured with a perforated polyethylene lid. Newly emerged *Trichogramma* adults reared from uninfected host eggs were aspirated individually into a number 2 gelatin capsule. The captured adults were sexed and a pair was introduced into each oviposition unit. Honey was provided as an adult food. On day seven after parasitization, the number of parasitized European corn borer eggs were recorded and held for *T. nubilale* emergence. After all progeny of each female had
emerged and died, individuals from each unit were counted and 
sexed. The parasitized European corn borer eggs were also 
checked for unemerged *T. nubilale*. The experiment was 
replicated three times with 10 pairs per replicate.

**Impact of *N. pyrausta* on the fecundity and longevity of 
*T. nubilale***: One pair of *N. pyrausta*-infected and one pair of 
uninfected *T. nubilale* (caged separately) were offered 
uninfected European corn borer egg masses for parasitization. 
Unless stated otherwise, the procedures were similar to those 
used in the emergence test. Ovipositing females were removed 
from the cage after death, smeared on glass slides and 
examined for microsporidiosis. The experiment was replicated 
four times with 10 pairs per replicate.

The possibility of transovarial transmission of 
*N. pyrausta* by *T. nubilale*, was determined by randomly 
selecting female and male progeny from infected *T. nubilale* 
adults and examining them for microsporidiosis. Twenty-five 
females and 25 males were selected per replicate. Data from 
all experiments were analyzed by analysis of variance.

**Histology**: Several *N. pyrausta*-infected European corn 
borer eggs were offered to adult female *T. nubilale* for 2 
hours for parasitization. The parasitized eggs were selected 
at intervals of 1 day post-parasitization for 10 consecutive 
days and fixed in alcoholic Bouin’s fluid for 24 hours. The 
specimens were dehydrated in a series of ethyl alcohol,
cleared in benzene and embedded in Paraplast\textsuperscript{R}. Serial sections were cut (5 um) and stained with a modified colophonium Giemsa stain (Shortt and Cooper, 1948).
RESULTS AND DISCUSSION

Analysis of the data showed that *T. nubilale* females parasitized an equal number of *N. pyrausta*-infected and -uninfected eggs (Table 11). The length of the development period of the parasitoid emerging from uninfected and infected eggs was not significantly different. There was however, a significant decrease in adult emergence of about 36% due to *N. pyrausta* but the sex ratio of the emerged adults did not differ significantly.

Histological studies showed that *T. nubilale* deposited more than one egg in the yolk of an European corn borer egg.

Table 11. Mean number of eggs parasitized, eclosion (day), number of adults and sex ratio of *Trichogramma nubilale* Ertle and Davis emerging from *Nosema pyrausta* (Paillot)-infected and -uninfected *Ostrinia nubilalis* (Hübner) egg masses

<table>
<thead>
<tr>
<th>Q. nubilalis egg masses</th>
<th>Uninfected</th>
<th>Infected^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eggs parasitized</td>
<td>51.00 a</td>
<td>48.00 a</td>
</tr>
<tr>
<td>Eclosion (Day)</td>
<td>12.00 a</td>
<td>12.00 a</td>
</tr>
<tr>
<td>Number of adults emerged</td>
<td>47.82 a</td>
<td>30.70 b</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>6.8:1 a</td>
<td>5.1:1 a</td>
</tr>
</tbody>
</table>

^aMeans in the same row followed by the same letter are not significant at 0.05 level of significance.
Fig. 27. Eggs of *Trichogramma nubilale* Ertle and Davis in the yolk of an *Ostrinia nubilalis* (Hübner) egg (250X)

