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THE OCCURRENCE AND EFFECTS
OF **ENTOMOPHAGA GRYLLI** ON
GRASSHOPPER POPULATIONS IN
SOUTH DAKOTA

By
Roger Allen Bohls

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science
Major in Entomology
South Dakota State University
1982
THE OCCURRENCE AND EFFECTS
OF ENTOMOPHAGA GRYLLI ON
GRASSHOPPER POPULATIONS IN
SOUTH DAKOTA

This thesis is approved as a creditable and
independent investigation by a candidate for the degree,
Master of Science, and is acceptable for meeting the thesis
requirements for this degree. Acceptance of this thesis
does not imply that the conclusions reached by the
candidate are necessarily the conclusions of the major
department.

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THE OCCURRENCE AND EFFECTS
OF ENTOMOPHAGA GRYLLI ON
GRASSHOPPER POPULATIONS IN
SOUTH DAKOTA

by
ROGER A. BOHLS

Under the guidance of Dr. Burruss McDaniel

ABSTRACT

The effect of Entomophaga grylli on grasshopper populations within South Dakota was investigated during a 2-year period. Field observations established that the state's 3 most important species of grasshoppers are susceptible to pathotype 2 infections. Outbreaks are believed to be initiated annually from the soil of undisturbed areas which act as the reservoir for Entomophaga grylli resting spores. The occurrence of the fungus in regions of the state receiving 355 mm of rain during the growing season indicate pathotype 2 infection is maintained in dry environments. However, detailed epizootiological studies of the fungus are needed before the potential of Entomophaga grylli, as a biological control agent, is established.
INTRODUCTION

Grasshoppers have posed a threat to man for centuries. Severe economic losses occur during outbreak years as grasshoppers compete with man and livestock for food. Grasshoppers are often the most destructive pests of cropland and rangeland in the United States (Cowan, 1958; Onsager and Hewitt, 1982; Parker and Shotwell, 1932; Pfadt, 1949). Annual average damage to crops and range in areas west of the Mississippi River was estimated at $75,000,000 during the 1950's (Cowan, 1958). Haeussler (1952) estimated that grasshoppers caused over $795 million damage to crops and rangelands from 1925 to 1950 in the United States. Hewitt (1977) gives a detailed review of forage losses caused by rangeland grasshoppers over a 40-year period.

A steady increase of grasshopper populations occurred on the Great Plains during the 1930's. Parker and Shotwell (1932) reported that during the spring and summer of 1931 there occurred in South Dakota and Nebraska the most serious grasshopper outbreak in the United States since the days of the Rocky Mountain locust. *Melanoplus bivittatus* and *Melanoplus differentialis* destroyed 75% of the crops in a 44,041 square km area and 25% of crops over an additional 33,678 square kms. Also, during the
5-year period of 1937-41, grasshoppers caused an estimated loss of $42,303,030 to cereal, forage, and truck crops in South Dakota (Spawn, 1945).

McGinnis (1959) reported that during the past 100 years of agricultural history in South Dakota, 45 years have been years in which grasshoppers have destroyed the bulk of crops in localized areas. Surveys (United States Department of Agriculture, APHIS) show that outbreaks do not occur every year in South Dakota. However, favorable conditions for grasshopper development and survival occur frequently within the state. Therefore, periodic outbreaks can be expected in future years.

Over the past few years (1976 to present) grasshoppers have once again devastated South Dakota. Davidson (1979) believes the outbreak may have been the largest in the history of the United States. Kantack (1981) reported the heaviest grasshopper infestation on cropland in the state in almost 20 years. These events led to the spraying of over 404,858 ha of cropland and almost 242,914 ha of rangeland in the state for 1981.

Within South Dakota there are two groups of injurious grasshoppers considered to be of economic importance. Species of importance are grouped into rangeland and cropland species (Appendix A). The most
damaging of these species is *M. differentialis*, *M. bivittatus*, and *M. femurrubrum*. Other species of occasional importance are *M. sanguinipes* and *C. pellucida*. Together these 5 species account for 90% of all grasshopper damage occurring within the United States. In South Dakota the average grasshopper hatch will begin by mid-May in the western and southern regions. There may be a variance in the hatching date of approximately one month between different regions of the state. Also, among the state's 3 most important species of grasshoppers there may be a 3-week difference in hatching dates. Of these, *M. bivittatus* is considered an early hatching species and may appear 2 to 3 weeks prior to the first emergence of either *M. differentialis* or *M. femurrubrum*.

