



Pathogenicity of *Vairimorpha* sp. (Nosematidae: Microsporida) in the Mormon cricket, *Anabrus simplex* (Tettigoniidae: Orthoptera)
by David Michael Currey

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 a pest of crops and rangelands in the western United States which frequently occurs endemically in environmentally sensitive areas. Biological control tactics are desirable in such areas. *Vairimorpha* sp., a protozoan known to infect Mormon crickets, appears useful as such a tactic.

The purpose of this study was to determine the potential of *Vairimorpha* sp. to adversely affect developing Mormon crickets. Field applications of 0.4×10^9 , 1.0×10^9 and 2.0×10^9 spores/ha were applied on 2 ha plots containing 2nd and 3rd instar Mormon crickets. Disease prevalence in treated plots ranged from 20.5-48.5%, with no infections in the untreated plots 2, 4 and 7 days post-treatment.

Laboratory bioassays involving 4th, 5th, 6th and 7th instar and adult Mormon crickets treated with 103, 104, 105, 106 or 107 spores/Mormon cricket resulted in infections, however, no significant difference in the mean number of days survived between untreated and treated Mormon crickets was observed. Delayed development in the treated 4th and 6th instars resulted from infections. Observable infections required more time to develop in older crickets compared with younger crickets.

Field cage studies of male and female 6th and 7th instar and adult Mormon crickets inoculated with 105, 106 and 107 spores/Mormon cricket resulted in reduced fecundity and evidence of venereal disease transmission. Egg production by females treated as 6th and 7th instars or mated with males infected as 6th instars was significantly lower when treated with 107 spores/Mormon cricket compared with the untreated Mormon crickets. Sixth and 7th instar males inoculated with 105-107 spores/Mormon cricket when mated with females caused significantly greater infection in the females.

The results showed that *Vairimorpha* sp. caused infections which resulted in retarded development in younger Mormon crickets in the laboratory bioassay and reduced egg production by adults in the field cage study. The development of infections requires more time and higher dosages in older Mormon crickets compared with younger Mormon crickets. Transmission was observed to occur from infected males to adult females via the spermatophore. The field application of wheat bran impregnated with *Vairimorpha* sp. spores established that epizootics can be induced in Mormon cricket populations. Accordingly, *Vairimorpha* sp. appears potentially useful for managing the densities of Mormon crickets.

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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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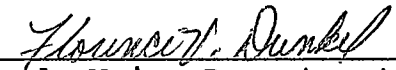
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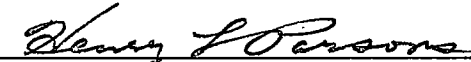
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ABSTRACT

The Mormon cricket, Anabrus simplex, is a pest of crops and rangelands in the western United States which frequently occurs endemically in environmentally sensitive areas. Biological control tactics are desirable in such areas. Vairimorpha sp., a protozoan known to infect Mormon crickets, appears useful as such a tactic.

The purpose of this study was to determine the potential of Vairimorpha sp. to adversely affect developing Mormon crickets. Field applications of 0.4×10^9 , 1.0×10^9 and 2.0×10^9 spores/ha were applied on 2 ha plots containing 2nd and 3rd instar Mormon crickets. Disease prevalence in treated plots ranged from 20.5-48.5%, with no infections in the untreated plots 2, 4 and 7 days post-treatment.

Laboratory bioassays involving 4th, 5th, 6th and 7th instar and adult Mormon crickets treated with 10^3 , 10^4 , 10^5 , 10^6 or 10^7 spores/Mormon cricket resulted in infections, however, no significant difference in the mean number of days survived between untreated and treated Mormon crickets was observed. Delayed development in the treated 4th and 6th instars resulted from infections. Observable infections required more time to develop in older crickets compared with younger crickets.

Field cage studies of male and female 6th and 7th instar and adult Mormon crickets inoculated with 10^5 , 10^6 and 10^7 spores/Mormon cricket resulted in reduced fecundity and evidence of venereal disease transmission. Egg production by females treated as 6th and 7th instars or mated with males infected as 6th instars was significantly lower when treated with 10^7 spores/Mormon cricket compared with the untreated Mormon crickets. Sixth and 7th instar males inoculated with 10^5 - 10^7 spores/Mormon cricket when mated with females caused significantly greater infection in the females.

The results showed that Vairimorpha sp. caused infections which resulted in retarded development in younger Mormon crickets in the laboratory bioassay and reduced egg production by adults in the field cage study. The development of infections requires more time and higher dosages in older Mormon crickets compared with younger Mormon crickets. Transmission was observed to occur from infected males to adult females via the spermatophore. The field application of wheat bran impregnated with Vairimorpha sp. spores established that epizootics can be induced in Mormon cricket populations. Accordingly, Vairimorpha sp. appears potentially useful for managing the densities of Mormon crickets.

CHAPTER 1

INTRODUCTION

The Mormon cricket, Anabrus simplex Haldeman, has been reported as a crop and rangeland pest in the Rocky Mountain region of the Western United States since the early Mormon settlements in Utah in 1848 (Corkins, 1923). Outbreaks, explosive increases in population numbers occurring in a short period of time (Berryman, 1987), have occurred in endemic habitats of the Mormon cricket. Mormon crickets often migrate from the endemic areas to surrounding range and crop lands where they may persist for many years. Outbreaks have occurred in Montana, Idaho, Wyoming, Nevada, Utah, and Colorado from 1879 to 1904, 1914 to 1939 and 1955 to 1962 (Corkins, 1923; Cowan et al., 1943; and Henry and Onsager, 1982). More recently, Mormon cricket populations have increased since 1972 with outbreak populations observed in Colorado during 1985-1988 and in Idaho and Nevada during 1990.

Historically, control methods included: 1) bran baits which initially were treated with sodium arsenite (1922-1935) or sodium fluosilicate (1935-1950s), and later (1950s-1960s) with aldrin, chlordane, or toxaphene; 2) kerosene or heavy crude oil application to irrigation ditches and streams; 3) barriers such as fences made of galvanized tin or trenches dug

in the ground; and 4) chemical dusts of sodium arsenite or calcium arsenite (Corkins, 1923; Cowan and McCampbell, 1929; Cowan, 1935; Wakeland and Shull, 1936; Wakeland et al., 1939; Cowan et al., 1943; and Cowan and Wakeland, 1962). Many of these methods were labor intensive and harmful to the environment (Corkins, 1923; Wakeland and Shull, 1936; and Cowan and Wakeland, 1962). Wheat bran bait treated with carbaryl has been used more recently to control Mormon cricket populations (personal observation).

Endemic cricket populations frequently occur on public lands. A large proportion of these lands are environmentally sensitive and applications of chemical insecticides are not appropriate. When outbreak populations occur in these areas, Mormon cricket bands often migrate to privately owned land where they can cause crop and range damage. The high cost of chemical control makes it difficult for landowners to protect their fields from the invading bands.

Most governmental agencies that manage public lands are interested in developing biological control protocols that prevent Mormon cricket outbreaks. Nosema locustae Canning, a microsporidium that infects many grasshopper (Orthoptera: Acrididae) species, has been tested on the Mormon cricket. Infection of young Mormon crickets resulted in increased mortality and reduced densities (Henry and Onsager, 1982). However, the increased mortality may have been due to bacterial septicemia because N. locustae does not infect older

crickets and does not produce high spore concentrations (Henry and Onsager, 1982), which limits its potential as a long-term control organism.

More recently, MacVean and Capinera (1991) isolated a microsporidium from Mormon crickets, which has been assigned to the genus Vairimorpha. They suggested that Vairimorpha sp. holds promise as a short-term and long-term biological control organism.

Statement of Objectives

It is hypothesized that infections by the protozoan Vairimorpha sp. hinders the growth and development of the Mormon cricket, Anabrus simplex. The objective of this study was to determine the effects of Vairimorpha sp. infections on the development, mortality and fecundity of the Mormon cricket. The prevalence and development of infections and the methods of transmission of the microsporidium also were studied.

CHAPTER 2

LITERATURE REVIEW

The Mormon Cricket (*Anabrus simplex*)History

The Mormon cricket is a long-horned grasshopper belonging to the family Tettigoniidae. Corkins (1923) noted that the Mormon cricket first was recorded as an agricultural pest in 1848 in Utah by the Mormon settlers. The common name Mormon cricket was probably adopted because of the above mentioned incident and the fact that it somewhat resembles the common black field cricket and produces a similar stridulation sound (Cowan, 1929).

