1984

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Harlan Gene Thorvilson

Iowa State University

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THE ECOLOGY OF NOMURAEA RILEYI (FUNGI: DEUTEROMYCOTINA) AND OTHER NATURAL ENEMIES OF THE GREEN CLOVERWORM IN IOWA AGROECOSYSTEMS

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The ecology of *Nomuraea rileyi* (Fungi: Deuteromycotina) and other natural enemies of the green cloverworm in Iowa agroecosystems

by

Harlan Gene Thorvilson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Entomology

Approved:

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For the Graduate College

Iowa State University
Ames, Iowa
1984
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\[
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GENERAL INTRODUCTION

The green cloverworm (GCW), *Plathypena scabra* (F.) (Lepidoptera: Noctuidae), is one of the most widespread soybean defoliators in North America (Pedigo, 1980). The insect is considered an "occasional" pest of soybeans in Iowa (Pedigo et al., 1973). Widespread larval outbreaks occurred in Iowa in 1966, 1968, and 1973 (Pedigo, 1974) and more local outbreaks were identified in 1975 in northern Iowa [Insect, Weed and Plant Disease Newsletter, IC-421 (20 and 21), 1975], 1977 in southeastern Iowa [Insect, Weed, and Plant Disease Newsletter, IC-434 (14 and 15), 1977], 1978, and 1979 (Pedigo et al., 1983). In Iowa, an outbreak population configuration is characterized by large numbers of immigrant moths that oviposit in soybeans in June and early July. The large numbers of resulting larvae may cause economic damage to full-bloom soybeans. If fewer moths immigrate, a noneconomic, endemic population exists. Life-table studies have revealed two GCW larval generations each year in central Iowa, the second of which rarely causes economic damage to soybeans (Pedigo et al., 1983).

The seasonal cycle of the GCW in Iowa includes early-season establishment on various legumes, especially alfalfa, before later-season colonization on soybeans. The GCW is not considered an important pest of alfalfa, and few larvae are believed to complete development in alfalfa fields because of frequent harvesting schedules. Therefore, alfalfa fields may act as "population sinks" for the GCW in Iowa and contribute few moths for colonization of soybean fields (Buntin and Pedigo, 1983). However, alfalfa may be a natural "nursery" for various predators and parasitoids (Lentz
and Pedigo, 1975; Roberts et al., 1977) that eventually migrate to soybean fields. The relatively uncultivated condition of alfalfa fields may also protect residual entomopathogens.

Predators and parasitoids contribute to GCW mortality throughout the season, especially during the late season in endemic years. In outbreak GCW years, predator and parasitoid activity cannot prevent the development of potentially damaging GCW populations in soybean, and their action seems dominated in late season by epizootics of the entomopathogenic fungus *Nomuraea rileyi* (Hyphomycetes:Moniliales) (Bechinski and Pedigo, 1981; Pedigo et al., 1983). The epizootics interfere with predator and parasitoid activity by reducing the numbers of suitable host larvae (Sloderbeck, 1981; Pedigo et al., 1983).

Natural *N. rileyi* epizootics among GCW larvae in Iowa were recognized in the mid-1960s [L. P. Pedigo, Dept. of Entomology, Iowa State University, Ames, IA, personal communication]. Pedigo et al. (1983) identified the pathogen as having characteristics of a key biological mortality factor in GCW populations in Iowa soybeans. During GCW outbreak years, *N. rileyi* epizootics develop late in larval generation one and cause late-season collapse of the second generation. The disease is usually enzootic during endemic GCW population years but causes some mortality in the second larval generation late in the season. Relationships between GCW population configuration and larval mortality caused by *N. rileyi* have been observed. However, critical host densities and environmental influences on disease occurrence have not been determined.

*Nomuraea rileyi* is able to maintain itself between seasons at low densities in host populations in southerly climates (Kish, 1975; Allen and
Kish, 1978). The pathogen has been reported to withstand the stresses of winter as far north as Missouri and still initiate epizootics the following season (Ignoffo et al., 1978). The regular annual occurrence of *N. rileyi*-infected GCW larvae in soybeans suggests that the pathogen has overwintering potential in Iowa, too.

The interrelationships of *N. rileyi* and other biotic mortality agents with GCW populations may be different under various types of soybean tillage systems. For example, fewer GCW larvae were collected in no-till soybeans than in tilled soybeans planted on the same date in Kentucky, but no differences in parasitoid and predator incidences were observed (Sloderbeck, 1981). GCW populations in no-till soybeans had greater incidence of *N. rileyi* infection than did populations in tilled soybeans in two of the three years of the study. Reduced-tillage crop production systems used to conserve topsoil have been widely adopted in Iowa in recent years. In the decade from 1970 to 1980, the number of reduced-tillage acres in Iowa increased by nearly seven times [Iowa Conservation Tillage Survey, USDA Soil Conservation Service, Des Moines, IA, 1980)]. Nonetheless, concerns about possible yield reductions under reduced-tillage remain. Among the concerns are the effects of reduced-tillage on pest insect populations and upon the activities of their biotic mortality agents. Research is needed to determine the relationships between tillage practices and pest insect populations.

Laboratory studies of the effects of biotic mortality agents on host larvae complement field observations. Because *N. rileyi* is considered a key mortality factor in GCW population dynamics (Pedigo et al., 1983), the development of the fungus in host larvae is of special interest. The pathogenic cycle within several noctuid species has been followed (Getzin, 1961;
Kish and Allen, 1978; Mohamed et al., 1978; and Boucias and Pendland, 1982). However, the histopathology of N. rileyi in GCW larvae has not yet been determined. Once larvae are inoculated with N. rileyi, several days elapse before they become sluggish, cease feeding, and die. During this period, the consumption patterns of infected larvae may differ from those of healthy larvae. Infected Heliothis virescens (F.) and H. zea (Boddie) larvae consumed substantially less cotton square tissue than healthy larvae in experiments by Mohamed (1982) and Mohamed et al. (1978b), respectively. The impact of N. rileyi infection on GCW larval consumption of soybean leaf tissue, however, has not been determined. Differences in consumption patterns of infected and healthy GCW larvae would affect models that predict the severity of defoliation by field populations.

Considering the foregoing, a better understanding of the relationship of GCW populations and their natural biotic mortality agents is necessary for the implementation of integrated management practices in soybean production. With these considerations in mind, the studies reported in this dissertation were conducted with the following objectives:

1. Determine if the total numbers of GCW larvae and the incidences of biotic mortality agents of the insect in four widely-used soybean tillage systems in Iowa are the same (Part I);
2. Differentiate between the consumption patterns and rates of N. rileyi-infected GCW larvae and those of healthy control larvae (Part II);
3. Assess the viability of N. rileyi in sclerotized cadavers and in conidia after exposure to an Iowa winter (Part III);
(4) Determine if alfalfa fields are early season nurturing centers for biotic mortality agents that later attack GCW populations in soybeans (Part IV);

(5) Characterize GCW larval population configuration and *N. rileyi* incidence and epizootic development (Part V);

(6) Trace the development of *N. rileyi* mycoses in infected GCW larvae (Part VI).
LITERATURE REVIEW

The Green Cloverworm

Biology

Distribution and pest status. The green cloverworm (GCW), *Plathy*-
*peny scabra* (F.) (Lepidoptera:Noctuidae), is one of the most widespread
soybean insects in North America (Pedigo, 1980). It is a potential pest of
economic legumes in the eastern two-thirds of the United States where epi-
phytotics on soybean have been reported since 1919 (Stone and Pedigo,
1972).

Hill (1925) was the first to publish detailed biological studies of
the GCW, describing the known geographic distribution, food plants, life
stages, and natural enemies. Pedigo et al. (1973) supplemented and up-
dated the biological synopsis and categorized the insect as an "occasion-
al" pest of soybeans in Iowa. Stone and Pedigo (1972) compiled a bibliog-
raphy of the GCW and Hammond (1975) briefly summarized the GCW literature.

GCW adult activity and oviposition begin ca. mid-May in central Iowa
(Pedigo et al., 1973). Extensive epiphytotics in soybean occurred in 1966,
1968, and 1973, in Iowa (Pedigo, 1974), and additional outbreaks occurred
in 1978 and 1979, in central Iowa (Pedigo et al., 1983).

Overwintering potential in Iowa. Stone and Pedigo (1974) tested the
overwintering potential of GCW larvae, pupae, and adults in field cages and
in the laboratory and found that the GCW is unlikely to survive the winter
in central Iowa litter habitats. Likewise, Myers and Pedigo (1978) found
that GCW moths, protected in cages covered with forest litter, were unable
to overwinter.
Myers and Pedigo (1977) determined from dissections that female GCW moths were unmated after late September in Iowa. Dissections of female moths collected in the fall and spring by Myers and Pedigo (1978) revealed that virtually all late fall females were unmated, whereas 96% of the spring-collected females were mated. This information, together with lack of experimental success in overwintering and observations of "tardiness" in first spring collections, led them to hypothesize that the GCW was a migratory pest in Iowa.

Buntin and Pedigo (1983) used ovarian dynamics to determine the mating status of GCW moths appearing during the spring in Iowa. A large percentage of female moths had mated at least once, especially those collected in alfalfa. Also, the predominance of unmated, dark-phase females in the fall and the lack of these moths in the spring suggested that the earliest spring flights were immigrant moths. They hypothesized that immigrant moths to central Iowa exhibit distinct endemic and outbreak population phenologies. Therefore, it might be possible to predict the type of GCW larval population within any given year on the basis of the nature of the immigrant flights.