Due to the possible widespread devastation caused by grasshoppers, the problem of control has been one not only of private individuals, but one of public responsibility. Federal funds are not available for spraying grasshoppers in cropland areas. However, organized control programs on rangeland have been supported by federal aid which paid one-third the total cost per acre of spraying. Nevertheless, such programs are usually crisis oriented and during the hot, dry years of grasshopper outbreaks, the benefits of using expensive chemicals is not justified from a farmer's standpoint.
Management of grasshoppers in the past 3 decades has relied heavily on broad-scale application of insecticides. These insecticides are not specific for grasshoppers and will kill the natural enemies of grasshoppers as well as other beneficial insects. Many insecticides are extremely toxic, limiting their use to commercial applicators only. Also, waiting periods may be required between the time of application and harvest or grazing of the treated area.

The development of a system utilizing biological control agents, selective pesticide use, and cultural control methods would help reduce grasshopper damage. Alternative control methods such as microbial pesticides, parasites, and predacious insects is well documented (Steinhaus, 1963; Burges, 1981). Advantages of these methods include the safety of their use, lack of harmful effects on beneficial insects, and possible long-term control with little or no resistance.

The use of the fungus *Entomophaga grylli* is currently being investigated as a tool in the management of grasshopper populations. Research conducted during the summers of 1980 and 1981 consisted of 3 separate studies. The primary focus of these preliminary investigations was to determine the influence of natural
outbreaks of *E. grylli* on grasshopper populations within South Dakota. The following specific objectives were formulated:

1) to locate the presence of *E. grylli* in South Dakota

2) to determine species of grasshoppers infected by *E. grylli*, and their population densities before and after infection

3) to determine the influence of host behavior and abiotic factors on the epizootiology of natural *E. grylli* outbreaks.

The occurrence of *E. grylli* within grasshopper populations is essentially controlled by 3 general factors: the pathogen, the susceptible host, and the proper climatic conditions. These general factors can be separated into more precise factors such as moisture, temperature, light, inoculum concentration, and host density. Natural *E. grylli* outbreaks were not correlated with the above factors due to difficulty in obtaining accurate measurements at disease locations.
LITERATURE REVIEW

FACTORS INFLUENCING GRASSHOPPER POPULATIONS

WEATHER

The most important factor influencing grasshopper populations is weather (Dempster, 1963; Edwards, 1960; Gage and Mukerji, 1977; Rodell, 1977). In general, warm, sunny, dry weather favors high survival, rapid development and maximum egg production (Parker, 1930; Pickford, 1966). Cool, wet conditions frequently increase mortality, delay development and maturation and reduce egg laying periods (Shotwell, 1941; Dempster, 1963; Pickford, 1966). Consequently, warm, dry summers with adequate food available for normal development and reproduction are important factors leading to grasshopper outbreaks. In addition, insects generally benefit from increased consumption of foods containing high levels of nitrogen (Slansky and Feeny 1977). During drought years amino acids increase in vegetation due to stress imposed upon plants (Hsiao, 1973). Haglund (1980) reports that grasshoppers detect and preferentially feed on grasses treated with amino acids. This ability to detect amino acids may lead to increased growth and survival of grasshopper populations that are concentrated on drought stressed plants.
HABITAT

Many environmental changes that alter grasshopper populations are directly related to agricultural practices. The breaking of native sod and drainage of marshy areas for agricultural development are directly related to grasshopper increases (Bird et al. 1966; Smith, 1969; Uvarov, 1956). New combinations or changes in the proportions of crops grown in an area can greatly increase or decrease grasshopper populations (Isely, 1942; Sanderson, 1939; Barnes, 1959). Allowing lands to become overgrazed enhances the population growth of grasshopper species normally associated with rangelands (Pepper, 1955; Scoggan and Brusven 1973). The accumulations of weeds within cultivated fields or nearby field margins provide grasshoppers with an extra source of available food (Barnes, 1959; Butcher, 1940; Smith, 1969). Ideal environments for the development of grasshopper populations were created when roads with steep ditches were built. These areas provided additional oviposition sites that contained adequate food and shelter for grasshopper development (Barnes, 1959; Butcher, 1940; Smith, 1969).

MICROORGANISMS

Members of the family Acrididae are infected by several species of fungi, bacteria, and protozoa (Steinhaus,
1949). Some of these microorganisms can cause severe population reductions within grasshopper populations. The use of *Aerobacter aerogens* var. *acridiiorium* (d'Her.) to control grasshoppers was the first well publicized event in which a bacterium was used to control an insect. The use of a protozoan, *Nosema locustae* Canning has been effective in reducing some grasshopper species (Henry, 1971; Henry et al. 1978; Henry and Onsager, 1982) and may also affect the fecundity of infected female survivors (Henry, 1981). After reviewing the works of others, Roffey (1968) stated that all stages of grasshopper development are susceptible to infection by fungi.

