Vertical transmission of a dimorphic microsporidium (Microspora) in the Mormon cricket, Anabrus simplex (Orthoptera: Tettigoniidae)
by Francoise Djibode

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:
The Mormon cricket, Anabrus simplex is an endemic pest of crops and rangelands in the western United States. It occurs mostly in environmentally sensitive areas where biological control options are desirable. A dimorphic microsporidium was found in this cricket and appears to be useful for such control.

My hypotheses state that this dimorphic microsporidium infects adult crickets and causes mortality. It also affects cricket fecundity and the viability of their progeny, and is vertically transmitted.

Increasing dosages of the spores were fed to young adult crickets, and the infection status of their progeny was checked by phase contrast microscopy. Reproductive organs from male and female crickets infected orally with 107 spores each were fixed after 40 and 49 days and checked for the presence of the pathogen.

Infection of young adult crickets ranged from 22.5% at 105 to 82.5% at 109 spores/cricket. The infection rate doubled and increased from 35% to 72.5% when 106 spores/cricket and 107 spores/cricket were applied, respectively. The dose required to infect 50% of adult crickets (ID50) was 106.4 spores/cricket. Mortality of the treated crickets increased from 30% to 82.5% for untreated versus treated with 109 spores/cricket.

The dimorphic microsporidium had a significant adverse effect on cricket fecundity and reduced the number of eggs produced by 57.6% when 105 and 109 spores were applied, respectively. This pathogen also affects crickets progeny viability. Fewer nymphs from the control parents were deformed and /or died when hatching compared to the nymphs from treated parents. This deformity or neonate death was due to the difficulty encountered by neonate nymphs when shedding their first skin; they died strangled by the exuviate.

Progeny of infected parents were not infected. However, histopathology studies show that crickets died before being able to lay any infected eggs. This study also showed a possibility of transovum transmission when each parent was infected with 107 spores.

The dimorphic microsporidium appeared potentially useful to control adult Mormon crickets. It affected cricket viability, and was vertically transmitted in adult crickets.

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IN THE MORMON CRICKET,
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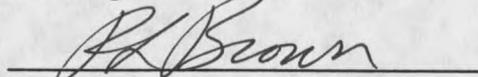_November 29, 1993_
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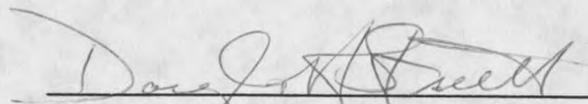
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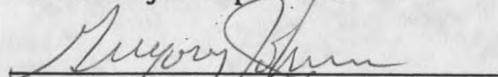
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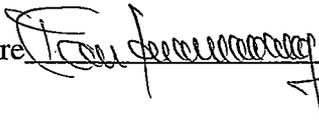Approved for the College of Graduate Studies

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ABSTRACT

The Mormon cricket, <u>Anabrus</u> <u>simplex</u> is an endemic pest of crops and rangelands in the western United States. It occurs mostly in environmentally sensitive areas where biological control options are desirable. A dimorphic microsporidium was found in this cricket and appears to be useful for such control.

My hypotheses state that this dimorphic microsporidium infects adult crickets and causes mortality. It also affects cricket fecundity and the viability of their progeny, and is vertically transmitted.

Increasing dosages of the spores were fed to young adult crickets, and the infection status of their progeny was checked by phase contrast microscopy. Reproductive organs from male and female crickets infected orally with 10^7 spores each were fixed after 40 and 49 days and checked for the presence of the pathogen.

Infection of young adult crickets ranged from 22.5% at 10^5 to 82.5% at 10^9 spores/cricket. The infection rate doubled and increased from 35% to 72.5% when 10^6 spores/cricket and 10^7 spores/cricket were applied, respectively. The dose required to infect 50% of adult crickets (ID_{50}) was $10^{6.4}$ spores/cricket. Mortality of the treated crickets increased from 30% to 82.5% for untreated versus treated with 10^9 spores/cricket.

The dimorphic microsporidium had a significant adverse effect on cricket fecundity and reduced the number of eggs produced by 57.6% when 10^5 and 10^9 spores were applied, respectively. This pathogen also affects crickets progeny viability. Fewer nymphs from the control parents were deformed and /or died when hatching compared to the nymphs from treated parents. This deformity or neonate death was due to the difficulty encountered by neonate nymphs when shedding their first skin; they died strangled by the exuviate.

Progeny of infected parents were not infected. However, histopathology studies show that crickets died before being able to lay any infected eggs. This study also showed a possibility of transovum transmission when each parent was infected with 10^7 spores.

The dimorphic microsporidium appeared potentially useful to control adult Mormon crickets. It affected cricket viability, and was vertically transmitted in adult crickets.

I. INTRODUCTION

Natural Habitat

The Mormon cricket, <u>Anabrus</u> <u>simplex</u> Haldeman, is a long-horned grasshopper belonging to the family Tettigoniidae. It is a flightless, shield-backed grasshopper which occurs primarily in the western United States and Canada and is best known for its huge migratory bands. These bands will typically develop in permanent breeding areas or in broken, mountain habitat, and then spread by walking to surrounding areas including lowlands and valleys (Wakeland and Shull, 1936).

History and Economic Importance

The name "Mormon cricket" originates from an early encounter in 1848 between hordes of this insect and Mormon settlers in the Salt Lake Valley. Outbreak populations built up in breeding areas during favorable years and then emigrated to cultivated (Cowan, 1929; Wakeland and Shull, 1936; Cowan <u>et al</u>. 1943) and grazed land (Wakeland and Parker, 1952).