*Life table studies* Pedigo et al. (1983) constructed partial life tables for GCW populations during two endemic and two outbreak years. Endemic years had characteristically low levels of immigrating moths in the spring, followed by a small first generation of larvae and a larger second larval generation. The outbreak configuration was characterized by abundant immigrant moths and a large first larval generation with the potential of causing economic damage to soybeans. The second generation of larvae was smaller than the first.
GCW population dynamics in Iowa

Pedigo et al. (1983) and Buntin and Pedigo (1983) suggested a working hypothesis to explain GCW population dynamics in Iowa soybeans. Four adult GCW flights and three larval generations typically occur in central Iowa. The first two adult flights emigrate from southern overwintering areas. Adult flight one emigrates from overwintering areas and oviposits almost exclusively on alfalfa. Few larvae survive alfalfa harvest to emerge as adults. The survivorship of subsequent larval generations in alfalfa is low, as is moth emergence. For these reasons, alfalfa fields act as GCW population "sinks" in Iowa and, therefore, contribute little to GCW invasion of soybeans.

GCW immigrant moth flight two not only oviposits in alfalfa fields, but also invades soybeans. If flight two is large, the resulting larval population may be sufficient to cause economic injury to soybeans and would represent an outbreak configuration. More typically, flight two is not large and produces an endemic, noneconomic larval population.

Flight three consists of indigenous moths which, in turn, produce the second larval generation in soybean. During outbreak years, larval generation two in soybean may be quickly and significantly reduced by Nomuraea rileyi epizootics. Endemic larval populations also are attacked by the fungus, although it may not become epizootic until later, as larval numbers increase.

During outbreak GCW years, a fourth adult flight may not be present because of the collapse of the second larval generation in soybean caused by N. rileyi epizootics. A small fourth adult flight occurs in endemic GCW years and its size depends on the magnitude of N. rileyi mortality in the second larval generation in soybean.
The female GCW moths of the fourth flight in Iowa are dark-colored and unmated. They fail to survive the winter and are unable to contribute to the next season's population.

*Nomuraea rileyi* is the dominant agent of natural control in GCW populations in outbreak years, especially in the second larval generation (Pedigo et al., 1983). Unfortunately, *N. rileyi* may not become epizootic in outbreak years until larval densities in soybean have exceeded a critical level and, perhaps, have begun to drop due to pupation. Therefore, *N. rileyi* epizootics fail to protect soybeans from defoliation by GCW larvae during the critical flowering stages. As the season progresses, *N. rileyi* epizootics intensify, causing collapse in GCW populations and preventing late-season soybean losses. Epizootic victims contribute fresh conidia and sclerotized cadavers to the "inoculum load" in fields. The load of inoculum has the potential to overwinter and be available for disease initiation the following season.

**Parasitoids and predators of the green cloverworm**

*Rankings of parasitoid abundance* Slodbeck (1981) compiled an extensive list of reported parasitoids of the GCW. The list of 53 primary parasitoids included representatives from three families of Diptera and six families of Hymenoptera.

Reports from several states have listed the parasitoid species reared from GCW larvae. The most abundantly reared species are presented in Table 1 and are ranked according to relative abundance. The most abundant species is ranked number one, with subsequent numbers indicating decreased species abundance. GCW larvae were collected from soybeans; however,
<table>
<thead>
<tr>
<th>Parasitoid</th>
<th>Iowa</th>
<th>Missouri</th>
<th>Arkansas</th>
<th>Delaware</th>
<th>N. Carolina</th>
<th>Kentucky</th>
<th>S. Carolina</th>
</tr>
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<tbody>
<tr>
<td><em>Hymenoptera:Braconidae</em></td>
<td></td>
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</tr>
<tr>
<td><em>Rogas nolophanae</em> Ashmead</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cotesia marginiventris</em> (Mason)</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Protomicropplitis facetosa</em> (Weed)</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>:Ichneumonidae</td>
<td></td>
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<tr>
<td><em>Venturia nigriscapus</em> (Viereck)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>:Diptera:Tachinidae</td>
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</tr>
<tr>
<td><em>Winthemia sinuata</em> Reinhard</td>
<td>2</td>
<td>3</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oswaldia assimilis</em> (Townsend)</td>
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<td>3</td>
</tr>
<tr>
<td><em>Chaetophlepsis plathypenae</em> Sabrosky</td>
<td></td>
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<td>1</td>
</tr>
</tbody>
</table>
reports from Iowa, Missouri, and Delaware also include collections from other legumes. In addition, Hill (1925) reported that *Rogas nolophanae* Ashmead was the most commonly reared parasitoid from GCW larvae in Tennessee in 1914. Roberts et al. (1977) reared four dipterous and six hymenopterous parasitoid species from GCW larvae collected in central Illinois soybeans, but the relative abundance of the parasitoids was not indicated.

Marston et al. (1979) collected adult hymenopterous parasitoids from Missouri soybeans with a vacuum-net technique. Although low in numbers, *Cotesia marginiventris* (Mason), *R. nolophanae*, and *Protomicroplitis facetosa* (Weed) were collected in descending order of abundance.

**Parasitism pattern and seasonal abundance** The pattern of parasitism may have significance to host population phenology. *Rogas nolophanae* attacks early stage GCW larvae (Whiteside et al., 1967; Mueller and Kunnala, 1979). Lentz and Pedigo (1974) found that female *R. nolophanae* attacked third instar GCW most frequently but also accepted second instars. Rarely, if ever, were fifth or sixth instars attacked.

Whiteside et al. (1967) felt that the numerical predominance of *R. nolophanae* was important, but the parasitoid had the added benefit of killing GCW larvae before their most injurious feeding stages. Lentz and Pedigo (1974) found that adult female *R. nolophanae* also inflicted predatory mutilation on GCW larvae, thus accounting for 30 to 70% mortality beyond observed parasitism.

The seasonal abundance of *R. nolophanae* was greatest in June in Missouri (Barry, 1970) and early in the season in Iowa (Lentz and Pedigo, 1975). The lowest percentage parasitization by *R. nolophanae* was in mid-season in Iowa, but it increased gradually to a fluctuating plateau for the
rest of the season. In contrast, *Winthemia sinuata* Reinhard parasitization was low in early season, increased to the first week of August and doubled again by early September. Seventy-nine percent of GCW larvae attacked by *W. sinuata* were in the sixth stage (Lentz and Pedigo, 1974).

Kunnalaca and Mueller (1979) reported that *Cotesia marginiventris* preferred to oviposit in first or second stage GCW larvae. McCutcheon and Turnipseed (1981) found that *C. marginiventris* emerged exclusively from small- to medium-sized GCW larvae, thus decreasing crop damage by the host population.

**Activity in various agroecosystems** Several studies have compared parasitization of GCW in alfalfa and soybeans. Lentz and Pedigo (1975) found ca. the same percentage of parasitization by *R. noilophanae* in the two crops, whereas *W. sinuata* accounted for 2½ times more parasitization in soybeans. Roberts et al. (1977) found a greater overall rate of parasitization in alfalfa than in soybeans (43.3% and 32.4%, respectively) by the entire parasitoid complex in Illinois. A greater number of parasitoid species were collected from soybeans than from alfalfa. The authors did not report the percentage parasitization by each species.

Sloderbeck (1981) found significantly higher combined mortality caused by pathogens and parasitoids in double-crop Kentucky soybeans than in either early- or late-planted, single-crop soybeans in 1978. He felt the difference was caused by significantly more parasitization by *P. facetosa* and increased incidence of *N. rileyi*. Also, *C. marginiventris* was more prevalent in double-crop soybeans than in early soybeans. Although McCutcheon and Turnipseed (1981) found fewer GCW larvae in Mexican bean
beetle-resistant soybeans than in a susceptible variety, they concluded that soybean genotype had no effect on the incidence of parasitization.

**Predators**  *Orius insidiosus* (Say) and *Nabis* spp. are important GCW egg and larval predators in Iowa (Pedigo et al., 1972a). Together with the Araneida, these predators account for ca. 90% of the foliage-inhabiting predator fauna in soybeans (Bechinski and Pedigo, 1981).

Barry (1973) made vacuum-net collections of predators from Missouri soybeans and found that *O. insidiosus* was the most abundant species, even though the population declined sharply in late summer. Spiders were the second most abundant group, with their number peaking in mid-summer. *Nabis* spp. were present all summer, and the fourth group, *Geocoris* spp., reached peak numbers in mid- to late-summer.

Shepard et al. (1974) ranked the abundance of predators in South Carolina soybeans from ground-cloth samples. In descending order, the rankings were: *Nabis* spp. nymphs, spiders, and *Geocoris* spp. nymphs. In general, the predatory species were most abundant when populations of Lepidoptera pest species were highest. For example, the presence of early-instar GCW preceded peak populations of *Nabis* spp. nymphs by ca. three weeks in 1972, and six weeks in 1973.

The stage of crop development may also influence predator abundance. Colonization of soybean fields by predators increases rapidly only after canopy closure (Price, 1976). In Missouri soybeans, total predator populations peaked during late flowering and early pod-fill stages (Marston et al., 1979). Sprenkel et al. (1979) reported that predator densities were greatest in dense soybean canopies in North Carolina. Predation was greater in early-planted than in late-planted soybeans. If late planting was
necessary, increasing plant densities by using narrow rows and high seeding rates maximized predation. The authors hypothesized that greater canopy density offered more abundant prey and a more favorable microhabitat. Bechinski and Pedigo (1981) indicated that predators were a stable source of late season larval mortality in soybeans but were not key regulators of GCW populations in Iowa.