Tn = *T. nubilale* egg; Yk = yolk

Fig. 28. A 1 day old *Trichogramma nubilale* Ertle and Davis embryo developing in a *Nosema pyrausta* (Paillot) -infected *Ostrinia nubilalis* (Hübner) egg (400X)

sp = spore; Tn = *T. nubilale*; Yk = yolk
Figures 27 and 28 show the eggs of *T. nubilale* and a *T. nubilale* embryo developing in a *N. pyrausta*-infected European corn borer egg respectively. As the embryo developed into a larva and feeding was initiated the microsporidian spores entered the alimentary system (Fig. 29). Additional spores were ingested with the host yolk as development progressed. Serial sections of a fully developed larva, 4 days old, shows that the larva had completely devoured the yolk of the host and the gut lumen was filled with the yolk and *N. pyrausta* spores (Fig. 30). No infection of the parasitoid tissues were seen at this stage of development. By day seven, the parasitoid had completed the larval stage and entered the pupal stage. At this time of development, presumably the parasitoid had ceased feeding. The spores that had been ingested during the larval stages were concentrated in the gut lumen and the gut epithelium (Fig. 31). By day 10, 2 days before imaginal eclosion, the microsporidian infection had spread from the midgut to adjacent tissues; gut epithelium, connective, muscle and neural (Figs. 32 and 33). A massive invasion by the microsporidium was observed in an adult. In a heavily infected adult, the whole abdomen was filled with spores (Fig. 34). This pattern of infection was similar to that of *N. pyrausta* in *T. evanescens* (Huger, 1984).

The reduction in the adult emergence rate from the infected host eggs was due to the microsporidian pathogen
Fig. 29. *Nosema pyrausta* (Paillot) spores in the gut lumen of a 2 day old *Trichogramma nubilale* Ertle and Davis larva (400X)

Gl = gut lumen; sp = spore

Fig. 30. *Nosema pyrausta* (Paillot) spores in the gut lumen of a 4 day old *Trichogramma nubilale* Ertle and Davis larva (400X)

sp = spore; Tn = *T. nubilale*
Fig. 31. *Nosema pyrausta* (Paillot) spores infecting gut epithelium of a *Trichogramma nubilale* Ertle and Davis pupa (400X)

G1 = gut lumen; sp = spore

Fig. 32. *Nosema pyrausta* (Paillot) spores infecting muscles of a *Trichogramma nubilale* Ertle and Davis pupa (400X)

M = muscle; sp = spore; Tn = *T. nubilale*
Fig. 33. *Nosema pyrausta* (Paillot) spores in the neural tissue in the head of *Trichogramma nubilale* Ertle and Davis pupa (400X)

Nt = neural tissue; sp = spore; Tn = *T. nubilale*

Fig. 34. Abdomen of *Trichogramma nubilale* Ertle and Davis adult filled with *Nosema pyrausta* (Paillot) spores (400X)

sp = spore
which impaired the larval-pupal development of the parasitoid. It is evident from the histopathology studies that *N. pyrausta* not only reduced the volume of food occupying the gut lumen of the parasitoid larva but also caused infections in the pupal stage resulting in reduced emergence. Similar results have been reported to occur in several host-parasitoid-microsporidium interactions such as *C. soronesis* (Brooks and Cranford, 1972) *M. grandii* (Andreadis, 1980; Cossentine, 1985), and *P. foveolatus* (Own and Brooks, 1986). In these cases, mortality occurred primarily in the pupal stage. Huger (1984) however, showed that development and emergence rates of *T. evanescens* were not affected by the host microsporidium.

Data on adult longevity, number of host eggs parasitized, adult emergence, progeny and sex-ratio are given in Table 12. There was a small, though statistically nonsignificant, decrease in the longevity of both infected females and males and the number of European corn borer eggs parasitized by infected females. The eggs parasitized by *N. pyrausta*-infected *T. nubilale* however, produced a significant reduction in the number of adults by 50% and in the number of progeny by 41%.

The reduction in the fecundity of infected *T. nubilale* females however, was not as great as that of *T. evanescens* infected with *N. pyrausta* (Huger, 1984). According to Own and Brooks (1986) the variation in fecundity of *Nosema-*
Table 12. Mean longevity, number of eggs parasitized, number of adults emerged, total progeny, and sex ratio of *Nosema pyrausta* (Paillot)-infected and -uninfected *Trichogramma nubilale* Ertle and Davis