Natural Habitat

The Mormon cricket prefers the high-elevation, rugged intermountain regions of the western United States. Gillette and Johnson (1905) reported large populations of Mormon crickets in the sage brush plains and hills in southern Idaho and northern Nevada and Utah. The broken, montane sagebrush-grass areas in the Rocky Mountains also represents their natural habitat (Cowan, 1929; Wakeland and Shull, 1936). These inter-mountain and montane habitats are areas in which endemic populations of Mormon crickets persist year after year

(Cowan, 1929; Wakeland and Shull, 1936; Cowan et al., 1943). Many of these endemic areas occur on public lands in remote mountainous or desert regions (Wakeland and Parker, 1953).

Distribution and Economic Importance

The Mormon crickets have been intermittent pests in the western United States and Canada since early settlement (Corkins, 1923). Wakeland and Shull (1936) reported economically important outbreaks in Colorado, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming. An outbreak of Mormon crickets occurred in South Dakota and to a lesser extent in Nebraska and North Dakota in 1937 and 1938 (Cowan et al., 1943). Endemic populations also occurred in southwestern Canada, Minnesota, and Arizona (Wakeland and Shull, 1936).

Outbreak populations build up in endemic areas during favorable years (Cowan, 1929; Wakeland and Shull, 1936; Cowan et al., 1943) and emigrate to cultivated (Cowan, 1929; Wakeland and Shull, 1936; Wakeland and Parker, 1953) and grazing lands (Wakeland and Parker, 1953) on which they are considered pests.

In their natural habitats, the Mormon cricket prefers plants with large succulent leaves such as balsamroot, mustard, dandelion, and bitterroot (Wakeland and Parker, 1953). 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). Before seed development,

cereal crops may be totally consumed; however, after seed production has begun, they tend to eat the kernels (Corkins, 1923; Cowan, 1929, 1935). Adult Mormon crickets often attack the cereal heads during the milk and dough stages, resulting in reduced yields of seeds (Cowan, 1929). Alfalfa, sweetclover and sugar beets also are preferred plants (Wakeland and Parker, 1953). Alfalfa, which probably is second to wheat in plant selection by the Mormon cricket, often is completely stripped of leaves (Cowan et al., 1943) and garden crops may be completely consumed by migrating bands (Corkins, 1923; Cowan, 1929).

Development

Copulation occurs about 10 days after the final molt to the adult (Cowan and McCampbell, 1929) and oviposition begins shortly thereafter. The onset of oviposition may occur as early as mid-July and often continues until mid-September (Corkins, 1923; Cowan, 1929; 1935; Wakeland and Shull, 1936). Each female deposits 25-50 eggs during a 1-2 day period followed by a period of 4-7 days during which the female mates and develops eggs for the next laying period (Cowan, 1935; Wakeland et al., 1939; Cowan et al., 1943). Females oviposit at about 7-8 day intervals during the reproductive period (Cowan, 1929).

Riley et al. (1880) noted that individual females laid from 50 to 75 eggs in a season. Gillette and Johnson (1905) have observed 133 eggs in different stages of

development in one female. In laboratory studies, Cowan (1929) and Cowan and McCampbell (1929) observed an average of 85 eggs with a high egg production of 160 eggs per female. Corkins (1923) suggested that several hundred eggs were laid while Cowan (1935) suggested that 150-200 eggs were laid by individual females. Wakeland et al. (1939), Cowan et al. (1943) and Cowan and Wakeland (1962) indicated that whereas individual females might produce about 300 eggs, the average production was about 150 eggs per female.

The eggs are laid singly about 0.65-2.54 cm deep in elevated and well-drained sandy or sandy loam soil devoid of plant roots (Corkins, 1923; Cowan, 1929; 1935). Gillette and Johnson (1905) observed females ovipositing on dry knolls of soft clay soil with very little vegetation. Riley et al. (1880) noted that the females did not oviposit in the fertile, moist, cultivated bench-lands of the Great Salt Lake region but preferred the dry bench-lands and higher elevated slopes of the foothills. Females tend to aggregate for egg laying, resulting in large numbers of eggs in confined areas (Cowan, 1935). Gillette and Johnson (1905) collected between 2000 to 3000 eggs from an ovipositional site 30.5 cm² in size.

Embryonic development occurs following deposition of the egg, proceeding into the fall and the embryo is fully developed before the onset of diapause prior to winter (Cowan, 1929; Cowan and McCampbell, 1929; Wakeland and Shull, 1936; Wakeland et al., 1939; Cowan et al., 1943).

Hatching occurs almost immediately after the snow has melted (Cowan, 1929; Cowan and McCampbell, 1929; Wakeland et al., 1939; Cowan et al., 1943). Mormon crickets develop through seven nymphal stages before the final molt to the adult (Cowan, 1929; Cowan, 1935; Wakeland and Shull, 1936; Wakeland et al., 1939; Cowan and Wakeland, 1962). The time required for development to the adult varies, depending on weather it may range from 60-100 days (Cowan, 1929; 1935; Cowan and McCampbell, 1929; Cowan and Wakeland, 1962; Cowan et al., 1943; Wakeland and Shull, 1936). Wakeland and Shull (1936) reported an average stadial duration of about 10 to 14 days in the field. Cowan (1929) reared crickets in the laboratory at room temperature (21-27°C) and observed average stadia of 5.1-10.4 days (n=16).

Reproduction

Gillette (1904) observed that a white mass was transferred from the male to the female while in copula. This white mass, the spermatophore, consists of an ampulla which contains sperm and a spermatophylax or protein mass (Gwynne, 1982a). Copulation begins with coupling and is completed with the transfer of the spermatophore (Gwynne, 1984a). After copulation, the spermatophore is generally visible externally to the female gonophore (Gwynne, 1982a). The large spermatophore produced by the male (Gillette, 1904; Gwynne,

1981) may represent up to 27% of the total body weight, but on average represents about 20% of the weight (Gwynne, 1981).

The spermatophylax contains a large amount of protein that appears important to females for reproduction. Most of the nutrients from the spermatophore are found in the ovaries and are used in egg production (Bowen et al., 1984). These nutrients enhance fecundity by increasing the number and size of eggs (Gwynne, 1984b). The female copulates several times during her adult life (Cowan and McCampbell, 1929). Gwynne (1984a) suggested that the spermatophore contains nutrients that might be limited or otherwise unavailable and that females will mate simply to obtain the spermatophylax even when food is available. These additional matings apparently do not influence the concentration of sperm in the spermatheca, but do increase fecundity (Gwynne, 1982b).

Migration

Major migrations often begin when Mormon crickets are fourth instar nymphs and continue during the remainder of their lives (Cowan, 1929). Gillette and Johnson (1905) and Cowan et al. (1943) have suggested that Mormon crickets in bands could travel up to one half mile per day. Assuming about 50 days between the beginning of migrations to the start of egg-laying, a band could move about 25 miles in a season (Cowan et al., 1943). Other reports of distances travelled in a day were 0.25 to 1.25 miles by Cowan (1929) and Cowan and McCampbell (1929) and 1.5 miles by Corkins (1923). The

direction of travel by migrating bands generally appears random (Cowan, 1929). However, during severe outbreaks, migrating bands coalesce to form massive bands which tend to move in a uniform direction and often cover thousands of acres (Cowan, 1929).

Cannibalism

Cannibalistic behavior is common among Mormon crickets (Cowan, 1929; Cowan and McCampbell, 1929). Deformed and moribund Mormon crickets are quickly consumed by the other members of the band (Riley *et al.*, 1880; Wakeland and Shull, 1936). Gillette and Johnson (1905), Corkins (1923) and Cowan and McCampbell (1929) have indicated that struggling or weakened Mormon crickets were preferred over other foods. The molting nymphs (Cowan and McCampbell, 1929) and ovipositing adult females (Cowan, 1929) also are susceptible to attacks. The Mormon cricket has few internal parasites which possibly is an outcome of this cannibalistic behavior (Cowan and McCampbell, 1929).

The Microsporida

Spore Structure

In a review of Microsporida, Larsson (1986) stated that the spore contains an extrusion apparatus consisting of the polar filament, which is attached to an anchoring apparatus, and the polaroplast. The polar filament develops from Golgi apparatuses within the sporoblast and form laminated tubular

structures that coil in the posterior half of the spore (Larsson, 1986). The polaroplast develops with the polar filament, forming membrane-bound sacs packed together in the anterior portion of the spore. In mature spores, the polaroplast completely surrounds the uncoiled portion of the polar filament.