The Entomopathogenic Fungus *Nomuraea rileyi*

(Farlow) Samson

The dynamics of entomopathogens and their effects upon insect populations were reviewed by Steinhaus (1954) in his classic paper. More specifically, Charles (1941) prepared a checklist of entomogenous fungi in North America. Madelin (1963, 1966) observed that most fungal pathogens of insects were hyphomycetous Deuteromycetes of the order Moniliales. Bell (1974) and Roberts and Humber (1981) reviewed the mycoses of insects, and Samson (1981) identified entomopathogenic Deuteromycetes, both macro- and microscopically.

**Taxonomic status and distribution of *Nomuraea rileyi***

Kish et al. (1974) transferred *Spicaria rileyi* (Farlow) Charles to *Nomuraea* and placed *Spicaria prasina* (Maublanc) in synonymy. Samson (1974), in a monograph on the genus *Paecilomyces* and some allied Hyphomycetes, retained the genus *Nomuraea* Maulblanc for *S. rileyi* and *Isaria atypicola* Yasuda. He followed the history of *Nomuraea*, gave a generic description, and provided a key to two species. Kish (1974) also described the systematics of *N. rileyi*.

Weiser (1981), in his presidential address to the Society of
Invertebrate Pathology, used a map showing *N. rileyi* geographically restricted to the southeastern United States. In contrast, Onions (1979) described the geographic distribution of the fungus as worldwide.

**Biology**

**Susceptible insect species**  The host range of *N. rileyi* is composed mainly of lepidopterans, most of them Noctuidae. Table 2 lists at least 44 insect species susceptible to *N. rileyi* infection; 38 of them are lepidopterans. Included are 27 noctuids, many of which are frequent economic pests of agronomic crops. Four Coleoptera species have been diagnosed as vulnerable to infection by *N. rileyi*. Ignoffo (1980) found susceptibility in an important dipteran, the seedcorn maggot, *Delia platura* (Meigen). Ignoffo (1981) compiled a list of susceptible insects that he called the host spectrum. He also presented a table of nonsusceptible insect species, including several predator and parasitoid species.

**Relative susceptibility of hosts**  Caterpillars differ in their susceptibility to *N. rileyi*. Puttler et al. (1976) tested conidia (produced on agar medium) of a strain originally isolated from *Heliothis zea* (Boddie) larvae against second instars of nine caterpillar species. The relative susceptibilities of the Lepidoptera species were ranked by percentage mortality. *Spodoptera exigua* (Hübner) was the most susceptible species. *Tri- choplusia ni* (Hübner), *Plathypena scabra*, *Heliothis virescens*, and *Heliothis zea* were all approximately equal in susceptibility. *Pseudoplusia includens* (Walker) and *Peridroma saucia* (Hübner) were less susceptible than the preceding species, and *Anticarsia gemmatalis* (Hübner) was the least susceptible to *N. rileyi* infection. The imported cabbageworm, *Artogeia*
Table 2. Insects susceptible to *Nomuraea rileyi* (Farlow) Samson infection

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name²</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achaea janata</em> Linnaeus</td>
<td>(castoroil looper)*</td>
<td>Phadke and Rao, 1978</td>
</tr>
<tr>
<td><em>Agrotis gladiaria</em> Morrison</td>
<td>claybacked cutworm</td>
<td>Ignoffo, 1980</td>
</tr>
<tr>
<td><em>Agrotis ipsilon</em> (Hufnagel)</td>
<td>black cutworm</td>
<td>Ignoffo and Garcia, 1979</td>
</tr>
<tr>
<td><em>Alabama argillacea</em> (Hübner)</td>
<td>cotton leafworm</td>
<td>Thomas and Poinar, 1973</td>
</tr>
<tr>
<td><em>Anagrapha falcifera</em> (Kirby)</td>
<td>celery looper</td>
<td>J. W. Mertins, 1982 (Dept. of Entomology, Iowa State University, Ames, Iowa, personal communication)</td>
</tr>
<tr>
<td><em>Anticarsia gemmatalis</em> (Hübner)</td>
<td>velvetbean caterpillar</td>
<td>Watson, 1916</td>
</tr>
<tr>
<td><em>Chrysodeixis eriosoma</em> Doubleday</td>
<td>(vegetable looper)*</td>
<td>Steinhaus and Marsh, 1962; Teakle, 1980</td>
</tr>
<tr>
<td><em>Feltia jaculifera</em> (Gueneé)</td>
<td>dingy cutworm</td>
<td>Crumb, 1929</td>
</tr>
<tr>
<td><em>Heliothis virescens</em> (Fabricius)</td>
<td>tobacco budworm</td>
<td>Chamberlin and Dutky, 1958</td>
</tr>
<tr>
<td><em>Heliothis zea</em> (Boddie)</td>
<td>corn earworm</td>
<td>Charles, 1941</td>
</tr>
<tr>
<td><em>Leucania latiuscula</em> Herrick-Schaeffer</td>
<td></td>
<td>Thomas and Poinar, 1973</td>
</tr>
<tr>
<td><em>Mamestra brassicae</em> Linnaeus</td>
<td></td>
<td>Rodrigues-Rueda and Fargues, 1980</td>
</tr>
</tbody>
</table>

²(1) Common names without parentheses are accepted common names by the Entomological Society of America (ESA).
(2) Common names in parentheses are not "accepted" common names by the ESA.
(4) Common names followed by ** are from Commonwealth Institute of Entomology, Distribution Maps of Pests. Series A (Agricultural).
Table 2. Continued

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common namea</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peridroma saucia</em> (Hubner)</td>
<td>variegated cutworm</td>
<td>Puttier et al., 1976</td>
</tr>
<tr>
<td><em>Plathypena scabra</em> (Fabricius)</td>
<td>green cloverworm</td>
<td>Hill, 1925</td>
</tr>
<tr>
<td><em>Pseudaletia unipuncta</em> (Haworth)</td>
<td>armyworm</td>
<td>Charles, 1941</td>
</tr>
<tr>
<td><em>Pseudoplusia includens</em> (Walker)</td>
<td>soybean looper</td>
<td>Gudauskas and Canerday, 1966</td>
</tr>
<tr>
<td><em>Rachiplusia ou</em> (Gueneé)</td>
<td>(a mint looper)</td>
<td>Getzin, 1961</td>
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<tr>
<td><em>Spodoptera eridania</em> (Cramer)</td>
<td>southern armyworm</td>
<td>Sutton, 1978</td>
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<tr>
<td><em>Spodoptera exigua</em> (Hübner)</td>
<td>beet armyworm</td>
<td>Puttier et al., 1976</td>
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<td><em>Spodoptera frugiperda</em> (J. E. Smith)</td>
<td>fall armyworm</td>
<td>Charles, 1941</td>
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<tr>
<td><em>Spodoptera littoralis</em> (Boisduval)</td>
<td>(Egyptian cotton worm)**</td>
<td>Thomas and Poinar, 1973; Fargues and Rodriguez-Rueda, 1980</td>
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<td><em>Spodoptera litura</em> (Fabricius)</td>
<td>(tobacco caterpillar)</td>
<td>Rao and Phadke, 1977</td>
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<td><em>Spodoptera mauritia</em> (Boisduval)</td>
<td>lawn armyworm</td>
<td>Thomas and Poinar, 1973</td>
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<td><em>Spodoptera ornithogalli</em> (Gueneé)</td>
<td>yellowstriped armyworm</td>
<td>Charles, 1941</td>
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<tr>
<td><em>Tiracola plagiata</em> (Walker)</td>
<td>(banana fruit caterpillar)*</td>
<td>Steinhaus and Marsh, 1962</td>
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<tr>
<td><em>Trichoplusia ni</em> (Hübner)</td>
<td>cabbage looper</td>
<td>Charles, 1941</td>
</tr>
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<td><em>Xestia badinodis</em> (Grote)</td>
<td>(spotted-sided cutworm)</td>
<td>Crumb, 1929</td>
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<td><strong>Lepidoptera</strong>:Bombycidae</td>
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<tr>
<td><em>Bombyx mori</em> (Linnaeus)</td>
<td>silkworm</td>
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<td><strong>Lepidoptera</strong>:Pyralidae</td>
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<tr>
<td><em>Evergestis forficalis</em> Linnaeus</td>
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<td>Maublanc, 1903</td>
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Table 2. Continued