The early taxonomic history of the Entomophthorales has been reviewed by MacLeod (1963) and Gustafsson (1965). The family Entomophthoraceae contains approximately 200 species. The first genus of the family, described by Cohn (1855), was named *Empusa*, and based on *Empusa muscae* Cohn, the type species. Fresenius (1956) proposed *Empusa* was unacceptable and that the genus should be named Entomophthora. Currently, several taxonomic controversies pertaining to this group of fungi remain unresolved. The most comprehensive taxonomic scheme was that proposed by Batko (1964 a-d, 1974; from Burges, 1981) which separated species into five genera and four subgenera. Following the proposals of Batko, the fungal pathogen of grasshoppers will be referred to in this study as *Entomophaga grylli*. 
Entomophaga grylli (Fresenius) was first described by Fresenius (1856) attacking a species of Gryllus near Frankfurt, Germany. *E. grylli* is best known as a pathogen of grasshoppers and locusts. The fungus is commonly found attacking insects throughout the world (Fresa, 1971; Gyllenber 1974; Mackie, 1923; MacLeod, 1956; Milner, 1978; Rao and Cherian, 1940; Roffey, 1968; Skaife, 1925). *E. grylli* has frequently been observed attacking susceptible species of grasshoppers across the United States (Charles 1941; Hayes and De Coursey 1938; Hutchison, 1963; Rockwood, 1950; Thaxter, 1888; Walton and Fenton, 1939).

In South Dakota, Severin and Gilbertson (1917) stated that at least two species of fungi were found to attack grasshoppers. Although these species were not identified in their report, collections were made of dead grasshoppers found clinging to the tops of alfalfa and grass plants. In addition, Riker mounts made by Severin include cadavers of *M. bivittatus* containing resting spores of *E. grylli*.

*E. grylli* plays a significant role in the natural control of grasshoppers and is the most important fungal pathogen of grasshoppers (Dempster, 1963). Both cropland and rangeland species of grasshoppers are susceptible to infection by *E. grylli* (Hayes and De Coursey 1938; Hewitt, 1979;
MacLeod and Muller-Kogler, 1973; Pickford and Riegert, 1964; Rockwood, 1950). Hewitt (1979) reported that E. grylli spreads swiftly and causes a rapid decline in grasshopper populations on Montana rangelands. Hayes and De Coursey (1938) considered the fungus the most effective natural control, killing large numbers of adult grasshoppers. Roffey (1968) and Kahn (1964) reported a high mortality among adult Patanga succincta in Thailand. Chapman and Page (1979) reported 10% of Zonocerus variegatus, in southern Nigeria, died overnight and this rate sometimes continued for 2 or 3 nights. In Canada, E. grylli caused high rates of mortality within Camnula pellucida populations (Pickford and Riegert, 1964). Natural outbreaks of the disease were responsible for reducing grasshopper populations to a very considerable extent during the 1933-34 period in Nyasaland (Smee, 1936).

PENETRATION AND DEVELOPMENT

Infections from fungi via the alimentary canal occur rarely (Steinhaus 1949). Infection is initiated by a germ tube from some type of spore penetrating the insect's integument. Penetration consists of a mechanical process and the secretion of enzymes that weaken or degrade the cuticle (Lefebvre, 1934; MacLeod, 1963; Sawyer, 1933; Samsinakova et al. 1971). Smith et al. (1981) suggest that the complexity of the insect integument would require more
than one enzyme for penetration to be successful and that these enzymes act in a sequential manner.

It has been reported that the interval between infection and death has a wide variation. A five to six day interval is reported by Schaefer (1936), Skaife (1925), and Roffey (1968). Whereas Pickford et al. (1964) suggest the fungus has a two-week incubation period within the host. Also artificial infection by injection of resting spores (Nelson et al. 1982) and protoplasts (MacLeod, et al. 1980) into the haemocoel caused death in approximately two weeks.

Once penetration has been completed, the invading hyphae usually separate into component cells known as hyphal bodies (MacLeod, 1963). However, several modes of vegetative growth occur among the Entomophthorales (Burges, 1981). The formation of hyphal bodies may begin at the initial phase of penetration of the insect cuticle by the fungus (Prasertphon and Tanada 1968) or be delayed until death of the host (Thaxter 1888; Ullyett and Schonken, 1940). Prasertphon and Tanada (1968) report that the size of the fungal bodies and sites of their multiplication may explain how fungi spread throughout the host. Hyphal bodies much larger than insect haemocytes would not circulate freely in the haemolymph, while relatively small hyphal bodies would be unimpeded as they circulate in the haemolymph. Skaife (1925) observed hyphal bodies of _E. gryll_ı_ı_ circulated to all parts
of the body via the haemolymph in the red locust, *Cyrtancanthacris septemfasciata*. After death of the host and with sufficient moisture, the hyphal bodies germinate giving rise to conidiophores (pathotype 1). These conidiophores emerge through the exoskeleton each bearing a single conidium (MacLeod, 1963). Conditions that are unfavorable for the development and survival of the fungus trigger the formation of resting spores which are adapted to withstand such conditions. In the case of *E. grylli*, hyphal bodies give rise to a resting spore form known as azygosporcs (Riddle, 1906).