Life Cycle

Spores usually are transmitted with the host's food (Weiser, 1976; Streett, 1987), with growth initiated after being consumed. The polar filament in each spore extrudes in the host midgut and penetrates an epithelial cell (Vavra, 1976). The sporoplasm is then transferred into the epithelial cell where the meront begins to develop (Vavra, 1976). Because vegetative growth, merogony, occurs within the host cell, microsporidia are considered strict intracellular parasites (Weiser, 1976).

The process by which meronts are transmitted to the other tissues within the body after the initial infection of the midgut epithelial cells is unknown (Vavra, 1976; Weiser, 1976). Weiser (1976) suggested that the meronts circulate through the host body in the hemolymph and are phagocytized by specific tissues which are suitable for growth of the microsporidium. He also noted that fat body, gut tissue, Malpighian tubules, or muscle tissue have been infected in some single tissue infections.

Meronts increase through binary and multiple fission (Vavra, 1976). The metabolic activity of the host cell affects the growth of the meront because microsporidian development is inhibited in diapausing cells (Vavra, 1976; Weiser, 1976). This characteristic may be important for microsporidian survival during transovarial transmission in diapausing eggs.

Transformation from merogony to sporogony is signaled by marked structural changes of the meront (Vavra, 1976). These structural changes include the appearance of a second membrane or patches of dense material around the microsporidian cell and the development of a clear structureless halo between the host cell cytoplasm and the microsporidium (Vavra, 1976). Environmental factors apparently influence this transformation (Vavra, 1976). Division of the nuclear component of the sporont occurs once to several times, with the final division producing single celled sporoblasts (Vavra, 1976). The sporoblast immediately precedes spore formation and is the stage in which spore organelles begin to form and the condensation of the cytoplasm occurs (Vavra, 1976).

Two types of sporogony have been observed in some Microsporida: disporoblastic sporogony and octosporoblastic sporogony (Malone and Canning, 1982). In disporoblastic sporogony, the sporont nucleus undergoes divisions to produce a tetranucleate sporont that ultimately undergoes a final

cytoplasmic cleavage to form two free binucleate sporoblasts. Each sporoblast eventually transforms into a binucleate spore.

In octosporoblastic sporogony, a sporophorous vesicle membrane forms to enclose the sporoblastic sporont (Malone and Canning, 1982). In some microsporidia, the sporophorous vesicle membrane blisters off the plasmalemma of the sporont, while in other species this membrane is generated de novo at the onset of sporogony. The formation of this vesicle may control the environment in which the spores are produced. The nucleus of the sporont undergoes divisions producing an octonucleate sporont (Pilley, 1976). The octonucleate sporont then undergoes a cytoplasmic division producing an eight unit pansporoblast which eventually becomes eight uninucleate spores within the sporophorous vesicle (Pilley, 1976).

Spore Development

The gradient of meronts, sporonts and sporoblasts suggest that the onset of sporulation may be influenced by some unidentified cue (Vavra, 1976). It may be triggered by saturation concentrations of meronts in infected tissue (Weiser, 1976) or by qualitative changes within the infected cell (Vavra, 1976). For some microsporidia, spore development does not occur until most of the susceptible host tissues are infected (Maddox and Sprenkel, 1978). The fast onset of sporulation observed in the genera Nosema and Thelohania suggests that the rapid change to sporulation protects the microsporidium from degradation during degeneration of the

infected tissues (Vavra, 1976). These degenerative changes may represent the onset of death of the host.

Hostounsky and Weiser (1972) observed that when small doses of Vairimorpha plodiae were administered to larvae of Mamestra brassicae, reproduction of the microsporidium appeared unrestricted; however, large doses inhibited reproductive activity. Sedlacek et al. (1985) observed that low doses of Vairimorpha sp. 696 in larvae of Heliothis virescens produced more spores in the infected tissues than did higher doses. The rate with which tissues are invaded may determine the progression of the disease.

Temperature also appears to influence the development of spores in microsporidian infections. Spores of N. locustae developed in 13 days in grasshoppers reared at 35°C (Henry, 1972). Spores of Vairimorpha necatrix developed in the fat body 10 days after inoculation of Ostrinia nubilalis held at 27°C at 70% relative humidity (Lewis et al., 1982). Spores of Vairimorpha sp. 696 developed in H. virescens in 8 days when reared at 32°C and in 12 days when reared at 19°C (Sedlacek et al., 1985). When Maddox and Sprenkel (1978) inoculated larvae of Plodia interpunctella with 3.9×10^5 spores of V. plodiae, spores developed in 26 days at 21°C and 117 days at 16°C.

Pathogenesis of Microsporida

Host morbidity often is the desired end product of infection by a control agent. The death of a host may occur

due to bacterial septicemia, which is induced by the infection, or by microsporidiosis. Bacterial septicemia occurs when the midgut epithelial cells are destroyed or disrupted by parasite invasion, allowing the entrance of bacteria into the hemocoel (Fuxa, 1981). Cossentine and Lewis (1984) observed that early instars of Agrotis ipsilon died as a result of gut damage following exposure to high inoculative dosages of Vairimorpha sp. (isolated from a cotton leafworm, Alabama argillacea) and V. necatrix. Chu and Jaques (1979) observed that when larvae of Trichoplusia ni developed acute symptoms of infection by V. necatrix, the infections were restricted to the midgut. High dosages of a microsporidium generally cause acute infections and shorter periods until morbidity (Tanada, 1976).

If bacterial septicemia does not result during invasion, the microsporidia will then develop in other susceptible tissues. Microsporidiosis results from pathogenesis and accumulation of parasites in the tissues. Morbidity of the insect coincides with sporulation, when the infected cells become filled with spores, thus impairing the proper function of the tissues.

Laboratory and field studies have provided insight on disease prevalence and resulting mortality caused by microsporidia in various lepidopterous and orthopterous pests. Pilley (1976) reared 1st and 5th instar larvae of Spodoptera exempta on media containing 6.75×10^5 spores of V. necatrix

and observed mortality at 1-2 days and 8-24 days, respectively. Wilson (1984) observed about 88% mortality among 3rd instar larvae of Malacosoma disstria inoculated with 5×10^3 spores/larva of V. necatrix and 78% mortality among 5th instar larvae inoculated with 5×10^4 spores/larva. Mortality among larvae of H. virescens was dose-responsive following infection with V. necatrix (Jordan and Noblet, 1982).

In field studies with N. locustae, Henry et al. (1973) demonstrated that increased spore concentrations between 5 to 140 spores/cm² reduced host densities and increased disease prevalence in grasshopper populations. They observed that the timing of applications of spores was important and that application which coincided with the appearance of 3rd instar nymphs maximized mortality and disease prevalence in the surviving grasshoppers. Henry and Onsager (1982) observed that N. locustae infected Mormon crickets. Aerial application of wheat bran formulated with 2.5×10^9 spores of N. locustae per kg at a rate of 1.86 kg/ha. resulted in infection of fewer than 20% and fewer than 33% of crickets collected 28 and 49 days, respectively, after treatment (Henry and Onsager, 1982).

Effects of Microsporida Infections on Fecundity

Veber and Jasic (1961) suggested that too much emphasis has been placed on lethal infections by pathogenic

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 microbial organism should reduce host fecundity. Reducing fecundity includes depressed egg production as well as decreased viability or hatchability of the eggs (Kellen and Lindegren, 1971).

Kramer (1959) observed that infection of females of O. nubilalis with Nosema pyrausta either inhibited egg production or reduced the number of egg masses. Veber and Jasic (1961) observed that infections by Nosema bombycis in Bombyx mori and Hyphantria cunea decreased egg production. They also found that higher dosages or earlier treatments caused a greater reduction in the number of eggs compared with lower dosages and later treatments. Infections of V. plodiae reduced egg production by 21% in P. interpunctella (Kellen and Lindegren, 1971). Increased infection of fat bodies in laboratory-reared females of Melanoplus differentialis by N. locustae resulted in decreased egg production (Henry and Oma, 1981). Infections by N. pyrausta in O. nubilalis also caused reduced fecundity (Lewis et al., 1982).

Henry and Oma (1981) suggested that fecundity among female M. differentialis may be influenced by the inability of

males to inseminate the female or to produce viable sperm due to infections by N. locustae.