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<th>Scientific name</th>
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<tr>
<td>Glyphodes phyloalis Walker</td>
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<tr>
<td></td>
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<td>(Ignoffo, 1981)</td>
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<tr>
<td>Ostrinia nubilalis (Hübner)</td>
<td>European corn borer</td>
<td>Thorvilson, 1979 (Dept. of Entomology, Iowa State Univ., unpubl.)</td>
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<td>Stenachroia elongella Hampson</td>
<td>(jowar web worm)</td>
<td>Phadke and Rao, 1978</td>
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<td>Susumia sp. near exigua Butler</td>
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<td>Steinhaus and Marsh, 1962</td>
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<td>Lepidoptera:Arctiidae</td>
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<td>Hyphantria cunea (Drury)</td>
<td>fall armyworm</td>
<td>Kawakami et al., 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Ignoffo, 1981)</td>
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<td>Lepidoptera:Lymantriidae</td>
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<td>Lymantria dispar (Linnaeus)</td>
<td>gypsy moth</td>
<td>Wasti and Hartmann, 1978</td>
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<td>Lepidoptera:Pieridae</td>
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<tr>
<td>Artogeia rapae (Linnaeus)</td>
<td>imported cabbageworm</td>
<td>Biever and Hostetter, unpubl. (Ignoffo, 1981)</td>
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<td>Lepidoptera:Plutellidae</td>
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<td>Plutella xylostella (Linnaeus)</td>
<td>diamondback moth</td>
<td>Robert and Marchal, 1980</td>
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<td>Lepidoptera:Hesperiidae</td>
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<td>unidentified specimen from East</td>
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<td>Thomas and Poinar, 1973</td>
</tr>
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<td>Malaysia</td>
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<td>Diptera</td>
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<td>Delia platura (Meigen)</td>
<td>seedcorn maggot</td>
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<td>unidentified specimen from Colombia</td>
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<td><em>Popillia japonica</em> Newman</td>
<td>Japanese beetle</td>
<td>St. Julian et al., 1982</td>
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<td><em>Hypera punctata</em> (Fabricius)</td>
<td>clover leaf weevil</td>
<td>Charles, 1941</td>
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<td>Coleoptera: Chrysomelidae</td>
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<tr>
<td><em>Leptinotarsa decemlineata</em> (Say)</td>
<td>Colorado potato beetle</td>
<td>Fargues, 1976</td>
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<td>Coleoptera: Elateridae</td>
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<tr>
<td>unidentified specimen</td>
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<td>Charles, 1941</td>
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</table>
*rapae* (L.), the only nonnoctuid, was not susceptible in this study.

Boucias et al. (1982) determined the relative susceptibility of six noctuid species to *N. rileyi* isolated from *A. gemmatalis* cadavers. *Pseudoplusia includens*, *T. ni*, and *H. zea* were very susceptible to the isolates, whereas *A. gemmatalis*, *S. exigua*, and *Spodoptera frugiperda* (J. E. Smith) were less susceptible. The black cutworm, *Agrotis ipsilon* (Hufnagel), was nearly 20 times less susceptible than was *T. ni* to two isolates of *N. rileyi* (Ignoffo and Garcia, 1979).

**Virulence of *N. rileyi* isolates** Considerable evidence suggests that various isolates of *N. rileyi* differ in virulence to insects. Puttler et al. (1976) utilized a *N. rileyi* isolate from *H. zea* for their bioassays against nine species of Lepidoptera larvae. Boucias et al. (1982) determined that two isolates from *A. gemmatalis* cadavers differed in virulence to six noctuid species after serial passage through *A. gemmatalis* and *P. includens* hosts.

Ignoffo et al. (1976c) found that isolates from Missouri, Mississippi, and Brazil were equally virulent to *T. ni*, but a Florida isolate was seven to 17 times less virulent than those from the other locations. *Anticarsia gemmatalis* larvae from various locations were very susceptible to the Brazilian isolate. Ignoffo and Garcia (1979) found that black cutworm larvae were not susceptible to a *N. rileyi* isolate from the Philippines.

Kawakami (1960) found that *N. rileyi* infectivity decreased with successive transfers on artificial media. However, virulence could be recovered if the isolate was passed through an insect host. Ignoffo (1981) claimed that, even after 15 successive transfers on Sabouraud maltose agar with 1% yeast extract, virulence was not lost. Apparently, the choice of
medium is important in maintaining infectivity of the fungus. Boucias et al. (1982) concluded that the recognition of such variability may necessitate the selection of specific *N. rileyi* strains and techniques in the preparation of commercial products for use against defined pest complexes.

**Larval age susceptibility** Small larvae are most often used in bioassays of fungal pathogens. Puttler et al. (1976) utilized second instars in comparing the relative susceptibility of nine caterpillar species. Boucias et al. (1982) also used second instars of six noctuid species.

Getzin (1961) showed that early instar *T. ni* were more susceptible to *N. rileyi* than were late instars. Mohamed et al. (1977) found that third to fifth instar *H. zea* were more susceptible to infection than were first and second instars. Possibly, conidia are lost with the exuviae before hyphal penetration of the integument can occur in rapidly growing larvae, accounting for the difference in susceptibility. Kish and Allen (1978) emphasized that large velvetbean caterpillars were nonsusceptible in field populations and that, normally, only first, second, and third instars were infected.

**Inoculation techniques** A number of techniques are used in bioassays of *N. rileyi*. Most involve some type of topical application. Examples of these procedures follow.

a) **Brushing**: Bell and Hamaille (1974) and Bell (1975) brushed dry conidia onto larvae, weighing groups of larvae before and after application to determine the weight of the dosage. A liquid slurry of blastospores budded from fragmented hyphae failed to cause mortality when brushed on larvae.
b) Immersion, "dipping:" Mohamed et al. (1977) and Mohamed (1982) prepared suspensions of conidia in water and wetting agent, immersed representative samples of larvae, washed off the conidia from larvae with a cyclo-mixer, and counted conidia with a hemocytometer.


d) Hand-atomized: Getzin (1961) and Gudauskas and Canerday (1966) used hand atomizers to apply suspensions of conidia and distilled water containing a wetting agent on larvae. Ignoffo et al. (1975a) hand-atomized conidial suspensions onto soybean leaves and allowed the leaves to dry. Leaves were sprayed with chemical pesticides and offered to T. ni larvae.

Many researchers consider the insect integument as the primary barrier to fungal diseases. A trophic response mechanism of conidia to insect cuticle was investigated by Kish (1975). He placed small pieces of P. includens larval integument in a drop of water to which N. rileyi conidia were added. Conidia germinated along a distance gradient; that is, those nearest the pieces of cuticle germinated first, whereas more distant conidia germinated later. Some soluble material apparently issues from the cuticle and stimulates conidial germination. Boucias and Pendland (1982) prepared a chloroform extract of sixth instar A. gemmatalis cuticle and demonstrated that it stimulated N. rileyi conidial germination.

Application of conidia to the food material of larvae has proven useful, but whether fungal invasion is via the integument or the gut remains to be determined. Ignoffo et al. (1976c) and Puttier et al. (1976) placed
quantified dilutions of conidia on soybean leaves, allowed larvae to feed for 48 hours, and then transferred the larvae to semisynthetic diet. Bell and Hamalle (1980) attempted per os treatment of larvae by applying microdrops of suspension on leaf disks. Larvae were allowed to feed for 18 hours, then were transferred to artificial diet. Garcia and Ignoffo (1978) applied conidia to the surface of firm, nonsterilized semisynthetic diet containing no antifungal agents. Larvae were allowed to feed for 24 hours after which they were transferred to sterilized diet containing antifungal chemicals. The diet treatment technique was a less sensitive bioassay than applying conidia to leaves, but it had the advantages of ease and quality control. Mohamed et al. (1982) obtained 90 to 100% mortality in third instar H. zea by feeding plugs of inoculated artificial diet.

Kish and Allen (1978) attempted to resolve the integument vs. gut-invasion-route problem by force-feeding larvae a conidial suspension with a finely drawn glass pipette. They found 30% mortality in eight days, identical to that found in larvae inoculated with a conidial suspension on the dorsum. They concluded that infection was probably a function of conidial concentration applied to larvae.

Intrahemocoelic injections of conidia and blastospores into T. ni and A. gemmatalis larvae were completed by Ignoffo et al. (1982). The antibiotics, penicillin and streptomycin, were simultaneously injected to prevent bacterial septicemia. Dose-mortality regression lines showed that resistance to conidia in A. gemmatalis was more than 100 times that in T. ni, and resistance to blastospores was more than 200 times greater than that in T. ni.
Rodriguez-Rueda and Fargues (1980) sprayed *N. rileyi* conidia onto egg masses of *Mamestra brassicae* Linnaeus and *Spodoptera littoralis* (Biosduval) but failed to cause mortality to the eggs. Larvae from treated eggs died later, perhaps as a result of ingestion of treated egg chorions. Another fungus tested, *Paecilomyces fumoso-roseus* (Wize) Brown and Smith, was highly virulent to eggs. Hyphomycetes pathogenicity to noctuid eggs may be a promising area for biological control workers to pursue.

Bell and Hamalle (1980) summarized the difficulties with fungal inoculation studies in insects. They hypothesized that cuticular invasion by fungi must rely on very specific microclimate variables, whereas per os infection routes have continuously favorable conditions. Mortality caused by oral introduction of inoculum placed on food disks is dosage-dependent, and, admittedly, this introduction method does not eliminate the possibility of cuticular contact with the conidia. Topical applications can be affected by larval molting. If fungal penetration into integument is only superficial, the insect might escape infection by ecdysis. *H. zea* larvae (and some others) have a second chance of inoculation by conidia because newly molted larvae quickly consume the shed integument that still harbors viable conidia.

**Cellular responses to *N. rileyi* invasion** Even though insect immune responses to *N. rileyi* are known, they are ineffective in suppressing mycoses. Sutton (1978) attempted to actively immunize *Spodoptera eridania* (Cramer) larvae by exposing them to heat-killed mycelia, but no significant decrease in mortality was shown. Passive immunization by transference of hemolymph from immunized larvae to infected larvae also failed to reduce
mortality. Sutton et al. (1981) observed that aggregations of hemocytes formed around \textit{N. rileyi} hyphae invading \textit{S. frugiperda} larvae. The encapsulations were ineffective, however, in halting disease development. It appears that once the insect cuticular barrier is breached, the victim succumbs quickly.