**DISEASE SYMPTOMS**

Grasshoppers succumbing to infection by *E. grylli* behave in a characteristic manner. Disease symptoms normally do not appear until the disease is in its advanced stages. Prior to death there may be a general restlessness, cessation of feeding, and loss of coordination (Madelin, 1963). Infected individuals tend to climb upwards on vegetation just before death and die with their legs wrapped around the plant. Following death there may be a distention of the abdomen to almost twice its original size. The grasshopper body becomes soft and pulpy and is easily broken up if disturbed. At this time the abdomen often curls upwards and forwards to the extent that it almost touches the pronotum.
Approximately one hour after death there may appear a white, furry growth covering the insect's body. The makeup of this growth is club-shaped sporagenous cells which project from the insect's integument like close set hairs (MacLeod and Muller-Kogler, 1973). This form of the fungus is equivalent to Soper's (1980) pathotype 1. Walton and Fenton (1939) and Skaife (1925) indicated an absence of a fine furry growth on infected grasshoppers. In such cases the insect is filled with azygospores, which Soper (1980) identified as pathotype 2.

STRAINS OF ENTOMOPHAGA GRYLLI

Certain species of grasshoppers appear to be unaffected by E. grylli. Skaife (1925) noted that Eugaster spp. were very abundant in disease locations; however, none became infected with the fungus. In Canada, Platybothrus brunneus did not succumb to the disease even though it was intimately associated with diseased C. pellucida (Treherne and Buckell, 1924). Also in Canada, Melanoplus sanguinipes appeared to be immune to E. grylli. Different strains of fungus were found to be lethal to different grasshopper species according to Pickford and Riegert (1964). Soper (1980) identified two different pathotypes of E. grylli. Pathotype 1 has a typical entomophthoralian life cycle with conidia.
and resting spore states. This pathotype has been isolated from field populations of *Dissostiera carolina* and *Camnula pellucida* and from laboratory infected *Locusta migratoria*. Pathotype 2 lacks the conidial stage in the field and is restricted to *Melanoplus* spp.

**EPIZOOTIOLOGY**

The precise factors controlling the occurrence of *E. grylli* outbreaks is unknown. Approximately two weeks of heavy and continuous rains are reported preceding outbreaks of *E. grylli* (Pickford and Riegert, 1964; Roffey, 1968). Skaife (1925) reported that a rainfall of 154 mm over a three month period was sufficient to start and maintain an epizootic of *E. grylli*. Development of the fungus has been reported as being dependent on rainfall which acts as an important source of surface water on the insect (Balfour-Browne, 1960; Chapman and Page, 1979). Apparently surface water enables spores to adhere long enough to the integument for the processes of germination and penetration to be completed. Epizootiological studies conducted within aphid populations revealed that inoculum levels and host density were of major importance; and disease incidence was not limited by climatic factors (Soper and MacLeod, 1981).

The spread of a pathogen within an insect population can be influenced to a large extent by environmental conditions. When initial infection is established, rainfall
is considered an essential element for the continued spread of the disease (Pickford and Riegert, 1964; Walton and Fenton, 1939). Dempster (1963) states that there is a positive correlation of maximum spread of fungal diseases with low wind velocities, high humidities and relatively high temperatures.

The normal habits of the host insect play an important role in the dissemination of insect pathogens. Tanada (1959) considers the movement of infected grasshoppers to be the primary method of disseminating the fungus. The habit of grasshoppers aggregating in small areas, creating dense populations, aids in the spread of the disease which can then reach epizootic proportions (Pickford and Riegert, 1964; Walton and Fenton, 1939). The positioning of _E. grylli_ cadavers high atop vegetation gives a wide dispersion of liberated fungal spores (MacBain Cameron, 1963; Thaxter, 1888). Healthy insects feeding on diseased cadavers is another factor contributing to the spread of some fungi (Sweetman, 1963).

**CONTROL ATTEMPTS WITH ENTOMOPHAGA GRYLLI**

Various attempts have been made to infect healthy grasshoppers with _E. grylli_. Skaife (1925) and McMartin (1935) conducted experiments in which _E. grylli_ resting spores, conidia, and hyphal bodies were fed to grasshoppers. They concluded that infection does not occur when grasshoppers
ingest *E. grylli*. Walton and Fenton (1939) reported that grasshoppers consuming hyphal bodies and resting spores in their diet have died from mycosis. However, *E. grylli* spores may have come in contact with the insect's integument as the bran spore mixture was left in the cages containing the grasshoppers for six days. MacLeod (1963) stated that obtaining infection by artificial inoculations with members of the Entomophthorales was very uncertain. No mortality was observed in experiments when spore sprays and resting spore pastes of *E. grylli* were applied to grasshoppers (Schaefer, 1936; Skaife, 1925; Smee, 1936). The use of old and fresh conidia collected from vegetation and diseased specimens failed to infect healthy grasshoppers (Schaefer, 1936). However, Petkov (1939) obtained a 83% kill of *Caloptenus italicus* in Bulgaria after scattering spores of *E. grylli* on plants. Furthermore the previously mentioned injection techniques of Nelson et al. (1982) and MacLeod et al. (1980) have proven to be successful.
MATERIALS AND METHODS