Transmission of Microsporida in Host Populations

Transmission within a host population is an important requirement of a long term microbial control organism (Streett, 1987). Normally per-oral transmission of Microsporida occurs as a result of contamination of food sources. Fat body or muscle cells often become filled with spores during sporulation (Weiser, 1976), forming a reservoir of spores for further infections. Venereal and transovarial transmission has been observed in microsporidian infections. Kellen and Lindegren (1971) reported that V. plodiae was transmitted venereally in P. interpunctella. The testes of Heliothis zea were heavily infected with spores of Nosema heliothidis (Brooks, 1968), which suggests venereal transmission. Instances of transovarial transmission have been observed with N. pyrausta in O. nubilalis (Kramer, 1959), Thelohania californica in Culex tarsalis (Kellen and Wills, 1962), N. heliothidis in H. zea (Brooks, 1968), V. plodiae in P. interpunctella (Kellen and Lindegren, 1971; 1973), and N. locustae in grasshoppers (Henry and Oma, 1981).

CHAPTER 3

MATERIALS AND METHODS

Source of Spores

Spores of Vairimorpha sp. were obtained from Mormon crickets collected from Dinosaur National Monument, Colorado during the summer 1988. Infected Mormon crickets were homogenized in distilled water and the solution was passed through cheesecloth to remove large tissue fragments. The suspension was centrifuged to separate the spores from smaller tissue fragments in the suspension. The pellet containing the spores was then resuspended and the spore concentrations were quantified by direct counts on a hemocytometer. Stock aliquots of 10^9 spores/ml were stored at 10°C .

Evaluation of Vairimorpha sp. in Field Applications

Four 2 ha (200mx100m) plots, comprising one replication, infested with 2nd and 3rd instar nymphs, were established in a mixed grass and sagebrush pasture in Moffat County, Colorado during the spring 1989. The three treated plots were contiguous with shared 200m borders while the control plot was located 2km from the treated plots. A pre-treatment collection of 200 Mormon crickets was obtained 1 day before

application of the treated wheat bran. Single plot treatments of 0.4×10^9 , 1.0×10^9 or 2.0×10^9 spores/ha on 4.5 kg/wheat bran/ha were applied with a mechanical spreader mounted on a four wheel ATV. The check plot received an application of unformulated wheat bran.

Two hundred Mormon crickets were collected from each plot at 2, 4 and 7 days after treatment, of which 50 were reared individually for 10 days and the remaining frozen for subsequent examination. The reared Mormon crickets were fed iceberg lettuce and wheat bran on alternate days. After 10 days, the time in which detectable concentrations of spores occur (Henry, pers. com.), the reared crickets were frozen for subsequent examinations. Microscopic examinations involved dissecting Mormon crickets for removal of fat body tissues from the abdominal cavity which then were examined under phase microscopy for spores at 400 diameter magnification.

Counts of infected and uninfected Mormon crickets for each plot collection were analyzed using 2x2 contingency tables adjusted for continuity with Yates' correction for continuity (Zar, 1974).

Laboratory Bioassay

Samples of Mormon crickets were collected from the sand dune areas in Fremont County, Idaho during the summer 1989. These crickets were examined for infections by Vairimorpha sp. preliminary to planned studies in 1990.

Mormon crickets were collected as 4th instar nymphs through adults from the sand dune areas in Fremont County, Idaho during the spring and summer 1990. They were segregated by age class and then randomly separated into the treatment groups. Each Mormon cricket was isolated in a vial, starved for about 24 hours, then fed a disc (7 mm dia.) of iceberg lettuce that was treated with a specified inoculum. Treatments were 10^3 , 10^4 , 10^5 , 10^6 or 10^7 spores/Mormon cricket with control groups treated with distilled water. Spore dosages were derived by serial dilution of the 10^9 spores/ml stock solution of Vairimorpha sp. Only Mormon crickets that consumed the entire disc were used in the tests.

After inoculation, Mormon crickets from each treatment group were placed in replicates of 5 insects/acetate tube (20 cm long by 5 cm dia.), for rearing at 24°C. The ends of the tubes were sealed with screened Kerr® rings. The number of replications (groups of 5 insects) for the 4th, 5th, 6th and 7th instar nymphs and adult stages were 8, 4, 14, 16 and 17, respectively. The grouped Mormon crickets were fed iceberg lettuce and wheat bran on alternating days. They were checked each day for mortality. Dead insects were removed and refrigerated until examined by phase microscopy for spores in the fat body. The developmental stage and infection status were recorded. The duration of bioassays of the 4th, 5th, 6th and 7th instar and adult Mormon crickets were 116, 78, 102, 82 and 59 days, respectively. These durations were determined by

the death of the last Mormon cricket in the treatment or the end of the field season on August 27, 1990.

Survival and Development of Mormon Crickets
Treated with Vairimorpha sp.

The mean number of days each Mormon cricket survived in each treatment was analyzed using the ANOVA procedure followed by a multiple range test on significant results using Tukey's studentized range test (SAS Institute Inc, 1989).

Cumulative mortality was calculated by totalling the number which died in each instar such that the treated 4th instar stage had a number x die in the 4th instar stage and a number y die in the 5th instar stage and the cumulative total for the 5th instar stage equals $x + y$. The percentage was determined by dividing the total dead by the total treated in the treatment (all replicates). Counts of dead and live Mormon crickets for each treatment were analyzed using 2x2 contingency tables adjusted for continuity with Yates' correction for continuity (Zar, 1974).

Development of Vairimorpha sp.
in Mormon Crickets

The Mormon crickets were divided into three arbitrary classes based on the onset of morbidity. The classes were 1 to 10 days, 11 to 20 days and 21 days to the end of each bioassay. The classes were selected to evaluate temporal aspects of spore formation. Readily detectible concentrations

of spores were not expected to occur before 10 days (Henry, pers. com.).

Counts of infected and uninfected cadavers for each treatment were evaluated using 2x2 contingency tables adjusted for continuity with Yates' correction for continuity (Zar, 1974).

Field Cage Study

Field tests were conducted in cages fabricated from 2 gallon plastic buckets. Several holes (7 cm dia.) 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 the ground. A circular screened lid was made from a plastic plant pot holder and was fitted into the hole in the base of each bucket. The soil under each cage was sifted and mixed with sand.

Mormon crickets were collected as 6th instar nymphs through adults from the sand dune areas in Fremont County, Idaho during the summer 1990. They were segregated by instar and sex, then grouped as 6th instar females, 6th instar males, 7th instar females, 7th instar males, adult females, and adult males. Mormon crickets were then randomly assigned to treatment groups. Treatments were 10^5 , 10^6 or 10^7 spores/Mormon cricket. Mormon crickets in control groups were treated with distilled water. Dosages were derived by serial dilution of the stock solutions of Vairimorpha sp. spores.

Each Mormon cricket was isolated in a vial, starved for about 24 hours, then fed a disc (7 mm dia.) of iceberg lettuce that was treated with a specified inoculum. Only Mormon crickets that consumed the entire disc were used in the tests.

After inoculations, Mormon crickets from each treatment group were placed in replicates of 5 Mormon crickets/cage. Four replicates were performed on each treatment group. The Mormon crickets were fed iceberg lettuce and wheat bran daily. They were examined daily for mortality and the females were observed for adult eclosion.

Effects of Vairimorpha sp. Infections on Mormon Cricket Fecundity

On June 27, 1990, after the treated crickets had become adults, the soil under each cage was sifted for eggs. At this time mates were added to the cages in equal proportion to the remaining crickets (i.e. 1:1). The soil under each cage was sifted weekly from July 4 to August 22, 1990. The eggs were placed in paper bags and transported to the laboratory where they were counted. The total adult female days for each cage was calculated from the time of adult eclosion to the time of death for each female in each cage of the 6th and 7th instar treated females, and from the time of addition to the cage to the time of death for the adults.

Mean number of eggs, mean number of adult days, and mean number of eggs per adult female day were analyzed using the ANOVA procedure and a multiple range test was performed on

significant results using Tukey's studentized range test (SAS Institute Inc, 1989).

Venereal Transmission
of Vairimorpha sp.

The female Mormon crickets that were mated with treated males were examined by phase microscopy for spores in the fat body tissues. Statistical analysis was performed on the counts of infected and uninfected female Mormon crickets for each treatment using 2x2 contingency tables which were adjusted for continuity using Yates' correction for continuity (Zar, 1974).

CHAPTER 4

RESULTS

Evaluation of *Vairimorpha* sp. in Field Applications

Examinations of Mormon crickets from pretreatment samples revealed no infections. Infections were observed in the Mormon crickets collected on 2, 4 and 7 days post-treatment in each treated plot and reared for 12 days, while infections were not observed in Mormon crickets collected in the control plot (Table 1). The Mormon crickets collected 4 days after treatment showed the highest prevalence in each of the three

Table 1. Prevalence of *Vairimorpha* sp. in Mormon crickets at 2, 4 and 7 days after treatment of 2 ha plots with 0.4×10^9 , 1.0×10^9 or 2.0×10^9 spores/ha on 4.5 kg/ha wheat bran.