**Histopathology** Madeline (1966) reviewed the cycles of insect-pathogenic Deuteromycetes, especially those of \textit{Beauveria bassiana} (Balsamo) Vuillemin and \textit{Metarhizium anisopliae} (Metchnikoff) Sorokin. Cuticular penetration by hyphae from germinating \textit{N. rileyi} conidia was noted by Getzin (1961) in \textit{T. ni} larvae. The ontogeny of \textit{N. rileyi} in \textit{P. includens} larvae was followed by Kish (1975) and detailed by Kish and Allen (1978). Mohamed et al. (1978b) published photomicrographs and described the histopathology of \textit{N. rileyi} in \textit{H. zea} larvae. Boucias and Pendland (1982) examined \textit{N. rileyi} as it infected \textit{A. gemmatalis} larvae by using scanning and transmission electron micrographs. Later, these researchers (Pendland and Boucias, 1982) published scanning electron micrographs of conidiogenesis. Pendland (1982) reported three types of resistant or resting structures found in \textit{N. rileyi}. Boucias and Pendland (1982) suggested that the initiation of the conidiophore stage of \textit{N. rileyi} was caused by nutrient depletion of host larval resources.

From the preceding references, a composite life cycle of \textit{N. rileyi} can be constructed.

a) Conidia germinate within ca. 48 hours under optimal conditions of greater than 90% microclimatic relative humidity at between 20° to 25°C.
b) Germ tube hyphae penetrate epicuticle and exocuticle, producing darkened, lysed cavities at the points of entry. Pores of sense organs and intersegmental areas also may be penetrated.

c) Laterally branched hyphae penetrate the endocuticle and hypodermis.

d) Hyphae reach the hemocoel where vegetative hyphal bodies are produced by budding. The short, thick hyphal bodies are one-, two-, or three-celled filaments with distinct nuclei. Hyphal bodies multiply dramatically and, suspended in the hemolymph, spread throughout the host larva.

e) Blood cells lyse and fat bodies are invaded. The host larva becomes sluggish. Hyphal bodies elongate and become fusiform. An anal secretion cements the larva to a plant substrate, the anterior portion of the body elevates in a typical "rearing" posture, and the larva dies.

f) Hyphae invade Malpighian tubules, muscles, mesenteron, and head capsule. A mycelial complex ramifies through the hypodermal and mesodermal tissues resulting in larval mummification.

g) Elongate hyphae emerge through the host cuticle. Erect, septate conidiophores are produced and form a white mat over the entire body surface of the cadaver.

h) Conidiophores mature, forming chains of pale green phialidic conidia. The cadaver appears covered by a velvety-green mat. Conidia are passively dispersed by wind, water, etc.

**Effects of entomopathogens on "nontarget" organisms**

**Parasitoids and predators** Insect pathogens may interfere with the full expression of the parasitoid complex. Steinhaus (1954) studied the decline in populations of diamondback moth, *Plutella xylostella* (L.), subjected to a fungal, *Zoophthora radicans* (Brefeld) Batko, epizootic. He felt that parasitoids and predators ordinarily responsible for regulating
larval populations were largely destroyed by the epizootic, probably because of the loss of available hosts. After the epizootic disappeared, the pest population resurged to higher levels than before, due to lack of sufficient natural enemies. Jaques and Morris (1981) reported from various sources that a reduced rate of parasitization resulted after applications of the bacterium, *Bacillus thuringiensis* Berliner, against an insect host population. They suggested that entomopathogens could also reduce parasitization by toxic effects directly upon the developing parasitoid or indirectly by killing the parasitized host before the parasitoid could complete its development.

Burleigh (1975) found that a reduced parasitization observed in *Heliothis* spp. larvae was at least partly caused by interference from *N. rileyi*. Host larvae succumbing to the fungus disease also may have been parasitized, but, because the disease expressed itself first, parasitism was obscured. Sloderbeck (1981) reported that a *N. rileyi* epizootic in 1978 double-crop soybeans killed many late-stage GCW, the preferred hosts of dipterous parasitoids. Thus, the disease negatively interfered with dipterous parasitism.

Laboratory studies found that *Microplitis croceipes* (Cresson) parasitism of third stage *H. zea* larvae increased their susceptibility to infection by *N. rileyi* (King and Bell, 1978). *Nomuraea rileyi* inoculations reduced parasitoid larval development whether the host was treated before or within one day after parasitism. Decreased suitability of host larvae or antagonism between the pathogen and parasitoid were hypothesized. Otherwise, both pathogen and parasitoid could complete development in the
same host. Host larvae attacked by either agent in the third instar failed to complete a fifth larval molt or to pupate before death.

Phadke and Rae (1978) showed that *N. rileyi* was nonpathogenic to *Tele-nomus proditor* Nixon, an egg parasitoid of *Achoea janata* L. in India. Ignoffo (1981) reported that four parasitoids of Lepidoptera (including *c. marginiventris*) were not susceptible to *N. rileyi*, even when exposed to rates ca. 25 times higher than those used in field experiments to induce epizootics in their hosts. Additionally, three important insect predators [*Hippodamia convergens* Guerin-Meneville, *Chrysopa carnea* Stephens, and *Podisus maculiventris* (Say)] were not susceptible. Ignoffo concluded that most beneficial insects would not be directly affected by *N. rileyi* applications.

Other entomopathogens Vago (1963) discussed the interrelations of microbial insect diseases. He noted that some fungal pathogens have a toxic effect on insects without killing them, and fatal symptoms are produced by rapid bacteriosis. Madelin (1963) gave examples of microbial infections that predisposed insects to invasion by other microorganisms.

MacLeod et al. (1966) suggested an interference between *Erynia neoaphidis* Remaudiere and Henriebert and *Triplosporium fresenii* (Nowakowski) Batko in infections of woolly pine needle aphids. Only a small percentage of insects showed a double infection by the two entomogenous fungi.

Harper and Carner (1973) observed the fungus *Entomophthora* sp. causing epizootics in *P. includens* and *T. ni* populations in Alabama soybeans in 1969 and 1970. *Nomuraea rileyi* attacked the greatly reduced residual
populations after the *Entomophthora* sp. epizootics. Sullivan (1981) observed that *Entomophthora* sp. and *N. rileyi* caused 39% and 28% mortality, respectively, in second generation *Heliothis* spp. populations. Together, the pathogens were largely responsible for preventing a buildup to damaging levels of second generation larvae.

Ahmadzabidi (1978) found that field applications of nuclear polyhedrosis virus (NPV) and *B. thuringiensis* very effectively reduced velvetbean caterpillar populations but also reduced the impact of *N. rileyi* epizootics. The reduction in *N. rileyi* demonstrated the host-density dependency of the fungal pathogen.

Moscardi (1977) found an antagonism between NPV and *N. rileyi* in *A. gemmatalis* populations. In dual exposure to the two pathogens, the virus infection predominated because of its rapid replication at the expense of *N. rileyi* expression. Moscardi (1977) and Moscardi et al. (1981) found that NPV greatly reduced the magnitude of *N. rileyi* epizootics by reducing the growth substrate, velvetbean caterpillars.

**Warm-blooded animals** Ignoffo et al. (1976a) studied the effects of temperature on the growth of *N. rileyi*. Mycelia *in vitro* did not grow at temperatures above 35°C, and probably would not develop at similar temperatures in living avian and mammalian species. Ignoffo and Garcia (1978) determined that *N. rileyi* conidia are inactivated by human gastric juice. Conidia exposed for more than 30 minutes did not grow when cultured on Sabouraud maltose agar plus yeast extract.

Ignoffo et al. (1979) found no deleterious effects on mice when conidia were administrated via gastric intubation. Greater than 99% of conidial
infectivity was lost during passage through the alimentary tract. No abnormalities were observed in white rat inhalation experiments. Likewise, the eyes and abraded skin of rabbits treated with conidia showed no abnormalities. *Nomuraea rileyi* should not pose a serious, acute hazard to mammals.

*Nomuraea rileyi* epizootiology

**Natural epizootics**

**Historical reports** Watson (1916) described the disease condition called "cholera" by Florida farmers; it often decimated populations of *A. gemmatalis* during September and early October. The causal organism, *N. rileyi*, was highly dependent on weather conditions and did not become epizootic until "cholera time" arrived. Hill (1925) reported great numbers of GCW larvae killed by *N. rileyi* in the fall of the year in Tennessee and Maryland.

Allen et al. (1971) reviewed historical and more recent outbreaks of *N. rileyi* in Florida, Alabama, Mississippi, Texas, Indiana, and Wisconsin. Carner et al. (1975) reported that *N. rileyi* was the most common pathogen affecting GCW larvae and other soybean Lepidoptera in South Carolina. Epizootics occur in Missouri (Ignoffo et al., 1975b), North Carolina (Dietz et al., 1976), and Illinois (Roberts et al., 1977). Fungal disease epizootics among GCW larvae in Iowa were recognized by extension personnel in the mid-1960s (Pedigo et al., 1973). The causal agents were identified as *Beauveria bassiana* and *Metarrhizium* sp., but the likely cause was *N. rileyi* (L. P. Pedigo, Department of Entomology, Iowa State University, Ames, IA, personal communication).
**Seasonal occurrence**  Once established in the field, *N. rileyi* is a very effective natural control agent of noctuid larvae. Unfortunately, epizootics often occur only after the pest population has seriously damaged plants (Kish et al., 1976). For example, the time delay between peak *A. gemmatalis* numbers and the occurrence of *N. rileyi* epizootics allows several days for severe soybean defoliation (Kish, 1975). This defoliation often occurs when soybean plants are in their very vulnerable flowering and pod formation stages (Ignoffo et al., 1975b).