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Infected(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female longevity</td>
<td>10.80 a</td>
<td>10.39 a</td>
</tr>
<tr>
<td>Male longevity</td>
<td>6.05 a</td>
<td>5.59 a</td>
</tr>
<tr>
<td>Number of ECB eggs parasitised</td>
<td>46.41 a</td>
<td>37.61 a</td>
</tr>
<tr>
<td>Number of adults emerged</td>
<td>52.76 a</td>
<td>26.63 b</td>
</tr>
<tr>
<td>Number failed to emerge</td>
<td>16.04 a</td>
<td>14.01 a</td>
</tr>
<tr>
<td>Total progeny</td>
<td>88.80 a</td>
<td>40.64 b</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>6.5:1 a</td>
<td>5.9:1 a</td>
</tr>
</tbody>
</table>

\(^a\)Means in the same row followed by the same letter are not significant at 0.05 level of significance.

Infected parasitoid females could be related to the intensity of host infections in which the parasitoids had developed. They showed that fecundity of *P. foveolatus* was inversely correlated with intensity of spores in the host. In this study all emerged progeny of infected females showed no incidence of microsporidiosis. As with *T. evanescens*, (Huger, 1984) transovarial transmission of *N. pyrausta* did not occur with *T. nubilale*.

*Trichogramma nubilale* are susceptible to *N. pyrausta* and this infection has an adverse effect on the establishment of
the parasitoid. If mass-reared T. nubilale are to be used in an inundative release program to control the European corn borer, the parasitoid must be reared on uninfected European corn borer egg masses or eggs of other lepidopterans free of a microsporidian infection.
PART III: THE IMPACT OF NOSEMA PYRAUSTA (PAILLOT) ON A PREDATOR, CHRYSOPERLA CARNEA (STEPHENS)
INTRODUCTION

Insect predators play an important role in regulating populations of the European corn borer, O. nubilalis (Sparks et al., 1966; Carlson and Chiang, 1973) utilizing both eggs and larvae as prey. A microsporidium, N. pyrausta is also a major factor in regulating populations of the European corn borer (Zimmack and Brindley, 1957). Nosema pyrausta is reported from the European corn borer from many corn agroecosystems (Kramer, 1959c; Van Denburgh and Burbutis, 1962; Hill and Gary, 1979). This microsporidium is an effective organism in integrated pest management programs to suppress the European corn borer. Maddox (1988) suggested several possibilities such as colonization, augmentation and manipulation of the insect host for the purpose of improving the effectiveness of N. pyrausta as a biological control agent. Lewis and Lynch (1978) and Lewis and Cossentine (1986) showed that foliar application of N. pyrausta on corn plants infested with the European corn borer, resulted in an increase of microsporidian infection and also a reduced number of larvae per plant.

If an organism such as N. pyrausta is to be manipulated or placed in the corn agroecosystem in an effort to control the European corn borer, one must first assess the impact of the microsporidium on nontarget beneficial insects. There are very few reports of microsporidia infecting predaceous
insects. Predators, like G. californica (Finney, 1950), L. erichsonii (Smirnoff and Eichhorn, 1970) and N. undulata (Van Essen and Anthony, 1976) are detrimentally affected by microsporidia. In the majority of the reports however, predacious insects are not affected (Smirnoff and Eichhorn, 1970; Van Essen and Anthony, 1976; Kaya, 1979; Young and Hamm, 1985). Instead, they disseminate the pathogens of their prey through their feces (Capinera and Barbosa, 1975; Kaya, 1979; Cooper, 1981; Young and Hamm, 1985).

Heretofore nothing was known about the interactions between N. pyrausta and insect predators associated with the European corn borer. In this study, a cosmopolitan predator, G. carnea which commonly occurs in various agroecosystems including corn was chosen. The objectives of the study were to determine the susceptibility of G. carnea to N. pyrausta, assess the effect on larval food consumption, developmental time and adult fecundity, and to measure the viability of N. pyrausta spores discharged in meconium of G. carnea.
MATERIALS AND METHODS

A laboratory colony of *C. carnea* was established from adults collected from cornfields during the month of June 1984. Two or three pairs of adults were kept in an ovipositional unit made of a cylindrical ice cream carton (170 x 175 mm) covered at the top with muslin cloth and lined with a strip of brown wrapping paper. The strip of brown paper served as an oviposition and feeding site. The *C. carnea* adults were provided with WheastR (a dairy by-product), smeared on the paper lining, and water in a cotton plugged vial protruding through the side of the carton. These units were kept in a chamber at 27 ± 1°C, 65 ± 5% RH and 16L : 8D photophase.