Determining the influence of *E. grylli* on grasshopper populations within the state was accomplished by the following specific objectives:

1) to locate the presence of *E. grylli* in South Dakota

2) to determine species of grasshoppers infected by *E. grylli*, and their population densities before and after infection

3) to determine the influence of host behavior and abiotic factors on the epizootiology of natural *E. grylli* outbreaks.

SURVEY OF NATURAL OUTBREAKS OF E. GRYLLI IN SOUTH DAKOTA

A survey was conducted to determine the natural distribution of *E. grylli* within grasshopper populations. Factors recorded throughout the course of the investigation are the general climatic conditions preceding outbreaks of the fungus, the species of grasshoppers succumbing to infection by *E. grylli*, and the ecological make-up of the areas in which natural outbreaks were found.

An examination of USDA-APHIS grasshopper surveys led to the establishment of 10 field stations in the following counties: Lyman, Todd, Mellette, Stanley and Jones. Grasshopper densities at each of these 10 stations were estimated weekly by taking 100 sweeps with a standard insect net sampler along each of 3 transect lines.
Vegetation at these sites consisted primarily of *Agropyron smithii* and *Buchloe dactyloides*. Grasshopper species encountered were those normally associated with grasslands as listed by Hewitt (1977) (Appendix B).

Additional information as to the whereabouts of *E. grylli* outbreaks within grasshopper populations was obtained by contacting county agents in regions heavily infested with grasshoppers.

After an initial outbreak was located, approximately 5-10 additional observations were made within that county. An equivalent number of observations was conducted in surrounding counties.

The majority of county records for *E. grylli* outbreaks were not discovered until the last summer of my research and, therefore, were not studied intensively. However, the Stadium Road site in Brookings County and White River sites in Mellette County were observed weekly.

The Mellette County outbreaks were located approximately 402 km southwest of the SDSU campus. This region of the state lies within the Pierre Hills and is characterized by smooth hills and ridges. Mellette County records are primarily based on observations at 1 of 2 alfalfa fields located along the edge of the Little White River. A deciduous growth consisting of Cottonwood,
Bur Oak, American Elm, and Chokecherry encompassed the east field, while the west field was bounded by trees along 3 of its 4 margins. Both fields contained substantial growth of Kochia, Russian Thistle, and Smooth Brome. The west field was harvested during the first week of July and, therefore, served as a collection site for *E. grylli* spores. The east field was not harvested during the 1980 or 1981 seasons and most observations were conducted at this location.

The Stadium Road-Brookings County site is located within the Coteau des Prairies and is an area dominated by highlands with low hills and numerous lakes. Observations conducted at the Stadium Road site are discussed in detail in the Nymphal Mortality and Persistence of Corpse studies.

The Pollock-Campbell County outbreak located in the northcentral region of the state is the largest outbreak observed. Pollock is located within the Coteau des Missouri and is characterized by highlands that are covered by glacial deposits and traversed by swales. The outbreak occurred on the North Dakota-South Dakota state line in barley fields. The adjacent barley fields were separated by a field border consisting of Kochia, Russian Thistle, and Annual Sunflower.
Annual average mean temperature and precipitation for South Dakota are presented in Appendix C.

NYMPHAL MORTALITY, BROOKINGS COUNTY, 1981

During the fall of 1980, a natural outbreak of *E. grylli* was observed on the campus of South Dakota State University (SDSU). This site (Stadium Road) is located approximately .2 km east and .2 km north of Coughlin-Alumni Stadium. The Stadium Road site consisted of 0.2 ha of alfalfa. Collections of adult *Melanoplus* spp. killed by the fungus were taken from 5 September until the first killing frost on 12 October. Observations were made daily due to the convenient location of the outbreak. Therefore, the Stadium Road site was selected as the area for the 1981 nymphal mortality study.

A drainage ditch at the Stadium Road site partitioned the field into 2 sections. From the southwest section of the field a 0.02 ha plot of alfalfa was selected as the area to be studied during 1981 (Fig. 1). The plot was bounded by a cornfield along the south and by a gravel road along the west. The remaining margins of the plot were separated from other areas of alfalfa by clearings of approximately 18-46 m.
Fig. 1. Brookings nymphal mortality study site.
(each division equals 9.1 m)
Field observations of mortality due to *E. gryllii* were based on weekly counts of live and dead grasshoppers. The number of live grasshoppers per square yard was estimated by using the pointer method described by Onsager (1977). Grasshoppers dying from the fungus were marked with colored flags. These flags were taped to the vegetation on which dead, clinging grasshoppers were found. Composition of economically important grasshoppers was estimated by taking 200 sweeps with a standard insect net sampler.