Mormon crickets prefer plants with large succulent leaves such as balsamroot, mustard, dandelion, and bitterroot in their natural habitats (Wakeland and Parker, 1952). However, they do consume seeds of range grasses, thus reducing natural

reseeding (Wakeland and Shull, 1936). In cultivated fields, cereals and forage crops are consumed by the Mormon cricket (Corkins, 1923). Crickets also exhibited marked preferences for flowers and seeds over vegetative tissue. In this regard, cricket injury to crops can often be distinguished from that of some Melanoplus grasshoppers which denude the leaves of wheat plants with intact culms (Swain, 1944; Wakeland, 1959; Cowan, 1929).

Sporadic outbreaks of the Mormon cricket cause severe damage to crops, especially wheat and alfalfa (Cowan, 1929; Wakeland, 1959). Cultivated crops are highly preferred though crickets normally feed on a wide diversity of rangeland plants (Swain, 1944). According to MacVean (1989) homesteaders were forced to abandon farming in northwest Colorado due to the yearly invasions of crickets during the 1920's. Damaging numbers of crickets persisted into the late thirties, with the peak of the epidemic occurring in 1938.

Ueckert and Hansen (1970) examined the digestive system (crop) contents of Mormon crickets from a population near Red Feather Lakes, Colorado, and found that the plants which comprised large proportions of the diet were made up of a small proportion of the total available herbage. Grasses, clubmoss, and grasslike plants made up only 8% of the diet. Forbs represented about 50% and fungi composed of 16% of the diet. The remaining 21% of the diet was made up of arthropod parts, most of them apparently the remains of small insects and other Mormon crickets. Diet composition varied during the season, with arthropods increasing from 10 to 20%

between July and September, and forbs decreasing from about 60 to 30% in the same period. This may reflect an increased protein requirement for mating and egg production.

Oviposition

Eggs are laid from mid-July through fall (Corkins, 1923). The long ovipositor of the female is worked into the ground to its full length, and the eggs are deposited singly, never in pods like grasshopper eggs (Wakeland et al. 1939).

The eggs of the Mormon cricket are about 1/2 cm long, rounded at the end, and slightly curved. They are chocolate brown when first laid, but in contact with the soil they become pearly gray. As the embryo develops, the egg appears dull gray and becomes enlarged at one end (Cowan, 1929).

The eggs are normally laid in groups of about 40 to 50 over a period of 1 to 3 days, followed by a non-oviposition period of 5 to 7 days (Wakeland et al. 1939). Female crickets prefer soil that is rather compact but not too hard and usually free of vegetation, or on sunny slopes where the soil is suitable for egg laying (Wakeland et al. 1939). They tend to aggregate for egg laying which results in a large number of eggs in a small areas (Cowan, 1935). Gillette and Johnson (1905) collected 2000 to 3000 eggs from an ovipositional site 30.5 cm^2 in size. Individual females lay 50 to 75 eggs in a season (Riley et al. 1880). One hundred and thirty-three eggs in different

stages of development have been observed within one female (Gillette and Johnson, 1905). Cowan (1935) suggested that 150-200 eggs were laid by individual females.

Embryonic Development

Embryonic development starts following deposition of the egg, and proceeds into the fall. The embryo is fully developed before the onset of diapause prior to winter (Cowan, 1929; Cowan and McCampbell, 1929; Wakeland and Shull, 1936). Shipman and Cowan (1940) showed, in a laboratory study, that a saturated soil and a constant temperature of 24°C was the best for embryonic growth. By the time the ground froze in the fall, embryonic development was fully completed (Cowan et al. 1943).

There are 23 stadia in the embryonic development of the Mormon cricket egg (Shipman and Cowan, 1940). The critical period occurs between the zygote and blastula stages, because too much moisture is harmful to embryonic development. Saturated soil conditions throughout the embryonic period retard it. However a return to lower moisture conditions, even for a short period, will allow complete development but the factors affecting this development are unknown (Shipman and Cowan, 1940).

Post Embryonic Development

Mormon crickets are univoltine insects. Hatching may begin as early as the last day of February, but normally does not start until approximately April 1. Fall hatching has never been observed under field conditions (Cowan et al. 1943). Typically, hatching starts after the snow has melted and ground temperature increases, depending upon elevation and weather conditions (Cowan, 1929).

The nymphs hatch together, and develop into the adult stage through successive molts. The average length of time for such development is 48 days at 31°C and 57 days at 25°C with a total lifespan of ca 100 days. The average length of time in each instar is 6.43 days at 32°C and 8.45 days at 27°C (Cowan, 1929).

Newly-hatched crickets are about 0.5 cm long. The color of immature stages varied from light green or tan to black, with various colors and shades for individual crickets (Cowan et al. 1943). They require 60 days, depending on weather conditions, to reach maturity. On reaching maturity, the adult female attains a length up to 5 cm including the swordlike egg-laying organ called the ovipositor (Cowan et al. 1943). The ovipositor of a mature female cricket is not longer than that of the seventh instar. However, the seventh instar can be distinguished from the adult because the stubby wing pads meet in the center of the thorax for the female, while the wing pads of the male overlap.

Diseases

The role of diseases in controlling natural cricket outbreaks is unknown. Attempts to introduce Entomophaga grylli Fresenius, a fungus found in many orthopterans, were unsuccessful in controlling outbreaks. Recent studies have focused on microsporidia and a number of authors have suggested that Nosema locustae Canning, may hold potential for long-term suppression of Mormon crickets (Henry and Onsager, 1982). This pathogen normally infects the fat body of grasshoppers but is only found in the gut of crickets (Henry and Onsager, 1982).

In 1985, a new microsporidium species was discovered in Mormon crickets near Dinosaur National Monument (Colorado-Utah) by MacVean and Capinera, and tentatively identified as a Vairimorpha sp. (MacVean, 1989).