**Developmental time and prey consumption:** Hundreds of *C. carnea* eggs were placed singly in cotton plugged glass vials (18 x 85 mm). Upon hatching, 200 larvae were randomly selected for experimentation. One hundred larvae were provided *N. pyrausta*-infected European corn borer egg masses and another 100 larvae were provided uninfected egg masses. The uneaten and empty egg masses were removed daily, frozen and later counted for number of eggs consumed. The vials were then replenished with fresh egg masses. Larval mortality and development were monitored daily. Molting was confirmed by examining vials for exuviae. The number of European corn borer eggs consumed daily was counted from 50 randomly
selected larvae from each treatment. All data were analyzed by one-way analysis of variance except the larval mortality data were analyzed by chi-square.

**Fecundity and fertility:** A total of 20 pairs of adults was randomly selected from each treatment from the previous experiment. One pair was placed in an individual ovipositional unit and these units were maintained as previously described. The brown paper strips were removed daily from the ovipositional units and the number of eggs laid was counted. Eggs laid on day 7 of the oviposition period were allowed to develop and hatch. Day 7 was chosen because all the chrysopid adults were actively laying their eggs by this time. The percentage of hatching was used as fertility index. A total of 350 eggs was observed from each treatment. The data obtained were analyzed by one-way analysis of variance.

**Histology:** Five 3 and 7 day old larvae, 2 day old pupae and newly emerged adults reared on infected and uninfected European corn borer eggs, were fixed in alcoholic Bouin’s fluid. These specimens were dehydrated in a series of ethyl alcohol, cleared in methyl benzoate and benzene, embedded in Paraplast®, sectioned (5 um) (Lewis et al., 1977) and stained in Giemsa colophonium (Shortt and Copper, 1945).

**Viability of N. pyrausta spores in meconium:** In this experiment, the viability of N. pyrausta spores from the
meconium was compared to the viability of *N. pyrausta* spores isolated from European corn borer larvae. Meconium discharged by newly emerged *C. carnea* adults which had been fed *N. pyrausta*-infected eggs during their larval stage, and European corn borer larvae infected with *N. pyrausta* were homogenized in distilled water using a tissue homogenizer and a Waring® blender respectively. Two-tenth ml of the homogenate (100 spores per mm² diet surface) was pipetted onto the surface of European corn borer diet in a 15 ml plastic cup. The cups were swirled and air-dried. One European corn borer neonate was placed in each cup. A total of 20 neonates per treatment per replication were used. The sealed cups were held in an incubator at 27°C and 70 ± 5 % R.H. After 48 hours, the larvae were transferred to cups of fresh untreated diet. On days 7 and 12, after initial infection, 10 larvae per treatment were randomly selected and examined for microsporidiosis (Raun et al., 1960). The experiment was arranged in a randomized block design with four replications and the data were analyzed by an analysis of variance.
RESULTS AND DISCUSSION

Preimaginal development: The microsporidium, *N. pyrausta* appeared to have no detrimental effect on the preimaginal development of *C. carnea* (Table 13). The total larval development period lasted ca. nine days with each stadium lasting ca. three days. The pupal period was ca. eight days.

Table 13. Developmental time and mortality of *Chrysoperla carnea* (Stephens) fed *Nosema pyrausta* (Paillot) -infected and -uninfected *Ostrinia nubilalis* (Hübner) eggs during the larval stage

<table>
<thead>
<tr>
<th>Prey</th>
<th>Uninfected eggs</th>
<th>Infected eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stadia (days)</td>
<td>Mortality</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Instar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>3.06 ± 0.02 a</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>2.58 ± 0.07 a</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>3.32 ± 0.08 a</td>
</tr>
<tr>
<td>Pupa</td>
<td>62</td>
<td>8.15 ± 0.09 a</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>17.19 ± 0.11 a</td>
</tr>
</tbody>
</table>

^aMeans in the same row followed by the same letter are not significant at 0.05 level of significance.