Dose (spores/ha)	Disease prevalence					
	Day 2		Day 4		Day 7	
	N	%	N	%	N	%
0	38	0.0a ¹	34	0.0a	12	0.0a
0.4×10^9	44	20.5b	45	37.8b	29	24.1a
1.0×10^9	30	26.7b	45	42.2b	29	34.5a
2.0×10^9	20	30.0b	33	48.5b	22	36.4a

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

treatments. The prevalence of infection in Mormon crickets collected on 2 and 4 days post-treatment in the treated plots were significantly higher than the prevalence of infection in Mormon crickets collected from the untreated plot ($v=1$, $\chi^2>3.841$, $P<0.05$). There were no significant differences among the treated and untreated groups collected 7 days post-treatment ($v=1$, $\chi^2<3.841$, $P>0.05$).

Laboratory Bioassay

Survival and Development of Mormon Crickets Treated with *Vairimorpha* sp.

The mean number of days survived by Mormon crickets inoculated with *Vairimorpha* sp. varied from 16.1 to 33.3 days compared to 23.8 to 38.3 days for untreated Mormon crickets (Table 2). However, significant differences were observed only between untreated 6th instar Mormon crickets and the 6th instar nymphs treated with 10^6 spores/each ($df=3$, $F=3.70$, $P=0.02$). The survival rates of 6th instar Mormon crickets treated with 10^4 or 10^5 spores each were not significantly different from that of untreated 6th instar nymphs.

Differences were observed in the temporal aspects of morbidity among Mormon crickets treated with *Vairimorpha* sp as 4th instar nymphs compared to untreated insects (Table 3). The cumulative mortalities during 4th and 5th instar stages of Mormon crickets treated with 10^5 spores each as 4th instar nymphs were significantly greater than morbidity among

Table 2. Mean number of days survived by 4th, 5th, 6th and 7th instar and adult Mormon crickets following treatment with Vairimorpha sp.

Dose (spores/cricket)	Days survived per instar									
	4th		5th		6th		7th		adult	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0	23.8	5.8	26.8	7.5	38.3a ¹	4.8	24.2	2.9	36.5	3.1
10 ³	16.1	3.6	----		----		----		----	
10 ⁴	19.4	5.6	23.3	1.5	26.7ab	2.1	----		----	
10 ⁵	18.6	4.9	27.4	4.6	28.4ab	3.3	18.5	2.2	32.3	2.4
10 ⁶	----		25.0	6.3	24.1 b	1.9	17.6	2.5	32.5	2.8
10 ⁷	----		----		----		19.1	1.9	33.3	3.3

¹Means followed by the same letter within a column indicate lack of significant difference between treatments using Tukey's studentized range test (alpha = 0.05).

Table 3. Percent mortality among 4th, 5th, 6th and 7th instar Mormon crickets following treatments as 4th instar nymphs with Vairimorpha sp.

Dose (spores/cricket)	Cumulative mortality by instar							
	4th		5th		6th		7th	
	N	%	N	%	N	%	N	%
0	33	6.1a ¹	33	42.4a	33	60.6a	33	81.8a
10 ³	24	25.0ab	24	62.5a	24	91.7b	24	95.8a
10 ⁴	23	26.1ab	23	65.2ab	23	95.7b	23	100.0a
10 ⁵	29	48.3 b	29	89.7 b	29	100.0b	29	-----

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

untreated Mormon crickets ($v=1$, $\chi^2>3.841$, $P<0.05$). The cumulative mortalities among 4th instar Mormon crickets treated with 10^3 , 10^4 or 10^5 spores each were significantly greater during the 6th instar stage than among the untreated Mormon crickets ($v=1$, $\chi^2>3.841$, $P<0.05$). There were no significant differences in cumulative mortality among the 7th instar nymphs ($v=1$, $\chi^2<3.841$, $P>0.05$).

There were no significant differences observed in the cumulative mortality of 5th, 6th and 7th instar nymphs among the Mormon crickets inoculated as 5th instar nymphs ($v=1$, $\chi^2<3.841$, $P>0.05$) (Table 4).

Table 4. Percent mortality among 5th, 6th and 7th instar Mormon crickets following treatments as 5th instar nymphs with Vairimorpha sp.

Dose (spores/cricket)	Cumulative mortality by instar					
	5th		6th		7th	
	N	%	N	%	N	%
0	10	22.2a ¹	10	55.6a	10	88.9a
10^4	13	33.3a	13	75.0a	13	100.0a
10^5	18	27.8a	18	88.9a	18	100.0a
10^6	11	18.2a	11	81.8a	11	90.9a

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

There were no significant differences ($v=1$, $\chi^2<3.841$, $P>0.05$) among the treated and untreated 6th instar Mormon

crickets as 6th instar nymphs (Table 5). The cumulative mortalities were significantly higher among 7th instar nymphs treated with 10^4 , 10^5 or 10^6 spores each as 6th instar nymphs when compared with the untreated Mormon crickets ($v=1$, $\chi^2>3.841$, $P<0.05$). The cumulative mortalities among Mormon crickets treated with 10^5 or 10^6 spores each as 6th instar nymphs also were significantly higher among 7th instar nymphs compared with dosages of 10^4 spores each ($v=1$, $\chi^2>3.841$, $P<0.05$).

Table 5. Percent mortality among 6th and 7th instar Mormon crickets following treatments as 6th instar nymphs with Vairimorpha sp.

Dose (spores/cricket)	Cumulative mortality by instar			
	6th		7th	
	N	%	N	%
0	59	25.4a ¹	59	50.8a
10^4	61	24.6a	61	75.4b
10^5	70	37.1a	70	94.3c
10^6	62	32.3a	62	93.5c

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

There were no significant differences ($v=1$, $\chi^2<3.841$, $P>0.05$) in cumulative mortality among 7th instar nymphs that were treated as 7th instar nymphs (Table 6).

Table 6. Percent mortality among 7th instar Mormon crickets following treatment as 7th instar nymphs with Vairimorpha sp.

Dose (spores/cricket)	<u>Cumulative mortality</u> 7th	
	N	%
0	77	32.5a ¹
10 ⁵	77	32.5a
10 ⁶	77	37.7a
10 ⁷	74	48.7a

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

Development of Vairimorpha sp.
in Mormon Crickets

Results relating the temporal aspects of development of spores of Vairimorpha sp. in cadavers from the bioassays indicated that spores developed more rapidly during early instars than in later instars of the host. When 4th instar nymphs were treated with 10³, 10⁴ or 10⁵ spores each, infections were not observed among the treatment or control Mormon crickets during the 1-10 day period (Table 7) and no significant differences ($v=1$, $\chi^2<3.841$, $P>0.05$) were observed in prevalence of infection during this time period. Between 11 and 20 days post-treatment, dosages of 10⁴ and 10⁵ spores/Mormon cricket caused significantly higher ($v=1$, $\chi^2>3.841$, $P<0.05$) disease prevalence when compared with

Table 7. Prevalence of *Vairimorpha* sp. in Mormon cricket cadavers during specific time periods following inoculation as 4th instar nymphs.

Dose (spores/cricket)	Percent of cadavers infected					
	Day 1-10		Day 11-20		Day 21-116	
	N	%	N	%	N	%
0	17	0.0a ¹	8	0.0a	15	26.7a
10 ³	19	0.0a	8	25.0ab	8	87.5b
10 ⁴	14	0.0a	6	66.7cb	10	100.0b
10 ⁵	23	0.0a	5	100.0c	12	100.0b

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

controls. After 21 days, treatments with dosages of 10³, 10⁴ or 10⁵ spores each were significantly higher ($v=1$, $\chi^2>3.841$, $P<0.05$) in disease prevalence compared with the untreated Mormon crickets.

Disease prevalence following inoculation of 5th instar nymphs with 10⁴, 10⁵ or 10⁶ spores/Mormon cricket varied substantially (Table 8). Accordingly, there were no significant differences observed between the treatments and control ($v=1$, $\chi^2<3.841$, $P>0.05$).

Likewise, no significant differences ($v=1$, $\chi^2<3.841$, $P>0.05$) were observed in the 1-10 day period for 6th instar nymphs treated with 10⁴, 10⁵ or 10⁶ spores each compared with the untreated Mormon crickets (Table 9). However, treatment of 10⁶ spores each resulted in significantly higher disease

Table 8. Prevalence of Vairimorpha sp. in Mormon cricket cadavers during specific time periods following inoculation as 5th instar nymphs.