An examination of weather records shows that substantial rainfalls (102 mm) occurred during the 22-day period preceding the first observed mortality on 10 August (Fig. 2). Most of the rainfall (77 mm) occurred during the first 5 days of the 22-day period. During this period daily maximum temperatures varied from 30°C and 18°C with a mean of 26°C. Daily minimum temperature ranged from 20°C to 7°C with a mean of 14°C.

**PERSISTENCE OF CLINGING-FUNGUS KILLED GRASSHOPPERS**

The objective of this study was to establish the whereabouts of *E. gryllii* spores formed in cadavers killed by the fungus. The persistence of *E. gryllii* cadavers found clinging to the upper regions of vegetation was monitored for a 2-week period during September at 2
Fig. 2. Precipitation preceding 1981 E. grylli outbreak at the Stadium Road site.
locations.

It was determined from field observations in South Dakota during 1980-1981 that the majority of *E. grylli* outbreaks were occurring in alfalfa fields and roadside ditches. Thus, areas chosen for the study were limited to these habitats. An interstate highway ditch (I-29) located 14 km south of SDSU and the Stadium Road site (see Nymphal Mortality) were chosen as the study sites.

Stadium Road observations were based on 64 adults and 21 nymphs, while the I-29 site contained 294 adults and 2 nymphs. Observations at both study areas were conducted daily and each cadaver was classified into one of three categories based on the following:

A - cadaver lost to the ground

B - cadaver clinging with spores exposed to the environment

C - cadaver clinging with no spores exposed.

Both sites received light rains and were subjected to strong winds. Weather information was obtained from the SDSU meteorological station. The Stadium Road and I-29 sites are located 0.4 km and 14 km from the weather station, respectively.
RESULTS

NYMPHAL MORTALITY

At the mortality site *M. femurrubrum* was the dominant species making up approximately 85% of the grasshopper population. The remaining 15% consisted of equal populations of *M. bivittatus* and *M. differentialis*. All stages of *M. femurrubrum*, except first instars, were found dying from the fungus. Fourth instars were the youngest stages of *M. bivittatus* and *M. differentialis* found succumbing to infection by *E. grylli*.

During the course of the study there was a decline in the grasshopper population from approximately 1.5 per square yard to .02 per square yard (Fig. 3). The total number of *E. grylli* cadavers flagged weekly also declined from 760 to only 2 during the last week of the study. The large number of immature grasshoppers flagged accounted for the majority of deaths due to *E. grylli* (Fig. 3).

Observations revealed that only resting spores were produced at the end of the infection cycle. Therefore, the form of *E. grylli* responsible for the recorded mortality at the Stadium Road site is equivalent to Sopers' (1980 pathotype 2.

PERSISTENCE OF CADAVERS

Fifty percent of the cadavers were lost to the
Fig. 3. Number of live grasshoppers (..) and corpses of *E. grylli* adults (—) and nymphs (--) at the Stadium Road site during 1981.
ground by day 7 at the Stadium Road site and by day 8 at the I-29 site (Fig. 4). Many cadavers that remained clinging to the vegetation had major body parts missing or portions of the integument broken to the extent that spores of *E. grylli* were lost to the ground and surrounding vegetation (Fig. 5).

Clinging cadavers failed to produce an external growth of conidiophores and conidia and the infection cycle terminated with the production of resting spores only. These results indicate a typical pathotype 2 (Soper 1980) outbreak of *E. grylli* at both the Stadium Road and I-29 study sites.

**SURVEY**

During the summers of 1980 and 1981 natural outbreaks of *E. grylli* were observed in 22 counties across the state (Fig. 6). A total of 7 species of grasshoppers were found dying from *E. grylli* infections (Fig. 6). Examinations of clinging cadavers at all locations revealed that only pathotype 2 was present as the infection cycle ended with the production of resting spores only.

*E. grylli* was observed in grasshopper populations as early as the last week in June in the southcentral part of the state. Earliest observation of the fungus in eastern South Dakota was 1 July. Deaths resulting
Fig. 4. Percent of clinging grasshoppers which were lost to the ground. Data for Stadium Road site based on 85 insects (—); data for I-29 site based on 296 insects (—).
Fig. 5. Percent of clinging grasshoppers with spores of E. grylli exposed to the environment. Data for Stadium Road site (—) based on 85 insects; data for I-29 site (--) based on 296 insects.
Fig. 6. Observed occurrence of *E. gryllli* in grasshoppers, 1980-81.
from *E. grylli* infections were occurring as late as mid-October and late September in eastern and central parts of the state, respectively.