There was a varying amount of mortality of the chrysopids during the preimaginal period. The highest mortality was observed during the pupal stage which accounted for 30% of the
total mortality. The difference in the mortality rate of individuals fed on infected and uninfected host eggs however, was nonsignificant.

Table 14. Mean number of *Nosema pyrausta* (Paillot)-infected and -uninfected *Ostrinia nubilalis* (Hübner) eggs consumed by *Chrysoperla carnea* (Stephens) during larval development

<table>
<thead>
<tr>
<th>Instar</th>
<th>Infected Eggs</th>
<th>Uninfected Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>20.36 ± 0.89 a</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>57.95 ± 2.98 a</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>330.51 ± 9.13 a</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>408.82 ± 8.95 a</td>
</tr>
</tbody>
</table>

*Means in the same row followed by the same letter are not significant at 0.05 level of significance.*

**Larval food consumption:** Regardless of the presence or absence of *N. pyrausta* a larva consumed about 400 eggs (Table 14). Prey consumption rate however, varied with the instar; 5, 15 and 80% of the total consumption occurred in the first, second and third stadium respectively.

**Fecundity:** The reproductive potential of chrysopids is particularly affected by the quality and quantity of food taken during the larval and adult stages (Principi and Canard, 1984). However, *N. pyrausta* in European corn borer eggs did
Table 15. Preovipositional period, egg production and female mortality of *Chrysoperla carnea* (Stephens) fed *Nosema pyrausta* (Paillot)-infected and -uninfected *Ostrinia nubilalis* (Hübner) eggs during larval development

<table>
<thead>
<tr>
<th>Prey</th>
<th>Uninfected Eggs</th>
<th>Infected Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Preovipositional period (day)</td>
<td>3.75 ± 0.13 a</td>
<td>4.20 ± 0.14 a</td>
</tr>
<tr>
<td>Mean total of eggs laid</td>
<td>451.25 ± 19.57 a</td>
<td>408.75 ± 19.57 a</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>5.00 a</td>
<td>5.00 a</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

*a* Means in the same row followed by the same letter are not significant at 0.05 level of significance.  
*b* First 31 days of oviposition.

not significantly influence the preovipositional period and total egg production of *C. carnea* adults fed infected eggs during the larval stage (Table 15). The female adult had a four day preovipositional period. The oviposition lasted for ca. 60 days. During the first 31 days of oviposition, adults developing from larvae fed uninfected European corn borer eggs laid a total of 451 eggs, 8% more eggs than those developing from larvae fed infected eggs. Figures 35 and 36 show the daily and cumulative egg production respectively. Both groups
Fig. 35. Daily egg production by *Chrysoperla carnea* (Stephens) fed *Nosema pyrausta* (Paillot) -infected and -uninfected *Ostrinia nubilalis* (Hübner) eggs during larval development.

Prey

+ = infected

• = uninfected
MEAN DAILY EGG PRODUCTION

DAY OF OVULATION

10 20 30 40 50 60 70 80
3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Fig. 36. Cumulative mean number of eggs produced by *Chrysoperla carnea* (Stephens) fed *Nosema pyrausta* (Paillot)-infected and -uninfected *Ostrinia nubilalis* (Hübner) eggs during larval development.

- Prey
  - + = infected
  - • = uninfected
CUMULATIVE MEAN OF EGG PRODUCTION

DAY OF OVPOSITION
of females exhibited a similar pattern of egg production, with the most number of eggs being laid during the first three days of oviposition. The hatching percentages of the eggs laid by females from both groups were 93.87 and 92.93% respectively.

**Histology:** Further evidence that *C. carnea* is not susceptible to *N. pyrausta* infection was gathered from histological studies. The microsporidian spores were confined to the midgut lumen (Figs. 37 and 38). The spores accumulated in the midgut throughout the larval feeding period because the midgut is functionally closed and there is no passage to the hindgut (Gaumont, 1976). In the pupae, the microsporidium appeared to be concentrated in the posterior midgut along with other fecal material (Fig. 39). The spores and fecal materials were eliminated in the meconium during imaginal ecdysis. Consequently the adult was devoid of *N. pyrausta* spores in its alimentary system (Fig. 40). The microsporidium failed to infect the alimentary system and thus no tissues of *C. carnea* were infected.