Microsporidia

The members of the phylum Microspora are commonly called microsporidia, and the disease they cause is called microsporidiosis. Microsporidia rank among the smallest of eukaryotes. They possess spores which contain a uninucleate or binucleate sporoplasm and an extrusion apparatus called polar filament and a polar cap. They do not have mitochondria. Stages within the life-cycle are ultrastructurally unique and distinct from other spore-forming protozoa and had played a critical role in taxonomic determinations (Larsson, 1986). Even though they are eukaryotic microorganisms,

their ribosomal RNAs have prokaryotic properties (Ishihara and Hayashi, 1968; Curgy

et al. 1980). Moreover, the sequence of ribosomal RNA suggests that the

microsporidia are extremely ancient eukaryotes and had separated very early from

other eukaryotes (Vossbrinck et al. 1987).

Taxonomy

The taxonomy of the Microsporida has undergone drastic revision during the

past two decades. Recognizing the uniqueness of microsporida, Weiser (1977, 1985)

and Sprague (1977, 1982) have elevated these organisms to the level of a distinct

phylum. Most microsporidiologists follow the system developed by Sprague (1977)

and the more recent revision by Sprague et al. (1992). The taxonomy of micro-

sporidian genera and species is based on differences in morphology, life cycle, and

parasite-host relationships. These may be inadequate in some cases, and there is need

for other criteria such as biochemical analyses and serology to differentiate genera and

species (Tanada and Kaya, 1993).

Life Cycles

The microsporidian life cycle has two distinct sequences: (1) merogony, the

vegetative phase; and (2) sporogony, the production of spores. The mother cell for

merogony is called the meront and for sporogony, the sporont. During merogony, the

microsporidium multiplies rapidly by binary fission, plasmotomy, or multiple budding.
Additional multiplication occurs during the sporulation phase and terminates in the
formation of spores. The stages in the merogonial sequence are (1) the sporoplasm,
the invasive stage, and (2) the meront, the main multiplicative stage. The daughter
cells may remain united as chains. The sporogonial stages are (1) the sporont, and (2)
the sporoblast, a nondividing morphogenic stage that forms the spore. Major
cytoplasmic reorganization takes place within the sporoblast to produce the spores. A
spore is surrounded by a two-layered wall which has a polar sac, a coiled polar
filament, and encloses two nuclei lying in the center of the spore. The cytoplasm also
contains numerous ribosomes and endoplasmic reticulum (Canning, 1977). A spore is
the resistant stage by which microsporidia are maintained in the environment. During
these stages, the cell may have one (unikaryon), two coupled nuclei (diplokaryon) that
are closely adjacent but separated by their membrane, or several nuclei in a
plasmodium (Tanada and Kaya, 1993).

Microsporidia have basically similar life cycles, but specific variations like the
mode of division and the number of daughter cells are of taxonomic value in classify-
ing these organisms (Vávra, 1976). Canning (1990) had reported that some genera
have three different sporogonic sequences leading to marked spore polymorphism.
She also described the complex and diverse types of sporogony found in different
microsporidia genera. Tanada and Kaya (1993) distinguished five developmental
stages, but for several microsporidia only a part of the life cycle is known:

1) All stages with nuclei in a diplokaryon arrangement.

2) Nuclei isolated (unikaryon) at all stages of development.

3) Merogonial stages are diplokaryotic. The diplokaryotic sporont gives rise to a sporogonial plasmodium with isolated nuclei and finally a sporoblast with a single nucleus.

4) Two-host life cycle. Diplokaryotic stages alternate with monokaryotic stages.

5) Dimorphic development with production of two different types of spores. In one sequence, all stages have coupled diplokaryotic nuclei. In the other sequence, merogonial stages and the sporont are diplokaryotic. The monokaryotic sporoblasts are formed after meiotic division, probably with karyogamy at the end of merogony. The following genera have such a life cycle: Spraguea, Goldbergia, Burenella, Hazardea, Edhazardia, Evlachovaia, Pilosporella, Culicospora and Vairimorpha.

The genus Vairimorpha has two developmental patterns that are temperature-dependant (Pilley, 1976). In pattern A, at a temperature greater than 26°C, it remains single-celled with paired nuclei which differentiate into a single spore. In pattern B, where growth occurs at a cooler temperature (20°C), the uninucleate schizonts differentiate into a spore-forming mother cell that in turn produces 8 sporoblasts which develop endogenously into eight spores (Pilley, 1976).

The demonstration of disporoblastic and octosporoblastic sequences in the same parasite has necessitated the designation of a new genus: Vairimorpha. This

name was derived from the Greek "vary" and "form". It can be diagnosed when a microsporidium has a dimorphic development through binary fission and disporoblastic sporogony dominant, and octosporoblastic sporogony occurring at low temperature. The type species is Vairimorpha necatrix (Kramer) and has as synonyms Thehalonia diazoma. Since Pilley's work, seven additional dimorphic species have been placed in this genus (Moore and Brooks, 1992).

Vairimorpha necatrix Kramer, is highly pathogenic among phytophagous Lepidoptera, many of which are major agricultural pests. Following infection with high doses of $8x10^3$ spores each for first instar larvae and $2x10^6$ spores each for sixth instar, 100% mortality is achieved within six days (Canning, 1982).

Adipose tissue is the principal target of the schizonts of this microsporidium, with the parasitized adipose cells hypertrophied due to the rapid propagation of V. necatrix schizonts using the lipid reserves in the adipose cell cytoplasm as an energy source (Darwish et al. 1989). The consequences of infection are frequently lethal because the fat body organ encompassing the adipose tissue is a center for insect larval intermediate metabolism. The total mass of adipose tissue varies significantly with the stage of larval development and accounts for over 50% of the insect's weight (Darwish et al. 1989). While V. necatrix could be effective as a larvicide, the lack of a vertical transmission component causes this pathogen to occur naturally at low prevalence levels (Canning, 1982).