The majority of grasshoppers dying from the fungus are found in areas not subjected to cultivation (field borders, roadside ditches, alfalfa fields). However, diseased grasshoppers were collected from the edges of cornfields (Brookings County) and soybean fields (Davison County).

*E. grylli* outbreaks were observed in the following counties during the summer and fall of 1980: Mellette, Tripp, Lyman, Meade, Charles Mix, Moody and Brookings. Additional outbreaks located in the southeastern and northcentral regions of the state during 1981 included the following counties: Davison, Hanson, McCook, Sanborn, Miner, Douglas, Hutchinson, Turner, Lincoln, Bon Homme, Yankton, Clay, Union, Campbell, and Walworth.

Grasshoppers dying from the fungus behaved in a characteristic manner. During the mid to late afternoon infected individuals could be located clinging to the tops of nearby vegetation and other structures such as barbed wire fences. Infected grasshoppers removed from their clinging positions exhibited slow, uncoordinated flexion and extension of the hind tibiae, while the first and second pairs of legs contracted as if the insect were
still clinging to the vegetation. The majority of clinging cadavers were found with their heads directed toward the sky. Other cadavers were found positioned similar to a moulting grasshopper with its head directed toward the ground. All cadavers failed to produce an external growth of conidiophores and conidia.

WHITE RIVER - MELLETTE COUNTY

At White River M. bivittatus and M. differentialis were the dominant species with small populations of the following; M. confusus, M. lakinus, and M. femurrubrum. Throughout the summer and fall of 1981, peaks of mortality occurred during the weeks of 6 August and 20 August. Deaths due to E. grylli were recorded from 25 June until 1 October when the last field observations were taken.

The normal inaccuracy encountered when estimating highly mobile insects such as grasshoppers, was further complicated due to the height of the unharvested vegetation. However, using an insect net sampler, the population was estimated to be reduced approximately 40% over the period of early June to late August.
POLLOCK - CAMPBELL COUNTY

In August the harvesting of adjacent barley fields drove large numbers of grasshoppers into vegetation along the field borders. During the last week of August large numbers of *M. bivittatus* and *M. differentialis* were found dying from *E. gryllii* infections. The majority of deaths due to the fungus occurred within the 0.8 km field borders; however, grasshoppers were found within the harvested barley fields and surrounding prairie, also.
DISCUSSION

Research conducted during the 1980, 1981 seasons established that the state's 3 most important species of grasshoppers are susceptible to pathotype 2 infections of *E. grylli*. In addition, other species of grasshoppers of importance to South Dakota's croplands and rangelands are dying from natural outbreaks of the fungus.

Field observations suggest that only pathotype 2 infections occur within the state. Grasshopper species within South Dakota that are known to be susceptible to pathotype 1 infections, apparently are not cross infected by the pathotype 2 form of *E. grylli*. This supports the findings of others (Pickford and Riegert, 1964; Milner, 1978; Soper, 1980) that determined different strains of the fungus are lethal to different species of grasshoppers. Nevertheless, from this investigation and that of others, it is evident that in South Dakota, all species of grasshoppers that are of immediate importance and those that have the potential to be destructive in the future are susceptible to either pathotype 1 or pathotype 2 infections of *E. grylli*.

The transmission of *E. grylli* can be expected to differ between pathotypes. Field observations have determined that clinging cadavers dying from pathotype 1
infections produce an external growth of conidiophores and conidia. Pathotype 1 cadavers producing and releasing conidia to the environment provide a mechanism for the rapid transmission of the disease. The lack of an external growth of the fungus from pathotype 2 clinging cadavers indicates that this form does not have the same potential for rapid transmission as pathotype 1. Although no production of conidia has been observed from clinging pathotype 2 cadavers, the possibility exists that these spores do not contribute to the spread of the disease until they reach the ground. Moisture levels are higher at soil level as compared to the elevated position of clinging cadavers. Therefore, spores at ground level are exposed to environmental conditions that are more favorable for the development of the fungus.

The short period that *E. grylli* cadavers remain clinging to the tops of vegetation indicates that a large percentage of *E. grylli* spores are located on the ground throughout the year. Studies have revealed a minimal movement of fungal spores through the soil of most undisturbed agricultural ecosystems (Ignoffo et al. 1977; Burges, 1950). Therefore, most *E. grylli* spores produced from diseased grasshoppers may be available in the top layer of soil in undisturbed habitats.
The majority of deaths due to *E. grylli* are found in uncultivated habitats. The location of *E. grylli* cadavers within cultivated fields probably resulted from the movement of infected hosts from a nearby undisturbed area. Therefore, it is likely that the mechanical cultivation of the soil disturbs the host-pathogen interaction as suggested by Kalmakoff and Miles, 1980). These findings suggest that the soil of undisturbed areas acts as the reservoir for *E. grylli* resting spores which initiate outbreaks annually. Grasshoppers would most likely come in contact with *E. grylli* spores at or near ground level. Peak periods of grasshopper activity at ground level take place in the early morning hours and during periods of cool, wet weather (Shotwell, 1941; Pickford and Riegert, 1964). Infection of aphid populations (Hall, 1981) by *Verticillium lecanii* and lepidopteran larvae with *Nomuraea rileyi* (Ignoffo et al. 1977) are also considered to be initiated from micro-organisms located on the soil.