Nonetheless, *N. rileyi* is effective in reducing late-stage larval numbers before pupation (Ignoffo et al., 1975b; Heinrichs et al., 1979). Burleigh (1975) observed that late-season *N. rileyi* epizootics reduced *Heliothis* spp. attack on late-maturing cotton bolls. Epizootics also destroyed larvae ready for diapause. Soper (1978) suggested that most fungus entomopathogens have evolved a survival strategy of delayed attack. Both host and pathogen species survive because the disease does not occur in epizootic proportions until late in the reproductive cycle of the insect.

**Inoculum reservoir**  *Nomuraea rileyi* is a deuteromycete with no known perfect stage, although Kish (1975) saw the formation of reddish, sterile ascocarp-like structures at elevated temperatures. He suggested that, under proper conditions (yet to be determined), the fungus may undergo sexual reproduction and ascocarp formation similar to that found in the Hypocreales. Pendland (1982) found three types of resistant structures that could allow survival through times of environmental stress. Sprenkel and Brooks (1977) found that the fungus was able to overwinter within sclerotized host cadavers. Ignoffo et al. (1975b) detected conidia on plant parts in Missouri on June 27, only 25 days after planting. Ignoffo
et al. (1977a) considered the soil as the source of inoculum. Plants are inoculated by soil-sheltered conidia as the seedlings emerge from the seed-bed or soon thereafter by wind and water agents. Ignoffo et al. (1977b) found that most conidia produced by diseased caterpillars remained in the top few cm of soil because of minimum vertical movement through undisturbed soils.

Once the fungus disease has killed insect hosts, newly produced conidia are disseminated and infect new hosts. The dispersal stage giving rise to subsequent infections is conidia, even though their viability and infectivity are highly dependent upon climatic factors. Ignoffo et al. (1976b) found that the half-life of *N. rileyi* conidia is only ca. two days under field conditions. Wind was the major dispersal agent and local dissemination led to epizootics (Kish and Allen, 1978). Hostetter and Ignoffo (1978) were able to detect gradual natural increases in *N. rileyi* incidences from the progressive outward spread of conidia from individual dead larvae. Kish (1975) observed conidia on the leg scales of velvetbean caterpillar adults during an epizootic in Florida. Allen and Kish (1978) postulated that *N. rileyi* maintains itself on alternative hosts and velvetbean caterpillars in southern regions of Florida and then is carried northward on migrating moths in the spring.

**Host density** Milne (1957) considered fungal pathogens as imperfectly host density-dependent mortality factors. Tanada (1963) believed environmental factors were more important than host density in the development of fungal epizootics. Thus, weather and climate should not be considered as causing disease to occur, but only as permitting or not permitting disease to occur (Tanada, 1964). The appearance of a fungal
disease may be host density independent, but the spread of the disease is host density dependent.

Tanada (1964) claimed that the relative spatial arrangement of a host population was more important in epizootic development than the actual number of individuals. He outlines the optimum conditions for spread of an insect pathogen. Spread is maximized when disease arises in the center of closely aggregated susceptible hosts, and then the hosts disperse widely. From the focal point of infection, the disease radiates throughout the local population and even expands to more distant populations.

Allen et al. (1971) reported that *N. rileyi* infections appear in velvetbean caterpillar populations at one larva/30 cm-row, and, later, an epizootic develops as the host population increases. Several "generations" of fungi are necessary before sufficient conidia become available to suppress the insect population.

Ignoffo et al. (1975b) estimated that a critical host density of ca. 0.1 larva/plant would sustain sufficient inoculum to suppress *P. scabra* and *T. ni* populations if a prophylactic application of *N. rileyi* was field-applied between soybean flowering and pod formation. Ignoffo et al. (1976b) reiterated an earlier estimate that 0.1 larva/plant (0.4 to 0.8 larva/30 cm-row) would initiate and sustain an epizootic. This low population is sufficient because one diseased caterpillar/m² in the autumn will contaminate the soil with enough conidia to survive winter and attack the next season's insect population.

Johnson et al. (1976) found that less than one *A. gemmatalis* larva/30 cm-row could not support a fungal epizootic following pesticide treatment,
whereas disease developed normally in untreated check plots with a larval density of 1.7 larvae/30 cm-row. Fungus development was delayed three weeks in the treated plots by lack of suitable host larvae.

**Environmental factors** Steinhaus (1954) recognized that the rate of spread of insect diseases can be increased or decreased by weather conditions. Fungus diseases are especially dependent on weather fluctuations in their development, but they still cannot be considered as independent of host density.

Madelin (1963) described the environmental factors that influence germination of hyphomycetous fungi and subsequent invasion of insects. Relative humidity has a more significant effect on fungal epizootics than temperature (MacLeod et al., 1966). The microclimate near the host insect integument is important to the fungal invasion process. For example, the host surface environment may have a higher relative humidity than that recorded from the macroclimate.

Kish (1975) and Kish and Allen (1978) outlined the effects of various environmental factors on the growth and development of *N. rileyi*. In the laboratory, *N. rileyi* grew slowly on Sabouraud maltose agar with 1% yeast extract. Optimum growth was between 15° to 30°C and at a pH from six to eight. Photoperiod was not a limiting factor in the life cycle. Infection of insect larvae was best at 70 to 90% relative humidity. Conidial production increased as relative humidity increased above 70%. Meteorological conditions in the field affect fungal development and epizootics, also. Dry, windy conditions promote conidial dispersion, but retard germination. Fungal ontogeny within the host is independent of weather conditions.
Conidiophore formation is independent of humidity as long as the host cadaver is moist. In general, an alternation between wet and dry field conditions is necessary for the spread of *N. rileyi* infections and the development of epizootics.

Kish (1978) listed several critical steps in the life cycle of entomogenous fungi, most of which were environmentally dependent. Conidial dissemination, germination, cuticle penetration, conidiophore formation, sporulation, and conidia viability are especially affected by relative humidity. Environmental conditions can be manipulated to encourage the successful development of entomopathogenic fungi. Passive manipulation might consist of predicting the direction and magnitude of epizootics. Such knowledge could define the proper conditions necessary for success of artificial dissemination of inoculum. Active manipulation of such agro-environmental factors as air movement, relative humidity, row spacing, soil tillage practices, and host densities could encourage epizootic development.

**Impact of pesticides on *N. rileyi***  
*In vitro* tests evaluated 44 pesticides, all of them registered for use in soybeans, against *N. rileyi* (Ignoffo et al., 1975a). Seven of the eight fungicides, four of 11 herbicides, and 13 of 25 insecticides showed inhibition of *N. rileyi* growth in culture. The fungicide benomyl is commonly sprayed on soybeans at early pod set with a second application 14 to 21 days later. This fungicide inhibited *N. rileyi* in the tests. Further, soybean leaves treated first with conidia and then sprayed with benomyl were fed to second instar *T. ni*. Mortality of larvae was reduced by the fungicide treatment.

Because soil is the natural reservoir for seasonal initiation of *N. rileyi* epizootics, the effects of pre-emergence herbicides on fungal
inoculum is of interest. Ignoffo et al. (1975a) sprayed the herbicide dinoseb on soil-conidial mixtures. Bioassays showed significant inhibition of caterpillar mortality from the entomopathogen. Laboratory tests by Sutton (1978), Gardner et al. (1979), and Jaques and Morris (1981) support and expand the known effects of pesticides on N. rileyi.

Sutton (1978) and Gardner et al. (1979) evaluated a new category of insecticide, represented by diflubenzuron (Dimilin®). This material is an insect growth regulator and acts by interfering with the deposition of chitin. Diflubenzuron was shown to promote N. rileyi mycelial growth in vitro. There may be some chemical relationship between this promotion of fungal growth, conidial germination, insect cuticle penetration, and the physiological activity of diflubenzuron.

Field trials of pesticides frequently used by producers and their effects upon N. rileyi epizootics have been approached in several studies. Laboratory inhibition by fungicides was substantiated in the field by Johnson et al. (1976), Livingston et al. (1978), and Horton et al. (1980). The combined use of fungicides and insecticides was particularly inhibitory. Apparently, the inhibitory effects of fungicides were compounded by a reduction in the insect host population available for inoculum buildup and development of epizootics.

Even insecticides alone applied against soybean pest caterpillars suppress N. rileyi epizootics. Johnson et al. (1976) believed the suppression was caused by reductions in suitable hosts, the substrate of disease development. Ahmadzabidi (1978) reported that carbaryl broke the developmental cycle of the fungus by reducing the velvetbean caterpillar population to below a critical density of one larva/30 cm-row of soybeans. The fungal
epizootic was delayed. Livingston et al. (1978) attributed a low incidence of *N. rileyi* infection to reduction in larval numbers rather than to direct suppression of the fungus by fungicides or insecticides. Moscardi (1977) and Moscardi et al. (1981) recommended applying insecticides at rates sufficient to reduce caterpillar populations below damaging levels but still leave enough larvae for *N. rileyi* development.