Infectivity

The infection process is frequently initiated in a new host by the ingestion of mature spores. Upon ingestion by a suitable insect host, the spores germinate and each extrudes a long hollow tube (the polar filament), through which the infective agent or " sporoplasm" travels. The polar filament is extruded with such force that it places the sporoplasm inside or in close proximity to a midgut epithelial cell. After this initial invasion, repeated binary and/or multiple fission-merogony is followed by sporogony which produces spores (Maddox, 1987). The route of invasion of host tissue from the gut epithelium has not been determined, but spores and developmental stages have been observed afterward in susceptible tissues of the host. It is likely that there is a passive transfer in blood or in migratory host cells to the final site of infection (Vávra, 1965; Canning, 1977). Because vegetative growth (merogony), occurs within the host cell, microsporidia are considered strict intracellular parasites (Canning, 1990).

Fecundity

Veber and Jasic (1961) suggested that too much emphasis has been placed on lethal infections by microsporidia and very little emphasis on functional damage of host organs from sublethal doses. They suggested that chronic effects of long lasting infections could be as important as the acute mortality. The effect of chronic

infections in reducing host fecundity is an important aspect of biological control.

Streett (1987) suggested that for long term control, a pathogen should reduce host

fecundity. Reducing fecundity includes depressed egg production as well as decreased

viability of the eggs (Kellen and Lindegren, 1971). The microsporidium Nosema

pyrausta (Paillot), either inhibits egg production or reduces the number of egg masses

of Ostrinia nubilalis (Hubner) (Kramer, 1959). Higher dosages or earlier treatments

caused a greater reduction in the number of eggs than lower dosages and later

treatments (Veber and Jasic, 1961). Henry and Oma (1981) found that increased

infection of the fat body in laboratory-reared females of Melanoplus differentialis

(Thomas) by Nosema locustae (Canning) resulted in decreased egg production. They

had suggested that fecundity among female M. differentialis may be influenced by the

inability of males to inseminate the female or to produce viable spermatozoids.

Reduced adult longevity and fecundity due to microsporidia infection are important

factors that reduce the rate of pest population growth, and play a significant role in

long-term control (Canning, 1982). Zimmack and Brindley (1957) reported that O.

nubilalis infected with N. pyrausta laid fewer eggs when compared to the uninfected

insects. N. algerae reduces the number of eggs laid by Anopheles albimanus

(Wiedmann) by 39% when exposed as 3rd and 4th instar larvae (Darrell et al. 1978).

Culex fatigans (Wiedmann) infected by Nosema stegomyiae Lutz and Splendore, laid

fewer eggs when compared to a population of non-infected flies (Reynolds, 1971).

Nosema heliothidis Lutz and Splendore reduces fecundity of Helicoverpa zea (Boddie),

from 20 to 50% (Gaugler and Brooks, 1975). <u>Vairimorpha</u> <u>necatrix</u> (Kramer) which

is higehly pathogenic to many phytophagous Lepidoptera causes mortality among

infected larvae and zero survived to adulthood. Currey (1991) studied the effects of

<u>Vairimorpha</u> sp. on the Mormon cricket. He observed that crickets inoculated during

early instars were less fecund when they reached the adult stage. The total number of

eggs produced by infected females was reduced from that observed for healthy

females.

<u>Viability</u>

Microsporidiosis has an adverse effect on egg viability and may induce

infertile eggs and/or infected eggs production by the host (Gaugler and Brooks, 1975).

Infected eggs have a lower hatch rate compared to the uninfected eggs (Gaugler and

Brooks, 1975). The factors affecting healthy egg production are multiple and could be

explained by the following:

1) Infected male fails to produce viable spermatozoids which result in non-

fertile egg production (Henry and Oma, 1981; Gaugler and Brooks, 1975).

2) Chronic infection prevents female hosts from producing healthy oocytes

which lead to the production of non-viable eggs (Snow <u>et al.</u> 1970; Darrell <u>et al.</u> 1978;

Windels <u>et al.</u> 1976).

Andreadis and Hall (1979) studied the effects of <u>Amblyospora</u> sp. Hazard and Oldacre in the mosquito <u>Culex salinarius</u> (Coquillet) and found a reduction of 52% in egg hatch from infected mosquitoes. <u>Nosema pyraustae</u> lowered the fertility rate of the European corn borer by 22% (Zimmack and Brindley, 1957). <u>Nosema algerae</u> (Vávra and Undeen) reduces the reproductive capacity of <u>Anopheles albimanus</u> (Wiedmann) regardless of the larval instar that became infected and varying numbers of eggs that failed to hatch were found to be infected with the pathogen. Infection rates of the unhatched eggs ranged from 10 to 70%, and appeared to be related to the intensity of the <u>Nosema</u> infection in the parent female (Darrell <u>et al</u>. 1978).

Vertical Transmission

Direct transfer of infection from parents to their progeny is an important mode of transmission of protozoa. In the majority of host insects, vertical transmission occurs entirely through the female line and is termed matroclinal or maternal-mediated (Andreadis, 1987). Such infections may arise in two distinct ways depending upon whether passage of the pathogen occurs within the ovary (transovarial) or on the surface of the egg (transovum).

Transovarial Transmission

Transovarial transmission occurs when the pathogen gains entry into the egg while within the female host via infection of the ovaries and the associated reproductive structures. Infection is achieved by direct invasion of the embryo or through oral ingestion of a pathogen located in the yolk by the embryo near the time of its hatching. The latter represents an important adaptation by the pathogen which ensures that hosts do not succumb to infection while still within the egg and thus defeat the purpose for which transovarial transmission has evolved (Canning, 1982). Nosema algerae is transovarially transmitted in A. albimanus to the eggs which fail to hatch and decay and this serves as a natural source of infection (Darrell et al. 1978). Zimmack and Bridley (1957) in his study of the infection of the European corn borer by N. pyrausta, found that infected females only transmit the disease to their progeny (i. e., are a matroclinal transmission). N. heliothidis infects the eggs laid by diseased Helicoverpa zea (Gaugler and Brooks, 1975).

Paternal-mediated vertical transmission has been observed, but is not common. In the case of microsporidiosis, it is often the venereal transfer of infection to the female parent during mating and the subsequent transfer to the egg (Andreadis, 1987). Transovarial transmission appears to be the principal method of vertical transfer of most microsporidia (Andreadis, 1987).