Other factors possibly contributing to the spread of the fungus are ants, flies, spiders, millipedes, and tettigoniids. All have been observed coming in contact with clinging *E. grylli* cadavers. Alternatively, *E. grylli* spores may be splash dispersed onto hosts and nearby vegetation by rainfall (Hirst and Stedman, 1963).
It is concluded that *E. grylli* is unlikely to play any major role in controlling grasshopper populations due to environmental requirements of the fungus (Pickford and Riegert, 1964; Riegert, 1968; Roffey, 1968). However, Chapman and Page (1979) reported *E. grylli* to be a key factor regulating grasshopper mortality from year to year, even though the development of the fungus was dependent on rainfall. In contrast, Schaefer, 1936 (from McMartin, 1935) reported that the development of the fungus was not dependent on rainfall. Conflicts over the environmental requirements for the development of *E. grylli* remain unsettled. Apparently unjustified conclusions have been reached about the development of *E. grylli*, since the factors controlling the fungus are not completely understood. County records for the occurrence of *E. grylli* show the fungus is widespread across the state. The precise factors controlling the occurrence of such outbreaks is still unknown. However, the occurrence of the fungus in regions of the state receiving only 355 mm of rain during the growing season (April - September) indicates the fungus is maintained in dry environments.

Field results obtained by distributing fungus from infected grasshoppers are contradictory (Smee, 1936; Schaefer, 1936; Petkov, 1939). Regardless of these field
studies, attempts should be directed toward determining the minimum quantity of fungus required to obtain adequate control of grasshopper populations. Accordingly, control methods should be directed toward immature grasshopper populations which are less harmful and occupy less area than adult grasshoppers. Also, with a 1- to 2-week interval between infection and death of the host (Pickford and Riegert, 1964; Skaife, 1925) it becomes necessary to initiate control attempts before economically damaging stages of grasshoppers are present. Thus, the application of E. grylli to nymphal grasshopper populations that are concentrated in hatching areas would be desirable. Fighting grasshoppers before or at the time they invade crops is one of the essentials of a successful campaign (Parker and Shotwell, 1932). Uncultivated land is one of the most common areas on which grasshoppers hatch (Butcher, 1940). Natural outbreaks of E. grylli are usually associated with uncultivated habitats. Therefore, the application of a pathogen in an undisturbed area is less likely to be subjected to factors that potentially alter the host-pathogen interaction.

Detailed epizootiological studies of E. grylli in grasshopper populations have not been conducted. The discovery of several natural E. grylli outbreaks
across the state provides an opportunity to observe the fungus under a variety of field conditions. The observation of natural outbreaks in combination with replicated experiments controlling abiotic and biotic factors is needed before the potential of *E. grylli*, as a biological control agent, is established.
LITERATURE CITED


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APPENDICES
Appendix A

Cropland and Rangeland Grasshopper Species of South Dakota

Ageneotettix deorum
Amphitornus coloradus
Aulocara elliotti
Camnula pellucida
Eritettix simplex
Melanoplus bivittatus
Melanoplus differentialis
Melanoplus femurrubrum
Melanoplus packardii
Melanoplus sanguinipes
Phoetaliotes nebrascensis
Trachyrachys kiowa
Appendix B

Destructive Rangeland Grasshopper Species Of The Great Plains (Hewitt, 1977)

Aeropedellus clavatus (Thomas)
Ageneotettix deorum (Scudder)
Amphitornus coloradus (Thomas)
Aulocara elliotti (Thomas)
Boopedon nubilum (Say)
Camnula pellucida (Scudder)
Chorthippus curtipennis (Harris)
Cordillacris spp.
Drepanopterna femoratum (Scudder)
Encoptolophus sordidus costalis (Scudder)
Eritettix simplex (Scudder)
Melanoplus infantilis (Scudder)
Melanoplus packardii (Scudder)
Melanoplus sanguinipes (F.)
Mermiria spp.
Morseiella flaviventris (Bruner)
Opeia obscura (Thomas)
Phlibostroma quadrimaculatum (Thomas)
Phoetaloites nebrascensis (Thomas)
Psoloessa spp.
Trachyrhachys kiowa (Thomas)
Trimerotropis pallidipennis (Burmeister)
APPENDIX C

ANNUAL AVERAGE MEAN TEMPERATURE (°C)

ANNUAL AVERAGE PRECIPITATION (mm)