In summary, it seems that consideration must be given to the choice of pesticide, the levels at which it is applied, and the timing of applications to insure the least inhibitory effect upon the development of *N. rileyi*. An integrated approach to weed control, plant disease suppression, and insect management would insure that *N. rileyi* epizootic development would not be disrupted. In this manner, the suppressive effects of *N. rileyi* infections on pest insect populations would be encouraged.

The epizootic cycle Pathogenic fungal diseases of insects create an "intermittant infection chain" in temperate zones because the winter season interrupts host availability and aerial transmission of inocula (MacLeod, 1966). Ignoffo et al. (1977a) identified the probable sequence of events that results in a seasonal epizootic of *N. rileyi*.

a) Conidia overwinter in soil and are transmitted to germinating soybean plants early in the season. Susceptible larvae become contaminated by *N. rileyi* during feeding activities. Diseased larvae disperse inoculum throughout the plants.

b) Deaths of larvae early in the season increase the inoculum load sufficiently to initiate an epizootic wave. Later in the season, more conidia are produced and are dispersed by wind and rain. Still larger numbers of larvae die until the local population is eliminated and no more hosts are available.
c) Dead larvae supply inoculum that overwinters on or in the soil. The date of the epizootic peak each season is determined by factors such as load of overwintering inoculum, availability of hosts during the early season, proper environmental conditions, and extent of conidial dispersal early in the season.

**Models of fungal epizootics** Kish and Allen (1978) developed a model for predicting the incidence of *N. rileyi* on velvetbean caterpillars in Florida soybeans. Three factors are needed to calculate the number of infections on a given day: (a) inoculum density, (b) weather effects upon inoculum, and (c) the relationship between inoculum density and infection levels. In two seasons of data, there was a fairly high correlation between predicted and observed infection levels, but refinement of the model continues. Allen and Kish (1978) listed 10 assumptions that formed the basis for development of the model.

Some additional work in modeling fungal pathogen effects was done by Brown and Nordin (1982), who studied the fungal pathogen *Erynia* sp. (Zygomycetes:Entomophthoraceae) of the alfalfa weevil, *Hypera postica* (Gyllenhal), in Kentucky. The model uses integro-differential equations to describe epizootics caused by the pathogen.

**Induced epizootics** Kish (1975) suggested that the time delay between pest population peaks, the accompanying severe defoliation, and the onset of *N. rileyi* epizootics shows that *N. rileyi* is an ineffective agent of natural pest control for crop protection. Others have attempted to induce earlier onsets of epizootics or to utilize the fungus inundatively. As early as 1916, Watson failed to start a premature *N. rileyi* "cholera"
epidemic in velvetbean caterpillar populations in Florida. Chamberlin and Dutky (1958) sprayed \textit{N. rileyi} conidia in water suspension on tobacco in the field but recorded only slight fungal mortality in budworms. Getzin (1961) sprayed \textit{N. rileyi} conidia in the field to induce earlier fungal disease development in cabbage looper larvae. Sprenkel and Brooks (1975) distributed pieces of \textit{N. rileyi}-caused cadavers among soybeans and induced earlier and more intense epizootics than those occurring naturally.

Ignoffo et al. (1976b) sprayed conidia on soybean test plots for infection of second instar GCW. \textit{Nomuraea rileyi} deaths began 14 days earlier than in untreated plots. The peak of the induced epizootic occurred before and during soybean flower initiation and pod formation, the stages most sensitive to the effects of defoliation. In that year, the natural epizootic in untreated plots occurred after the damage at the sensitive plant stages had been done. Prophylactic use of \textit{N. rileyi} is feasible if it reduces larval populations below the economic injury levels during the most sensitive soybean growth stages.

Mohamed et al. (1978a) sprayed \textit{N. rileyi} conidia on sweet corn to evaluate their effectiveness in protecting ears from \textit{H. zea} damage. The pathogen caused high mortality in larvae but was unable to prevent economic damage. Such applications to corn at the whorl stage could reduce \textit{H. zea} populations that later migrate to cotton and, therefore, may be important in an integrated pest management system.

Hostetter and Ignoffo (1978) suggested that epizootics also could be induced by agents other than the fungal pathogen. Stressors or incitants, selective use of fungicides, and environmental modification might be used to induce early initiation of an epizootic.
Ignoffo (1980) proposed a program to protect soybeans from caterpillar pests by utilizing entomopathogens in an integrated, three-pronged attack. First, *N. rileyi* conidia would be applied to soybeans at initial flowering or when about one-half of the plants had flowers. This would specifically apply to midwestern indeterminate soybeans. If an *N. rileyi* epizootic failed to develop because of environmental conditions, the second phase of the program, application of *B. thuringiensis*, would proceed. The last phase of the program would be the use of specific nucleopolyhedrosis viruses against such pests as *Heliothis* spp.

### Tillage Practices and Soybean Insects

#### Reduced tillage in Iowa

Interest in reduced-tillage crop production in Iowa centers mainly on lessening soil erosion. In 1970, there were 29,218 ha. under chisel-plow systems, 85,909 ha. under till-plant systems, and 20,218 ha. under no-till systems. By 1980, there were 2,683,455 ha. under chisel-plow systems, 107,497 ha. under till-plant systems, and 45,197 ha. under no-till systems in Iowa (USDA, Soil Conservation Service, Des Moines, IA). Nonetheless, there are still concerns about possible yield reductions under reduced-tillage, and insects are one of those concerns. Reduced tillage could influence pest insect populations as well as effectiveness of their biotic mortality agents.

#### Effects of agronomic practices on pest Lepidoptera populations

Agronomic practices affect insect populations. *Heliothis zea* moths emerging from corn during late July and early August in North Carolina
preferred to oviposit in soybean fields with open canopies, especially those at peak flowering (Dietz et al., 1976). Funderburk et al. (1983) found greatest seedcorn maggot, *Delia platura*, adult emergence in soybeans from plots that had been fall chisel plowed. Mayse (1978) observed the highest GCW density in 24 cm-wide row soybean plots for most of the season in Illinois, but the density became greatest in plots planted to 96 cm-wide rows later in the season. Sprenkel et al. (1979) found that population densities of phytophagous Lepidoptera in North Carolina were higher in early-planted than in late-planted soybeans. Narrow rows and high seeding rates also positively affected insect population density. Of the cultural variables, planting date had the greatest and seeding rate the least effect upon pest population density.

Buschman et al. (1981) found higher GCW populations in Mississippi soybeans planted in June or July than in those planted in April or May. Also, GCW larvae were more abundant in late-maturing soybean cultivars. GCW populations were more dense in soybeans drilled into 18 cm-wide rows than in more conventional 76, 91, and 102 cm-wide rows. Sloderbeck (1981) found higher GCW egg densities in double-crop, no-till soybeans than in tilled, late-planted soybeans planted on the same date.

**Relation of agronomic practices to biotic mortality agents**

Even though pest population densities may be potentially greater under specific soybean cultural methods, natural mortality factors may affect the final infestation level. The contributions of predators, parasitoids, and pathogens have been addressed by several researchers.
Predators and parasitoids

Price (1976) observed that colonization by predators increased rapidly only after canopy closure in soybean fields. Mayse (1978) found that the numbers of various predators and parasitoids were significantly greater in soybeans planted in narrow (24-cm) rows. This greater number corresponded with higher GCW densities in narrow rows early in the season. Significantly, the time of canopy closure in narrow-row plots occurred six weeks before it occurred in plots planted in 96 cm-wide rows.

Sloderbeck (1981) observed that high GCW egg densities did not necessarily translate into high larval densities. Egg densities were higher in double-crop, no-till soybeans, perhaps because of ovipositional preference. But, larval populations were generally higher in late-planted, tilled soybeans. Greater larval mortality in double-crop, no-till plots probably caused the disparity. Rates of predator and parasitoid incidence showed no clear relationship to cropping system, but the fungal pathogen *N. rileyi* was more prevalent in two of the three years of the study in the double-crop, no-till soybeans.

Entomopathogens

Fungal insect pathogens and their expression in insect populations may also be affected by agronomic practices. For example, Lewis (L. Lewis, Insect Pathologist, USDA Laboratory, Ankeny, IA, personal communication, 1983) reported a greater incidence of *Beauveria bassiana* in European corn borer larvae in reduced tillage corn plots.

Many entomopathogens persist in the soil or upon plant debris on the soil surface. Soil disturbance by rain, wind, and cultivation may disperse the inoculum to crop leaf surfaces, thereby exposing host insects to
disease. Jaques (1978) recognized that shallow tillage is less disruptive to the layer of inoculum concentrated at or near the soil surface. Deep tillage would dilute or bury inoculum necessary for epizootic initiation. Kish and Allen (1978) used overhead irrigation as a rain simulator and found that an average of 90% of the conidia present on \( N. rileyi \) cadavers was washed off after two hours and 1.8 cm of precipitation. Cadavers not exposed to irrigation lost an average of 49% of their conidial load. Ignoffo et al. (1977b) studied the vertical movement of \( N. rileyi \) conidia through soil in undisturbed agroecosystems. There was minimal vertical movement and plentiful availability of inoculum in the top few cm of soil. Vertical movement was greatest in soils with little organic material.

Dietz et al. (1976) concluded that canopy closure produced favorable micro-climatic conditions for pathogenic fungus development. This was supported by Mayse (1978), who showed that the soil surface of soybean plots planted at high density remained moist longer after rain and retained higher levels of relative humidity than did soil in low density plots. Jaques (1978) concluded that high density planting of soybeans and the resultant earlier canopy cover also provided suitable temperature for development of \( N. rileyi \) and protection from solar radiation.