Transovum Transmission

In this route, infective stages contaminate the external surface of the egg and are consumed by host larvae at eclosion. The egg chorion is contaminated by fecal or meconial discharges or substances used to cover the egg. The pathogen gains entry into the egg through the micropyles. N. algerae contaminates externally eggs laid by H. zea and may be a natural source of infection to newly-hatched larvae (Darrell et al. 1978). In some cases, transovarial transmission is similar to that of a larva feeding on spores contaminating the surface of the eggshell. As an example, in the winter moth, Operophtera brumata, the embryo within an egg is not infected nor is the larva one day after emergence. But the larva subsequently becomes infected when the microsporidian spores are ingested together with the remains of the yolk, as the larva eats its way through the eggshell (Tanada and Kaya, 1993).

Hypotheses

MacVean (1989) isolated a dimorphic microsporidium from Mormon crickets, that was tentatively identified as a Vairimorpha sp. MacVean (1989) tested this microsporidium for both host-density reduction and long-term sublethal effects in a laboratory study. He reported that 1st to 3rd instar nymphs were infected when fed with 5×10^6 spores and rapid mortality occurred within 21 days. In contrast, the parasite did not significantly reduce survival when fed to 7th instar and adult crickets

compared to the control group. This same study also stated that the microsporidium cannot significantly affect cricket fecundity when 10^6 spores were fed to either 4th-5th instar or 7th instar to adult crickets.

Currey (1991) also studied the effects of the dimorphic microsporidium on the development, mortality and fecundity of the Mormon cricket, and stated that the parasite in laboratory studies caused infections which resulted in retarded development in younger crickets. Field cage studies of males and females, 6th and 7th instars and adults, inoculated with 10^6 and 10^7 spores/cricket resulted in reduced fecundity. Egg production by females treated with 10^7 spores as 6th and 7th instars or had mated with males infected as 6th instars was significantly lower when compared to eggs laid by the healthy crickets.

Henry and Onsager (1989) collected 300 eggs during March 1989, from a location in Utah where prevalence of the dimorphic microsporidium exceeded 85% during August 1989. Following individual incubation of each egg, 95 eggs hatched and crickets were reared individually for 30 days. Examination of these crickets revealed that 41.1% were infected with the parasite. the pathogen is, therefore, vertically transmitted in the Mormon cricket.

The term dimorphic microsporidum will be used in my thesis until a scientific name is approved in the literature. I am proposing the following hypotheses for that dimorphic microsporidium found in the Mormon cricket:

1) infects and causes mortality in adult female crickets.

2) affects adult female Mormon cricket fecundity.

3) affects the viability of the progeny from infected adult female crickets.

4) is vertically transmitted to the progeny of infected female adult crickets.

II. MATERIALS AND METHODS

Source of Spores

Mormon crickets naturally infected with dimorphic microsporidium were collected from Fremont County, Idaho, in 1990. Infected crickets were homogenized in distilled water and filtered through nylon organdy fabric. By centrifugation spores were isolated on July 8th, 1991. Spore preparations in distilled water were quantified with a hemacytometer, refrigerated, and then used for the 1991 experiment.

Field Cage Design

Infectivity and Mortality

Young adult Mormon crickets were collected from the sand dune areas in Fremont County, Idaho, on July 10, 1991. Crickets were individually isolated, in acetate tube (10 cm long x 4 cm diam.) and starved for 24 hours. Both ends of the tubes were sealed with Kerr or Mason wide-mouth rings with screen inserts.

Crickets were fed with 4 cm^2 of organically grown iceberg lettuce previously treated with 10^5, 10^6, 10^7, 10^8, and 10^9 spores. Control groups were given untreated

lettuce. Each treatment had four replicates, and each replicate was made up of ten inoculated females, and five inoculated males. Each replicate was put in a cage randomly set up in an area located behind the Fieldhouse of Montana State University.

The cages were fabricated from 2 gallon plastic buckets. Several holes (7 cm diam.) were cut around the top of each bucket and each hole was covered with screening. The base of each bucket was partially removed and the bucket was inverted on top of a hole dug in the ground and filled with sand collected from Idaho. These crickets were reared until August 29th on a diet of wheat bran, iceberg lettuce, alfalfa leaves, and yellow clover plants.

Cadavers were collected during week days (from Monday to Friday every week) to assess timing of cricket mortality. Remaining crickets were terminated August 29th. The infection status of the crickets which died during the trial or were terminated at the end of the experiment was checked using phase contrast microscopy.

The frequency of mortality and infectivity per treatment was computed four weeks post-inoculation. Logit analysis was done to assess the effects of dimorphic microsporidium on the infectivity and mortality.

Fecundity

The sand from the bottom of the cages was sifted in September of 1991 and the total number of eggs per treatment replicate was counted in the laboratory. The

eggs were stored at 5°C in plastic cups containing moistened vermiculite. The total number of female days for each cage was calculated from the time of treatment to the time of death or termination of the adult females. PROC ANOVA with Tukey comparison was used to show the effect of dimorphic microsporidium on egg production. The variables "mean number of eggs", "mean number of adult days" and "mean number of eggs per female days" were analyzed using the ANOVA (SAS Institute Inc, 1989).

Viability

From 54,000 eggs collected during the field cage study, 24,400 eggs were randomly chosen, and the number of fully developed eggs was counted and the number of newly hatched crickets was determined per treatment replicate. Many deformed newly-hatched offspring were observed, and data were taken to see if the deformity was related to the treatment. Frequency distributions were compiled for hatching and for crippled neonate nymphs per treatment replicate. Logit analysis was used to assess the effects of dimorphic microsporidium on viability (SAS Institute, Inc, 1989).