Dose (spores/cricket)	Percent of cadavers infected					
	Day 1-10		Day 11-20		Day 21-78	
	N	%	N	%	N	%
0	5	0.0a ¹	2	50.0a	6	66.7a
10 ⁴	5	0.0a	4	0.0a	5	100.0a
10 ⁵	5	20.0a	4	50.0a	9	100.0a
10 ⁶	7	0.0a	2	100.0a	3	66.7a

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

Table 9. Prevalence of Vairimorpha sp. in Mormon cricket cadavers during specific time periods following inoculation as 6th instar nymphs.

Dose (spores/cricket)	Percent of cadavers infected					
	Day 1-10		Day 11-20		Day 21-102	
	N	%	N	%	N	%
0	19	0.0a ¹	10	10.0a	41	14.6a
10 ⁴	23	4.3a	23	17.4a	24	75.0 b
10 ⁵	23	8.7a	20	35.0a	32	96.9c
10 ⁶	36	5.6a	15	86.7b	24	83.3cb

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

prevalence ($v=1$, $\chi^2>3.841$, $P<0.05$) during the 11-20 day and 21-102 day periods when compared with the untreated Mormon

crickets. Treatments of 10^4 or 10^5 spores each caused significantly higher disease prevalence ($v=1$, $\chi^2>3.841$, $P<0.05$) compared with the control Mormon crickets after 20 days inoculation.

Development of infections appeared to be slowed among 7th instar nymphs treated with 10^5 , 10^6 or 10^7 spores each (Table 10). There were no significant differences in the prevalence of infection ($v=1$, $\chi^2<3.841$, $P>0.05$) observed during the 1-10 day period. Even though the prevalence of infection was low during the 11-20 day period, it was significantly higher ($v=1$, $\chi^2>3.841$, $P<0.05$) among those treated with 10^7 spores each compared with the untreated Mormon crickets. The prevalence of infection during the 21-82 day period following inoculation with 10^7 spores each was significantly higher ($v=1$, $\chi^2>3.841$,

Table 10. Prevalence of Vairimorpha sp. in Mormon cricket cadavers during specific time periods following inoculation as 7th instar nymphs.

Dose (spores/cricket)	Percent of cadavers infected					
	Day 1-10		Day 11-20		Day 21-82	
	N	%	N	%	N	%
0	25	8.0a ¹	28	3.6a	27	7.4a
10^5	31	3.2a	29	10.3ab	20	20.0ab
10^6	36	8.3a	28	21.4ab	16	56.2 b
10^7	31	0.0a	25	36.0 b	24	95.8c

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

$P < 0.05$) than that after treatments of 10^5 and 10^6 spores each and of untreated Mormon crickets. The prevalence of infection during the 21-82 day period following treatment with 10^6 spores each also was significantly higher ($v=1$, $\chi^2 > 3.841$, $P < 0.05$) than that among the untreated Mormon crickets.

Following treatment of adult crickets with 10^5 , 10^6 or 10^7 spores each, disease prevalence remained very low through the first 20 days for all three treatment groups (Table 11). There were no significant differences ($v=1$, $\chi^2 < 3.841$, $P > 0.05$) among the treated and the control Mormon crickets during the 1-10 day and 11-20 day periods. During the 21-59 day period, treatments of 10^6 or 10^7 spores each resulted in significantly higher disease prevalence ($v=1$, $\chi^2 > 3.841$, $P < 0.05$) than that among treatments of 10^5 spores each and untreated Mormon

Table 11. Prevalence of Vairimorpha sp. in Mormon cricket cadavers during specific time periods following inoculation as adults.

Dose (spores/crickets)	Percent of cadavers infected					
	Day 1-10		Day 11-20		Day 21-59	
	N	%	N	%	N	%
0	6	0.0a ¹	14	0.0a	61	9.8a
10^5	8	0.0a	15	6.7a	60	26.7b
10^6	7	0.0a	17	0.0a	61	50.8c
10^7	10	10.0a	12	0.0a	57	70.2c

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

crickets. The prevalence of infection during the 21-59 day period among Mormon crickets treated with 10^5 spores each was significantly higher ($v=1$, $\chi^2>3,841$, $P<0.05$) than that among untreated Mormon crickets.

Field Cage Study

Effects of Vairimorpha sp. Infections on Mormon Cricket Fecundity

Egg production by females inoculated as 6th and 7th instar nymphs and adults with 10^5 , 10^6 or 10^7 spores each, ranged from 0.3 to 6.3 eggs per female day compared with 5.5 to 6.6 eggs per female day produced by untreated females. Only the 6th instar females inoculated with 10^7 spores each produced significantly fewer eggs ($df=3$, $F=5.47$, $P=0.0133$) than the untreated Mormon crickets (Table 12). Likewise, the

Table 12. Mean egg production by Mormon crickets inoculated with Vairimorpha sp. as 6th instar nymphs.

Dose (spores/cricket)	Mean number					
	Eggs		Female days		Eggs per female days	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0	933.8a ¹	218.7	161.8	21.8	5.5a	0.8
10^5	340.5ab	105.9	108.8	9.6	3.4ab	1.2
10^6	718.2ab	245.6	131.5	39.2	5.5a	0.9
10^7	21.2 b	11.1	64.5	13.8	0.3 b	0.1

¹Means followed by the same letter within a column indicate lack of significant difference between treatments using Tukey's studentized range test ($\alpha = 0.05$).

number of eggs produced per female day by crickets inoculated with 10^7 spores each was significantly lower ($df=3$, $F=8.08$, $P=0.0033$) than the untreated and treatment of 10^6 spores each. There were no significant differences ($df=3$, $F=2.93$, $P=0.0770$) in the number of female days among the treated and untreated Mormon crickets.

The 7th instar females inoculated with 10^6 or 10^7 spores each produced significantly fewer eggs ($df=3$, $F=11.70$, $P=0.0007$) than the untreated females (Table 13). The females inoculated with 10^7 spores each also produced significantly fewer eggs than those inoculated with 10^5 spores each. The female Mormon crickets inoculated with 10^6 or 10^7 spores each survived a significantly shorter period of time ($df=3$, $F=12.31$, $P=0.0006$) than untreated insects and those treated

Table 13. Mean egg production by Mormon crickets inoculated with Vairimorpha sp. as 7th instar nymphs.

Dose (spores/cricket)	Mean number					
	Eggs		Female days		Eggs per Female day	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0	1585.2a ¹	133.5	241.5a	16.6	6.6a	0.4
10^5	1107.5ab	198.3	254.8a	12.3	4.4ab	0.8
10^6	473.2cb	284.9	140.8b	25.8	2.7 b	1.4
10^7	150.0c	51.9	141.5b	12.6	1.0 b	0.4

¹Means followed by the same letter within a column indicate lack of significant difference between treatments using Tukey's studentized range test ($\alpha = 0.05$).

with 10^5 spore each. The number of eggs produced per adult female day by female Mormon crickets inoculated with 10^6 or 10^7 spores each were significantly lower ($df=3$, $F=7.47$, $P=0.0044$) than the number produced by the untreated Mormon crickets.

There were no significant differences observed in the number of eggs produced ($df=3$, $F=1.74$, $P=0.2123$), the number of adult female days survived ($df=3$, $F=0.63$, $P=0.6112$), or the number of eggs produced per female day ($df=3$, $F=4.47$, $P=0.0250$) among untreated Mormon crickets and those treated with 10^5 , 10^6 or 10^7 spores each (Table 14).

Table 14. Mean egg production by Mormon crickets inoculated with Vairimorpha sp. as adults.

Dose (spores/cricket)	Mean number					
	$\bar{X}$	Eggs SE	$\bar{X}$	Female days SE	Eggs per female day $\bar{X}$	SE
0	1378.8	121.2	217.0	18.9	6.4a ¹	0.2
10^5	1367.0	127.3	214.8	13.5	6.3a	0.2
10^6	1159.5	70.5	220.8	10.2	5.3a	0.4
10^7	1052.5	152.3	193.0	18.8	5.4a	0.3

¹Means followed by the same letter within a column indicate lack of significant difference between treatments using Tukey's studentized range test ($\alpha = 0.05$).