**Viability of *N. pyrausta* spores in the meconium of *C. carnea***: The results from this experiment demonstrate that some *N. pyrausta* spores which accumulated in the alimentary system and were eliminated in the meconium remained viable and infective to European corn borer (Table 16). The viability however, declined by 50% when compared to fresh spores from European corn larval tissue. The intensity of infection
Figs. 37 and 38. *Nosema pyrausta* (Paillot) spores in the midgut lumen of *Chrysoperla carnea* (Stephens) larva

Ec = epithelial cell; Gl = gut lumen; sp = spore

Fig. 37: 250X
Fig. 38: 400X
Fig. 39. *Nosema pyrausta* (Paillot) spores in the midgut lumen of *Chrysoperla carnea* (Stephens) pupa (400X)

*sp* = spore

Fig. 40. Midgut of *Chrysoperla carnea* (Stephens) adult devoid of *Nosema pyrausta* (Paillot) spores (400X)

*Ec* = epithelial cell; *G1* = gut lumen
Table 16. Percentage and intensity of an infection of *Ostrinia nubilalis* (Hübner) larvae by *Nosema pyrausta* (Paillot) obtained from *O. nubilalis* larval tissues and meconium of *Chrysoperla carnea* (Stephens) fed *N. pyrausta* spores 7 days after initial ingestion

<table>
<thead>
<tr>
<th>Source of spores</th>
<th>7 days after initial ingestion</th>
<th>12 days after initial ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% larvae infected</td>
<td>Spores/mg of tissue (x10^4)</td>
</tr>
<tr>
<td>Meconium</td>
<td>55.00 a</td>
<td>6.35 a</td>
</tr>
<tr>
<td><em>Ostrinia nubilalis</em></td>
<td>100.00 b</td>
<td>11.08 a</td>
</tr>
</tbody>
</table>

*Means in the same column followed by the same letter are not significant at 0.05 level of significance.*

produced by the spores from meconium on day 7 was not significantly different from the intensity of infection caused by spores from European corn borer larval tissue although *N. pyrausta* from the meconium yielded 50% fewer total spores per mg of tissue. On day 12, the differences in percent of infected larvae and intensity of infection between the two treatments remained unchanged. The intensity of infection within treatment however, increased proportionately by three fold within a period of 5 days indicating that once an infection has occurred microsporidian development is normal.

Unlike *C. californica* and *C. carnea* which were detrimentally affected respectively by a microsporidium, *P. californica* (Finney, 1950) and a bacterium, *Bacillus*...
thuringiensis Berliner (Eubacteriales: Bacillaceae) (Salama et al., 1982), *C. carnea* evidently is not affected to *N. pyrausta* infection. The larvae fed and developed normally and were able to produce adults with unimpaired fecundity, fertility and longevity.

From the analysis of data from this study it is evident that *C. carnea* was neither directly nor indirectly affected by the accumulation of *N. pyrausta* spores in the alimentary system. The presence of spores in the gut lumen apparently did not cause a nutritional imbalance. The apparent failure of the spores to produce an infection could be due to:

(i) failure of spores to germinate in the digestive tract because of rate of flow, pH, or the lack of adequate digestion of the seal covering the polar filament, or (ii) host tissues are not susceptible to the microsporidium (Weiser, 1976).

*Chrysoperla carnea* is resistant to *N. pyrausta* and it can disseminate viable *N. pyrausta* spores in the meconium at least 7 days after they are ingested. This phenomenon of nonsusceptibility to a pathogen and the passage of viable infective stages in the meconium is known from other predators. The carabid, *Calosoma sycophanta* (Linneaus) (Coleoptera: Carabidae) excreted fecal deposits containing polyhedral bodies of an insect virus which were infective to its prey but had no deleterious effect on itself (Capinera and Barbosa, 1975). Young and Hamm (1985) reported that *Calosoma*
Savi DeJean voided infective *Vairimorpha necatrix* (Kramer) spores 13 days after ingesting infected prey (Kaya, 1979). The assassin bug, *Zelus exsanguis* (Stål) (Hemiptera: Reduviidae) excreted infective spores of *V. necatrix* and *Pleistophora* sp. after feeding on infected prey. These spores however, were virulent for only 3 days after ingestion.