Vertical Transmission

Eggs collected from the field cage experiment were randomly selected per treatment replicate (100 eggs), and then immersed in a petri dish filled with 25 ml of sodium hypochlorite 2.5% for 15 min. The chorion is dissolved and we can evaluate the level of embryonic development. Because eggs without embryos cannot hatch, only fully developed eggs were chosen for the hatching experiment. Fully developed eggs were chosen out of 1000 to 1400 eggs per treatment replicate and put in an incubator in a cup filled with moistened vermiculite. The incubator was set at 25°C ±2°C days and 15°C±2°C in dark phase with L/D 12:12 photoperiod. Hatching started three days later and lasted for eight days. Crickets were reared individually for thirty days. Cadavers of crickets which died during the trial and the terminated crickets were frozen. The infection status of 350 non-deformed nymphs and one hundred deformed nymphs were later checked, using phase contrast microscopy.

Laboratory Studies

Histopathology

Transovarial Transmission: Females inoculated with 10^7 spores developed chronic infections. This dosage was therefore used to infect 120 young adult Mormon crickets of each sex. Male and female crickets were reared separately to avoid

contrast microscopy. Ovaries and testes of infected crickets were fixed in alcoholic Bouin's fluid, dehydrated through a graded series of ethanol, cleared in toluene and embedded in paraffin. The embedded specimens were serially sectioned and stained with hematoxylin-eosin. Photomicrographs of the infected tissues were taken with a Nikon microscope equipped with a 35mm camera.

Ten fully-developed eggs were randomly chosen per treatment replicate from eggs collected from the cage experiment of 1991. These eggs were dehydrated through a graded series of ethanol, cleared in toluene and embedded in paraffin. The embedded specimens were serially sectioned and stained with hematoxylin-eosin. Photomicrographs of the infected tissues were taken with a Nikon microscope equipped with a 35mm camera.

Mated pairs were assigned to one of four combinations of healthy (H) and infected (I). "Infected" was defined as 10^7 spores per individual adult. Twelve pairs per treatment replicate were established. Each pair was maintained in a rearing tube of sheet acetate that was 20 cm in length by 10 cm in diameter. The top end was sealed with Kerr® and Mason® wide mouth rings with screen inserts, and the bottom ends were inserted into styrofoam cups (12 cm diameter). The cups were filled with moistened sand collected from Idaho, because crickets lay eggs better in native soil, and the crickets used in the test are collected from Idaho. Eggs and cadavers were collected daily over a two week period. Paired crickets were discarded if one of them

died before laying the first batch of eggs. Fourteen pairs were kept for the overall treatment. Ten eggs were randomly chosen per treatment replicate, and washed one time with 5 cc of detergent solution (1ml of dishwashing liquid in 20 ml of distilled water). They were then rinsed three times with 10 cc distilled water, and immersed in 2.5% sodium hypochlorite for five minutes to remove the chorion. The yolk from five eggs per treatment replicate was stained with Giemsa's and checked for different developmental stages of the dimorphic microsporidium. The remaining five eggs were checked for the presence of spores in the yolk using tissue culture buffer as a dilution solution.

Transovum Transmission: Five eggs from the 1992 experiment were randomly chosen per treatment replicate before the onset of embryonic development. They were washed in 10 ml of distilled water, and the pellet obtained from centrifugation at 235g for 15 minutes in an IEC clinical centrifuge. Pelleted samples were resuspended in water and examined for the presence of spores by phase contrast microscopy.

III. RESULTS

Field Cage Design

Infectivity and Mortality

None of the crickets from the control treatment were infected. The percentage of infected females increased steadily from 22.5% in the population infected with 10^5 spores/female to 82.5% in those infected with 10^9 spores/female (Table 1). The rate of infection of adult Mormon cricket with the dimorphic microsporidium was dose dependent. The percentage of infected crickets increased from 35% to 72.5% when the dose was increased from 10^6/female to 10^7/female.

Table 1: Infectivity of Dimorphic Microsporidium to Adult Female Crickets

Dose	Total No.	Infection (%)
0	40	0.0
10^5	40	22.5
10^6	40	35.0
10^7	40	72.5
10^8	40	80.0
10^9	40	82.5

Logit analysis of the infectivity of the dimorphic microsporidium to female crickets showed a positive relationship between infectivity and the administered dose . Criteria for assessing model fit was significant ($X^2 = 42.32$, df = 1, $P = 0.0001$). Infective dose required to infect 50% of any population of adult crickets was $10^{6.3}$ spores (Table 2). The equation was: y = -4.8 + 0.76D (where D = dose).

Table 2:   Logit Analysis of Infectivity of Dimorphic Microsporidium Against Adult Female Mormon Crickets.

Analysis of Maximum Likelihood Etstimates				
Variable	Parameter Estimate	Standard Error	X^2	P
Intercept	-4.86	0.894	29.56	0.0001
LOGDOSE	0.758	0.1305	33.77	0.0001

Mortality

Mortality of adult female Mormon crickets treated with the dimorphic microsporidium began seven days post-inoculation when at least 10^6 spores were inoculated per cricket. Mortality rate increased over time and reached 88.3% for 10^9 spores/ cricket. Mortality of crickets began 21 days postinoculation for 10^5 spore/ adult cricket (Table 3).

Table 3:   Timing of Female Mormon Cricket Mortality in Relation to Dimorphic
Microsporidium Infection.

Dose	No. of dead crickets Week				No.[a] Eaten	No.[b] Alive	Mortal- ity (%)
	4	5	6	7			
0	2	3	2	1	4	28	30
10^5	0	1	2	4	1	32	20
10^6	1	4	1	10	3	21	47.5
10^7	0	7	2	6	7	18	55
10^8	9	14	3	5	5	4	90
10^9	5	5	7	14	2	7	82.5

[a] number of crickets cannibalized

[b] number of crickets which survived to termination after 7 weeks

Logit analysis of female mortality related to dose of the dimorphic microsporidium showed a significant overall fit of the model ($X^2 = 96.31$, df = 5, $P = 0.0001$). The combined effect of dose and treatment applied significantly affected cricket mortality, with a positive relationship between dose applied and cricket mortality. There was also a significant difference between treatment ($X^2 = 49.77$, df = 3, $P = 0.00001$) (Table 4).