Egg production by females mated with males inoculated as 6th or 7th instar nymphs and adults with 10^5 , 10^6 or 10^7 spores each, ranged from 3.6 to 6.6 eggs per female day

compared with 5.8 to 7.3 eggs per female day produced by those mated to untreated males. The number of eggs produced by female Mormon crickets mated with males inoculated as 6th instars with 10^5 or 10^7 spores/each were significantly lower ($df=3$, $F=5.41$, $P=0.0138$) than the number of eggs produced by those mated with untreated males (Table 15). There were no significant differences ($df=3$, $F=0.32$, $P=0.8086$) in the number of female days survived by females mated with treated and untreated 6th instar males. The females mated with males treated with 10^5 or 10^7 spores each as 6th instar nymphs produced significantly fewer ($df=3$, $F=4.90$, $P=0.0189$) eggs per female day compared with females mated with untreated males.

Table 15. Mean egg production by female Mormon crickets mated with males that were inoculated with Vairimorpha sp. as 6th instar nymphs.

Dose (spores/cricket)	Mean number					
	Eggs		Female days		Eggs per female day	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
0	1093.2a ¹	92.9	158.2	21.8	7.3a	1.0
10^5	479.8 b	158.8	130.5	37.1	3.8 b	0.6
10^6	557.5ab	97.0	138.2	10.0	4.1ab	0.8
10^7	468.5 b	150.3	123.8	28.5	3.6 b	0.6

¹Means followed by the same letter within a column indicate lack of significant difference between treatments using Tukey's studentized range test ($\alpha = 0.05$).

There were no significant differences observed in the number of eggs produced ($df=3$, $F=1.78$, $P=0.2041$), the number of female days survived ($df=3$, $F=1.82$, $P=0.1964$), or the number of eggs produced per female day ($df=3$, $F=1.28$, $P=0.3271$) among females mated with untreated males or those inoculated with 10^5 , 10^6 or 10^7 spores each as 7th instar nymphs (Table 16). Also, significant differences were not evident in the number of eggs produced ($df=3$, $F=0.24$, $P=0.8665$), the number of female days survived ($df=3$, $F=0.40$, $P=0.7532$), and the number of eggs produced per female day ($df=3$, $F=0.03$, $P=0.9912$) among females mated with untreated males or those treated with 10^5 , 10^6 or 10^7 spores each as adults (Table 17).

Table 16. Mean egg production by female Mormon crickets mated with males that were inoculated with Vairimorpha sp. as 7th instar nymphs.

Dose (spores/cricket)	Mean number					
	Eggs $\bar{x}$	SE	Female days $\bar{x}$	SE	Eggs per female day $\bar{x}$	SE
0	1012.2	254.6	164.0	30.7	5.8	0.9
10^5	1118.8	68.7	189.8	7.7	5.9	0.4
10^6	915.5	118.0	190.2	22.2	4.8	0.3
10^7	635.2	114.7	133.8	8.9	4.6	0.6

Venereal Transmission
Of Vairimorpha sp.

The prevalence of infections in female Mormon crickets

mated with males inoculated with 10^5 , 10^6 or 10^7 spores each as 6th instars were significantly higher ($v=1$, $\chi^2 > 3.841$, $P < 0.05$) than among females mated with untreated males (Table 18). Also, the prevalence of infections among females mated

Table 17. Mean egg production by female Mormon crickets mated with males that were inoculated with Vairimorpha sp. as adults.

Dose (spores/cricket)	Mean number					
	$\bar{X}$	Eggs SE	$\bar{X}$	Female days SE	Eggs per female day $\bar{X}$	SE
0	1288.0	148.8	196.0	24.5	6.6	0.2
10^5	1407.8	254.5	211.0	22.7	6.5	0.7
10^6	1224.2	227.7	182.5	21.6	6.6	0.6
10^7	1188.5	125.2	184.2	11.7	6.4	0.4

Table 18. Transmission of Vairimorpha sp. from male Mormon crickets inoculated as 6th and 7th instar nymphs and adults to untreated females.

Dose (spores/cricket)	Prevalence of infection in females					
	6th		7th		adult	
	N	%	N	%	N	%
0	15	13.3a ¹	17	17.6a	19	15.8a
10^5	10	80.0b	17	82.4b	20	10.0a
10^6	14	92.9b	18	77.8b	18	11.1a
10^7	13	61.5b	12	75.0b	19	31.6a

¹Percentages followed by the same letter within a column indicate lack of significant difference between treatments using the Chi square statistic from a 2x2 contingency table ($v=1$, $\chi^2=3.841$, $P=0.05$).

with males inoculated with 10^5 , 10^6 or 10^7 spores each as 7th instar nymphs were significantly higher ($v=1$, $\chi^2 > 3.841$, $P < 0.05$) than among females mated with untreated males. The prevalence of infection was not significantly different ($v=1$, $\chi^2 < 3.841$, $P > 0.05$) among female Mormon crickets that were mated with either untreated or those treated as adults.

CHAPTER 5

DISCUSSION

Evaluation of *Vairimorpha* sp. in Field Applications

MacVean and Capinera (1991) observed a dramatic reduction in survival of 1st, 2nd and 3rd instar Mormon crickets when treated with *Vairimorpha* sp. at a rate of 2.0×10^{12} spores/ha. They suggested that lower concentrations may reduce mortality and increase sublethal infection levels. During the present study, the prevalence of infection among 2nd and 3rd instar nymphs collected 2 and 4 days after the treatment of plots with 0.4×10^9 , 1.0×10^9 and 2.0×10^9 spores/ha was significantly higher than the prevalence of infection in the Mormon crickets collected from the untreated plots (Table 1). No infections were observed in the Mormon crickets collected from the untreated plot. The prevalence of infection observed in the 2 and 4 day post-treatment collections may suggest that the lower dosages (0.4×10^9 , 1.0×10^9 and 2.0×10^9 spores/ha), may have caused chronic infections in these 2nd and 3rd instar Mormon crickets rather than acute infections such as seen by MacVean and Capinera (1991).

The prevalence of infection in Mormon crickets collected 7 days after the treatment of plots with 0.4×10^9 ($P=0.226$),

1.0×10^9 ($P=0.077$) and 2.0×10^9 ($P=0.071$) spores/ha was not significantly different from the prevalence of infection among those collected from the untreated plot at the $P=0.05$ level. However, treatments of 1.0×10^9 and 2.0×10^9 spores/ha resulted in significantly higher levels, compared with untreated nymphs at the $P=0.10$ level. The reduction in disease prevalence may have resulted from either mortality among the insects in treated plots, to migration out of the treated plots, or the combined effects of both.

The occurrence of infections in the treated plots and the lack of infections in the control plots suggest that epizootics are inducible in Mormon cricket populations by the application of wheat bran impregnated with spores of Vairimorpha sp.

Survival and Development of Mormon Cricket
Treated with Vairimorpha sp.

MacVean and Capinera (1991) observed a high rapid mortality in 1st-3rd instar Mormon crickets when inoculated with about 5.0×10^6 spores each. They observed no significant differences in the survival of 7th instar nymphs and adults inoculated with about 8.5×10^7 spores each. During the present study, no significant differences were observed in the number of days survived by Mormon crickets treated with 10^5 , 10^6 , 10^7 or 10^7 spores of Vairimorpha sp. each as 4th, 5th or 7th instar nymphs and adults, respectively, compared with the

untreated Mormon crickets (Table 2). The 6th instar Mormon crickets treated with 10^6 spores each survived significantly fewer days compared with the untreated 6th instar Mormon crickets. It would appear that these treatments did not cause acute infections as observed in the treatment of 1st-3rd instar Mormon crickets by MacVean and Capinera (1991), but rather resulted in chronic infections resulting in disease manifestations other than death. The treatment of 6th instar Mormon crickets with 10^6 spores each appears to have been sufficiently high to cause acute infection resulting in a significant difference in mortality between these treated and the untreated Mormon crickets. The dosages used to inoculate the 4th and 5th instar Mormon crickets in this test were lower than the dosages used by MacVean and Capinera (1991) in treating 1st-3rd instar Mormon crickets. It is likely that reduced survival was not observed in the treatments of 4th and 5th instar nymphs because the dosages were too low to initiate acute infections.

Chu and Jaques (1979) observed that inoculation of T. ni with low dosages of V. necatrix caused chronic infections that were restricted to the fat body and muscle tissues. When the larvae of A. ipsilon were inoculated with V. necatrix or Vairimorpha sp. (isolated from a cotton leafworm, Alabama argillacea) either in low concentrations or late in their larval development, microsporidiosis resulted, causing reduced feeding and increased morbidity (Cossentine and Lewis, 1984).

MacVean and Capinera (1991) observed that low dosages of the Mormon cricket infecting Vairimorpha sp. (about 5×10^2 and 5×10^4 spores each) retarded nymphal development of 1st-3rd instar Mormon crickets and promoted heavy infections. The occurrence of relatively high disease prevalence in conjunction with low mortality following treatment of later instars with relatively low spore dosages suggests the initiation of chronic or sublethal infections.