The results of this laboratory study show that *C. carnea* plays an important role in the extra-corporeal ecology of *N. pyrausta*. The impact of this relationship on the epizootiology of *N. pyrausta* in a European corn population is yet to be determined.
SUMMARY AND CONCLUSIONS

This dissertation reports data from studies on the impact of a microsporidium, *N. pyrausta* on certain biological parameters of the female European corn borer, *Q. nubilalis*. Investigations were conducted on the success of vertical transmission (transovarial and transovum) of the microsporidium when different instars of the host were exposed to varying concentrations of microsporidian spores. Also studies were made on an egg parasitoid, *T. nubilale* and a predator, *C. carnea* when a *N. pyrausta*-infected egg is utilized as a host or as prey.

*Nosema pyrausta* detrimentally affects the development of its host. European corn borer larvae when exposed during the first two instars to 100 to 800 spores per mm² diet surface for 48 hours, failed to pupate or emerge as morphologically normal adults. Infections of the older instars reduced the female adult longevity by 2 days and fecundity by at least 50%. These infected adults laid eggs which were internally and/or externally contaminated with the microsporidium. The prevalence of these infections, determined by the presence of spores in the eggs or the emerging larvae, varied with the concentrations to which the larvae were exposed and with the time when the eggs were laid. The differences due to age of larvae when they were exposed to the *N. pyrausta* spores however, were not significant. The overall rate of
transovarial infection increased by 15% when the spore concentrations were increased by a factor of eight. Also, the infection in the eggs increased from 37 to 54% from day 1 to day 7 of the ovipositional period. The pattern of infection in the eggs and subsequent larvae was similar; however, it was 18% higher in the larvae.

Histological studies show that all parts of the reproductive organs, regardless of the stage of development, were susceptible to infection by N. pyrausta. In immature ovaries, the microsporidium infected the epithelial cells, stroma and germ cells. This infection subsequently spread to follicle cells and finally to the yolk. Thus vertical transmission from parent to progeny was achieved.

In experiments to assess the impact of N. pyrausta on T. nubilale and C. carnea, it was found that N. pyrausta detrimentally affected T. nubilale but not C. carnea. Nosema pyrausta infected the T. nubilale and decreased the number of parasitoid adults emerging from infected hosts. Even though the infection did not adversely affect the adult longevity, the fecundity was significantly reduced. Histological sections of parasitoids within the eggs showed that the microsporidian spores, which were confined to the gut lumen in the larval stage, caused extensive infection to the gut epithelial, connective, neural and muscle tissues of the pupal and the adult stages. The significance of such infections of
beneficial insects, as indicated by Tanada (1976), will depend on the relative importance of the parasitoid as compared to that of the microsporidium in regulating the population of the host insect.

The *C. carnea* larvae fed *N. pyrausta*-infected eggs developed normally and were able to produce adults with unimpaired fecundity, fertility and longevity. The microsporidium apparently failed to cause any infection. The spores remained in the midgut throughout the larval feeding period and were eliminated in meconium during imaginal eclosion. At this time the spores had been within *C. carnea* for at least 7 days. However, they remained infective in bioassays against European corn borer larvae. In nature, this voracious predator serves as a population regulator not only by suppressing the pest insect, but also by dispersing microsporidian spores.
LITERATURE CITED


Cossentine, J. E. 1985. Compatibility of parasitism by Bonnetia comta (Fallen), Lydella thompsoni Hertig, and Macrocentrus grandii Goidanich, and a bacterial, viral, or microsporidian infection in larvae of Agrotis ipsilon (Hufnagel) and Ostrinia nubilalis (Hübner). Ph.D. Thesis. Iowa State University, Ames. 137 pp.


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