Table 4: Logit Analysis of Adult Female Mortality in Relation to Dimorphic
Microsporidium Infection.

Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimates	Standard Error	X^2	P
Intercept	-2.37	0.30	61.90	0.0001
TRT	-2.33	0.64	13.09	0.0003
LOGDOSE	0.45	0.08	35.04	0.0001

Effects on Fecundity

Table 5 shows the total number of eggs laid by adult female crickets inoculated with the dimorphic microsporidium.

Table 5: Frequency of Egg Laid by Adult Female Crickets Infected with the Dimorphic Microsporidium

Dose	No. of Eggs[1] per Replicate				Total No.
	A	B	C	D	
0	2129	2095	3054	2770	10,048
10^5	2784	2480	2610	3498	11,372
10^6	2444	2428	2474	2149	9,495
10^7	1512	1933	2445	2507	8,397
10^8	1575	1810	1236	1918	6,539
10^9	1364	1795	1870	2049	7,078

[1] = number of eggs produced by 10 female crickets.

ANOVA analysis was performed on the number of eggs collected and showed a significant decrease in the total number of eggs as dose increased (F = 5.96, df = 5, P = 0.0020). However, a Tukey range test comparison between the mean number of eggs laid in relation to dose applied only showed a significant difference for the 10^8 spores/cricket compared to the control (Table 6).

Table 6:   Effect of the Dimorphic Microsporidium on Adult Female Mormon Cricket
Fecundity.

Dose	Replicate[a]	$\bar{X}$[b]	±SE
0	4	2512 ab	476
10^5	4	2843 a	454
10^6	4	2374 abc	151
10^7	4	2099 abc	468
10^8	4·	1634 c	301
10^9	4	1770 bc	291

[a] there were 10 female crickets per treatment.
[b] means follow by the same number are not significantly different

ANOVA analysis in Table 7 showed that the dimorphic microsporidium had a

significant effect on the number of female-days (y = 2512 -288.6D + 1652T (where D

= dose and T = treatment));   (F = 12.91, df = 2, P = 0.0003). It also had a significant

effect on the mean number of eggs/female/day (y = 441.73 - 23.41 D + 140 T (F =

13.27, df = 2, P = 0.0002)), and the number of eggs/female day (y = 5.66 - 0.35 D +

1.96T); (F = 5.80, df = 2, P = 0.0099).

The parameters that compose these equations showed that the combined effect of

dose and treatment was important to reduce cricket fecundity. The number of female-

days, the number of eggs produced, and the number of eggs per female-day were

significantly reduced when 10^9 spores/cricket was applied versus 10^5 spores/cricket.

There was a reduction of 20% in female-days (465 at 10^5 and 371 at 10^9); 57.6% for

the number of eggs produced (2721.25 at 10^5 and 1566.85 at 10^9), and 23.89% for the

mean number of eggs produced per female-day (5.86 at 10^5 and 4.46 at 10^9).

Table 7:   ANOVA Analysis of Dimorphic Microsporidium Effects on Adult Female
Mormon Cricket Fecundity.

Parameter	Estimate	T-Value	P
Number of Eggs			
Intercept	2512	13.31	0.0001
LOGDOSE	-288.6	-4.83	0.0001
TRT	1652.25	3.54	0.0019
Number of Female Days			
Intercept	441.75	29.57	0.0001
LOGDOSE	-23.41	-4.89	0.0001
TRT	140.061	3.78	0.0012
Number of Eggs Per Female Day			
Intercept	5.66	15.93	0.0001
LOGDOSE	-0.353	-3.14	0.0050
TRT	1.96	2.23	0.0371

Viability

The effects of the dimorphic microsporidium on embryonic development, egg

hatch, and nymphal deformity are shown in Table 8. A higher hatch rate was

observed for the treated groups when compared to the control group. Fewer nymphs

from control parents were deformed at hatching compared to the nymphs from the

treated group. The neonatal death and the deformity of the survived crickets were due

to the difficulty encountered by neonate nymphs while shedding their first skin.

Table 8: Effects of the Dimorphic Microsporidium on Progeny Viability

Dose	Rep.	Total No.	DVP[a] %	HTD[b] No.	ANN[c] %
0	4	4400	27.75	287	27
10^5	4	4400	26.85	469	37
10^6	4	4000	37.55	463	41
10^7	4	4000	25.20	436	47
10^8	4	4000	34.70	636	45
10^9	4	4000	32.47	529	45

[a] = % fully developed eggs
[b] = No. of eggs hatched
[c] = % abnormal or deformed nymphs

Logit analysis of the data from Table 9 showed a significant difference between

treatments ($X^2 = 127.33$, df = 3, $P = 0.0001$), and a significant overall fit ($X^2 = 34.14$,

df = 2, P = 0.0001). There was a negative relationship between dose applied and embryonic development of the eggs as shown in the equation:

y = 0.96 - 0.106D + 0.039T (where D = dose and T = treatment).

Table 9: Logit Analysis of the Effects of Dimorphic Microsporidium on Egg Development.

Embryonic Development			
Analysis of Maximum Likelihood Estimates			
Variable	Parameter Estimate	Standard Error	X^2	P
Intercept	-0.957	0.034	807.76	0.0001
TRT	-0.1067	0.084	1.59	0.2061
LOGDOSE	-0.0391	0.011	13.13	0.0003

Logit analysis in Table 10 of the sublethal effects of this microsporidium on Mormon cricket progeny had an overall fit (X^2 = 36.74, df = 2, P = 0.0001). Progeny survival was influenced by the combined effects of treatment and dose of dimorphic microsporidium. There was a negative relationship between the dose applied and nymphal development. The equation was:

y = 1.003 - 0.0766D - 0.18T (where D = dose and T = treatment).