The results presented in Tables 3 and 5 suggest that Mormon crickets infected with Vairimorpha sp. do not develop at the same rate as do untreated Mormon crickets. In Table 3, the mortality that occurred among 4th instar nymphs that had been treated with 10^5 spores during the 4th instar stage was significantly greater than among the untreated Mormon crickets. Also, mortality among 6th instar nymphs that had been treated with 10^3 and 10^4 spores during the 4th instar stage was significantly greater than among the untreated Mormon crickets. In Table 5, the mortality that occurred among 7th instar nymphs that had been treated with 10^4 or 10^5 spores during the 6th instar stage was significantly greater than among the untreated Mormon crickets. However, there were no significant differences in the number of days survived among the treated or untreated Mormon crickets. This mortality occurred during the earlier instars, which suggests that the development of infected Mormon crickets was delayed. These results concur with MacVean and Capinera's (1991)

observation that the development of the young Mormon crickets is retarded by the development of chronic infections of Vairimorpha sp.

Development of Vairimorpha sp. in Mormon Crickets

The results demonstrate that sporulation of Vairimorpha sp. in the Mormon cricket is dependent on both the age of the Mormon cricket and the dose of Vairimorpha sp. Although some spore formation was observed during the 11-20 day post-inoculation period for all the treatments, differences in the temporal aspects of spore development also were evident. The prevalence of infection, as indicated by presence of spores in Mormon crickets which died during the 11-20 day period, was significantly higher among Mormon crickets inoculated with dosages of 10^4 , 10^6 or 10^7 spores each during 4th, 6th or 7th instars, respectively, than that among untreated Mormon crickets (Tables 7, 9 and 10). The prevalence of infection among adults inoculated with 10^7 spores was not significantly greater during the 11-20 day period than that observed among untreated insects (Table 11).

As the Mormon cricket molts from one instar to the next it increases its body size and likely increases the amount of fat body tissue. If the initiation of sporulation is dependent on the available resources, a low initial infection will take longer to utilize the fat body resources than a high initial infection. As the infected tissues become saturated,

sporulation will occur and the entire infected tissue may be replaced by spores. The age of the host and the size of the initial dose may influence the sequential development of merogony and sporogony.

Effects of *Vairimorpha* sp. Infections
on Mormon Cricket Fecundity

Vairimorpha sp. primarily infects the fat body but also infects gonads, Malpighian tubules and midgut epithelial cells of the Mormon cricket. In a review of fat body function, Keeley (1978) noted that this tissue was active in intermediate metabolism. He also noted that in adult mosquitoes, beta-ecdysone initiated protein synthesis in the fat body which was necessary for oocyte maturation. Infections of Vairimorpha sp. likely inhibit the proper function of fat body tissues and thus, inhibit egg production by infected Mormon crickets. This inhibition would likely be greater among Mormon crickets infected earlier in their development.

The production of the protein mass or spermatophylax of the spermatophore also probably is affected by infections of the fat body. The spermatophore is an important contribution to egg development and production (Bowen et al., 1984). Undoubtedly, this accounts in part for the observed reduced fecundity of females that were mated with infected males.

The fecundity of the Mormon cricket was reduced by infections of Vairimorpha sp. Based on results in Tables 12

and 13, the mean number of eggs and the mean number of eggs per female day for the females inoculated with 10^7 spores each as 6th instar nymphs and 10^6 or 10^7 spores each as 7th instar nymphs were significantly lower than the mean number of eggs and the mean number of eggs per female day for the untreated females. A similar pattern was observed from the results in Table 15 in which the mean number of eggs and the mean number of eggs per female day for the females mated with males inoculated with 10^5 or 10^7 spores each as 6th instar Mormon crickets were significantly lower than the mean number of eggs and the mean number of eggs per female day for the females mated with untreated males. The Mormon crickets inoculated with 10^6 spores each as 6th instar males and females did not respond as expected. It is assumed that experimental error in preparation of the inoculant resulted in administration of improper dosages.

MacVean and Capinera (1991) treated 7th instar and adult Mormon crickets by placing 25 insects in a 29x29x29cm aluminum-screen cage and adding 85 mg of wheat bran per cricket. The wheat bran was formulated with 10^4 or 10^6 spores/mg of wheat bran. They observed that the mean number of eggs per female day was not significantly different from the untreated crickets. The untreated Mormon crickets and those treated with 10^4 or 10^6 spores/mg produced 3.74 (SE=2.06), 4.51 (SE=2.34) and 3.05 (SE=1.88) eggs per female day, respectively. I observed a higher mean number of eggs

per female day of 5.8 (SE=0.9) and 6.6 (SE=0.4) by untreated 7th instar Mormon crickets and of 6.4 (SE=0.2) and 6.6 (SE=0.2) by untreated adult Mormon crickets. This suggests that Mormon crickets maintained in field cages and provided with sandy soil for oviposition possibly were more fecund than when maintained in laboratory cages and provided with an artificial substrate for oviposition. The natural fluctuations in light and temperature, which could not be duplicated in the laboratory, may also enhance egg production.

Venereal Transmission of *Vairimorpha* sp.

Vairimorpha sp. spores have been observed in the spermatophore produced by male Mormon cricket and infections may be transmitted venereally. Infections in the female may also result when she feeds on the spermatophore. Spores of Vairimorpha sp. formed within 20 days of inoculation of 6th instar Mormon crickets when inoculated with 10^6 spores each and after 20 days when inoculated with 10^5 spores each (Table 9). Spores of Vairimorpha sp. formed within 20 days of inoculation of 7th instar Mormon crickets when inoculated with 10^7 spores each and after 20 days when inoculated with 10^6 spores each (Table 10). Therefore, mature spores are likely to be present in the treated 6th and 7th instar male Mormon crickets before spermatophore production would begin.

Disease transmission to untreated females was observed from adult male Mormon crickets that had been inoculated as

6th and 7th instar nymphs (Table 18). The prevalence of infection in adult female Mormon crickets mated with males that were inoculated with 10^5 , 10^6 or 10^7 spores each as 6th and 7th instar nymphs was significantly higher than observed with females mated with uninfected males. The percentages of infection among adult female Mormon crickets mated with males inoculated with 10^5 , 10^6 or 10^7 spores each as adults were not significantly different compared with females mated with uninfected males. Infections, therefore, were transmitted from infected males to uninfected females. Venereal transmission does occur although contamination and cannibalism also may have contributed to the observed transmission.

CHAPTER 6

SUMMARY

Vairimorpha sp. readily infects Mormon crickets, A. simplex, resulting in chronic infections, causing retarded development and reduced fecundity. The timing of disease development and sporulation appeared dependent on the age of the host and the inoculative dosage received by the Mormon cricket. Transmission was observed to occur through cannibalism and venereally from males to females.

From field applications and laboratory inoculations it was observed that the Mormon cricket is easily infected by application of spores of Vairimorpha sp. The results of the field applications suggest that epizootics can be induced by inoculation of young Mormon crickets with low dosages of spores.

The mortality observed among the 6th instar Mormon crickets treated with 10^6 spores each was the only instance where significant differences were observed between treated and untreated Mormon crickets. Accordingly, chronic infections or microsporidiosis were observed in Mormon crickets as a result of lower dosages of Vairimorpha sp. These chronic infections resulted in slowed development of young treated Mormon crickets. The Mormon crickets

inoculated during early instars, but which successfully reached the adult stage, were less fecund. The total number of eggs produced by females was reduced from that observed among healthy females.

Transmission occurs both horizontally and vertically. Horizontal transmission has been observed through the cannibalism of infected crickets. Infected crickets can produce in excess of a billion spores. They become moribund and are quickly attacked by healthy crickets. The spores that are consumed during cannibalism represent high levels of inocula. This is likely the major method of spread during outbreaks. Venereal transmission has been observed. The spermatophore from infected males contains spores that either are drawn into the spermatheca or consumed by the female.

Long-term control may be achieved by treating 5th, 6th and 7th instar nymphs with moderate doses of spores. Disease development reduces activity and hinders development of the infected crickets. This translates into a negative effect on fecundity, thus reducing the subsequent generation. Horizontal transmission would be enhanced as moribund crickets are cannibalized.

These results support the contention that infections by Vairimorpha sp. are detrimental to the growth and development of Mormon crickets. Accordingly, it appears to be a viable tactic for managing the densities of these insects.

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