Table 10: Logit Analysis of the Sublethal Effect of Dimorphic Microsporidium on
Progeny.

Deformed Nymphs				
Analysis of Maximum Likelihood Estimates				
Variable	Parameter Estimate	Standard Error	X^2	P
Intercept	1.0033	0.0133	56.71	0.0001
TRT	- 0.1804	0.2465	0.054	0.464
LOGDOSE	- 0.0766	0.0285	7.21	0.0072

Laboratory Studies

Vertical Transmission

Transovarial Transmission

Three hundred and fifty out of 1651 (21%) non-deformed nymphs hatched from
eggs laid by infected female crickets and 100 deformed nymphs out of 1169 (8.5%)
were randomly chosen and examined for infection. None of the non-deformed or
deformed nymphs were infected.

Histopathology studies of ovaries from infected crickets showed developmental
stages or meronts in the ovary of the infected cricket fixed on July 1st, 1992.
Evidence of infection was found in the ovaries one month post-inoculation (Figure 1).

Meronts in "chains" were predominantly in the yolk of the oocyte. Ovaries from one female cricket out of two that were dissected contained contaminated oocytes.

None of the developmental stages of the dimorphic microsporidium were found in cricket testes from either July 1st, 1992 or July 10th, 1992 samples.

Longitudinal sections of fully developed embryos of eggs collected in the field cage study showed no stages of the dimorphic microsporidium. This was corroborated by the absence of infection in the hatched crickets.

Transovum Transmission

Spores were present on the shells of eggs when both the male and female crickets were infected. The number of spores varied from 12 to 20 spores/ 5 eggs (Table 11).

Table 11: Presence of Spores on the Eggshells of Infected Mormon Crickets.

Infection status of Pair	No. of Pairs	No. of Replicate	No. of eggs Examined/ Replicate	Mean No. of spores/Field of Pellet	
				x ±	SE
H ♂ x H ♀	3	3	5	0	0
I ♂ x H ♀	3	3	5	0	0
H ♂ x I ♀	3	3	5	0	0
I ♂ x I ♀	5	3	5	16.2	3.19

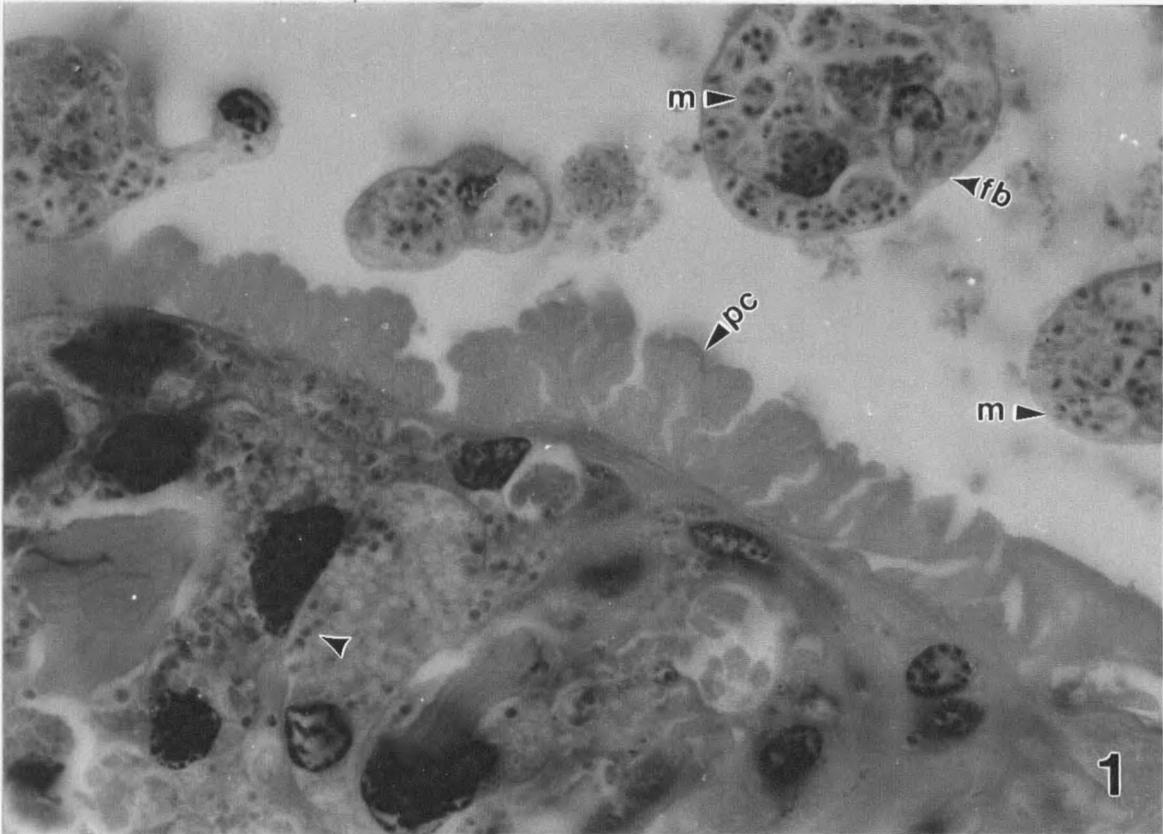# VERTICAL TRANSMISSION OF A DIMORPHIC MICROSPORIDIUM IN MOR-MON CRICKET OVARIOLE.

Figure 1: Section through an ovariole and fat bodies surrounding the ovariole of Mormon cricket infected with a dimorphic microsporidium (Hematoxylin-eosin stain). Fat body (fb) infected with meront (m). Outside the ovariole is the non-infected peritoneal coat (pc). Inside the ovariole, infected follicles containing chain groups of trophozoites and meronts (arrows).