Burleigh (1975) recognized significantly more infection of \( Heliothis \) spp. larvae by \( N. rileyi \) in closed canopy cotton than in open canopy cotton. More \( N. rileyi \) mycoses of fall armyworms were found in corn fields near to or in rotation with soybeans than in isolated fields in Brazil (Habib and Patel, 1980).
Optimizing natural mortality of soybean Lepidoptera

Agroecosystems can be managed to maximize the effects of natural pest control agents. Sprenkel et al. (1979) suggested planting soybean early to optimize yields and maximize predation and N. rileyi incidence on key insect pests. If soybeans are planted late as a second crop following small grain, narrow rows and high seeding rate will help maximize the effects of N. rileyi and key predators.

Ignoffo (1981) recommended that an early planting of soybeans should be alternated with ca. 100 late-planted rows to induce an earlier appearance of an N. rileyi epizootic. A trap-crop planting to attract host insects and create an early focus on development for entomopathogens was proposed by Jaques (1978). A naturally-induced or man-augmented epizootic could spread from the trap plot to adjoining croplands.

An integrated approach to weed control, plant disease suppression, and pest insect management is necessary to insure that the life cycles of N. rileyi and other biotic mortality agents will not be disrupted. Consideration must be given to the choice of pesticide, the levels at which it is applied, and the timing of application to provide the least inhibitory effects upon natural enemies of insect pests.
PART I. GREEN CLOVERWORM POPULATIONS AND THE INCIDENCES OF BIOTIC AGENTS OF NATURAL MORTALITY IN FOUR SOYBEAN TILLAGE SYSTEMS - A COMPARISON
INTRODUCTION

The acceptance of reduced-tillage production systems to control soil erosion has been slowed by concerns about possible yield reductions. Among the concerns are the effects of reduced-tillage upon pest insect populations and upon the activities of their biotic mortality agents. Research will yield better understanding of the relationships between tillage and insect populations.

Several cultural practices affect Lepidoptera populations in soybeans. Higher green cloverworm (GCW), *Plathypena scabra* (F.), numbers occur in narrow row plantings (Buschman et al., 1981; Mayse, 1978; Sprenkel et al., 1979). Buschman et al. (1981) found GCW larvae more abundant in late-maturing soybean cultivars and in soybeans planted in mid-summer in Mississippi. Sprenkel et al. (1979) observed higher densities of phytophagous Lepidoptera in early-planted than in later-planted soybeans in North Carolina. Higher seeding rate also positively affected the population density. Sloderbeck (1981) found fewer GCW larvae in no-till soybeans planted as a double-crop after winter wheat than in tilled soybeans planted on the same date.

Biotic mortality factors acting as regulators of insect populations may be affected by soybean cultural techniques. Various predator and parasitoid populations were significantly more dense in narrow-row soybeans (Mayse, 1978). Soybean canopy closure provided favorable microclimatic conditions for predators (Price, 1976) and entomopathogenic fungi (Dietz et al., 1976; Jaques, 1978). Incidences of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson were greater in GCW populations in no-till
soybeans than in tilled soybeans planted on the same date in two of the three years of study in Kentucky (Sloderbeck, 1981). No differences in parasitoid and predator incidence between tillage practices were observed.

The following study aims to increase our understanding of the effects of reduced-tillage production systems upon pest insect populations. The objectives of the study were to correlate the total numbers of GCW larvae, important defoliators of soybeans in the Midwest, and the incidences of biological mortality agents of the insect in widely-used soybean tillage systems in Iowa.
METHODS AND PROCEDURES

The study was conducted in soybeans at the Northeast Iowa Research Farm near Nashua with four different tillage regimes following corn. The tillage plots were first established in 1977. The regimes investigated were: (1) fall moldboard plow, (2) fall chisel plow, (3) till-plant, and (4) no-till. A replication consisted of a 1.6-ha block within which each tillage system was used to plant a 0.4-ha plot. Soybeans were planted in 76-cm rows on 14 May (var. 'Vickery') and 8 June (var. 'Corsoy 79') in 1981 and 1982, respectively. Plots were arranged in a randomized complete block design and replicated three times.

The shake-sampling technique, previously found to be efficient for establishing GCW larval population estimates in soybeans (Hammond and Pedigo, 1976), was used in the study. A ground cloth was placed between two soybean rows and 30-cm sections of soybeans from each adjacent row were shaken over the cloth for ca. 25 seconds. GCW larvae and predators that fell onto the cloth were field-identified, counted, and the data were recorded.

During each season of the study, five shake samples were taken in each tillage plot on each sampling date. In 1981, collections were made on six dates: 14 and 23 July, 7, 18, and 25 August, and 1 September. In 1982, a total of nine sample dates were utilized. Seven weekly-collections were taken between 14 July and 25 August, with an additional shake sample on 7 September. The soybeans were too small for shake-sampling on 1 July, and only a visual check of the plants was made.

Each collected GCW larva was placed in a separate 7-dram plastic snap-top vial with a soybean leaflet. Each vial was labeled as to date, tillage
plot, and estimated stage of the larva. The vials were returned to the laboratory, maintained at room temperature, and the fates of the larvae were recorded. When GCW killed by N. rileyi were encountered, they were counted and left in the field. Predators shaken from the soybeans were field-identified and their numbers recorded in 1982 collections.

In 1982, GCW adult populations were sampled using a flushing technique (Pedigo et al., 1982). With this technique, one sampler walked between two soybean rows, and vigorously brushed the canopy on either side with two 92-cm aluminum rods. A recorder-observer followed behind by ca. five to six paces. Ten passes were made through each plot with ca. six rows separating each pass. The total area flush-sampled in each plot on each date was ca. 0.1 ha. As GCW moths flew up from the sampled rows, sightings were called out and the number was recorded. Sampling began on the downwind side of each plot so that flushed moths would fly into previously sampled areas and not be counted again.
RESULTS AND DISCUSSION

1981

A total of 1305 GCW larvae was collected from the Nashua soybean tillage plots in 1981. Figure 1 shows the distribution of numbers by tillage system through the season. The peak GCW larval populations occurred in mid- to late-August. The graphs suggest differences in the number of GCW larvae among tillage regimes. August 18 and 25 collections from fall moldboard plow (plow) and fall chisel plow (chisel) plots seem substantially larger than those from till-plant and no-till plots. However, according to the analysis of variance (ANOVA), only on 18 August did a statistically significant difference occur. On 18 August, fewer GCW larvae were collected from no-till plots than from plow and chisel plots (PR>F=0.037).

ANOVA for the total numbers of GCW larvae collected over the entire season revealed significant differences. There were significantly fewer GCW larvae collected in the till-plant and no-till plots as compared to the plow and chisel plots (PR>F<0.5).

The differences in numbers of GCW larvae collected among tillage systems were not explained by analysis of biotic mortality factors. Of the nine different biotic mortality agents detected in GCW larvae in 1981 (Table 1), *N. rileyi* caused the greatest overall proportion of deaths (71.3%). The disease first appeared in 23 July no-till collections, and it increased in frequency in all tillage plots for the remainder of the season (Figure 2). On 1 September, the disease caused mortality in more than 90% of the larvae in each plot. ANOVA showed no significant differences (P>0.05) in disease incidences among tillage regimes.
Figure 1. Mean number of GCW larvae collected from each soybean tillage system. Nashua, Iowa, 1981
Table 1. Fates of GCW larvae collected from Nashua tillage plots, 1981

<table>
<thead>
<tr>
<th>Fate</th>
<th>Tillage systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall moldboard plow</td>
</tr>
<tr>
<td>Diptera:Tachinidae</td>
<td></td>
</tr>
<tr>
<td>Winthemia sinuata Reinhard</td>
<td>1</td>
</tr>
<tr>
<td>Oswaldia assimilis (Townsend)</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
</tr>
<tr>
<td>Hymenoptera:Braconidae</td>
<td></td>
</tr>
<tr>
<td>Rogas nolophanae Ashmead</td>
<td>20</td>
</tr>
<tr>
<td>Cotesia marginiventris (Cresson)</td>
<td>7</td>
</tr>
<tr>
<td>Meteorus Sp.</td>
<td>1</td>
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<tr>
<td>Apanteles Sp.</td>
<td>0</td>
</tr>
<tr>
<td>Pathogens:</td>
<td></td>
</tr>
<tr>
<td>Nomuraea rileyi (Farlow) Samson</td>
<td>245</td>
</tr>
<tr>
<td>Unknown deaths(^a)</td>
<td>65</td>
</tr>
<tr>
<td>Pupated</td>
<td>21</td>
</tr>
<tr>
<td>Totals</td>
<td>367</td>
</tr>
</tbody>
</table>

\(^a\)Dead larvae and prepupaes.
Figure 2. Mortality of GCW larvae caused by Nomuraea rileyi. Nashua, Iowa, 1981
In each tillage system, the population peak of living larvae preceded by one sample date (ca. one week) the peak number of *N. rileyi* cadavers shaken from the soybean canopy. Peak number of cadavers corresponded with the collapse of the larval generation. Even though the economic injury level for GCW larvae was never reached in 1981, the peak number of larvae in the tillage plots ranged from ca. 5 to 8/60 cm-row. These numbers were great enough to trigger a late-season epizootic of *N. rileyi*.

The parasitoid most frequently reared from GCW larvae was *Rogas nolophanae* Ashmead, and it accounted for total seasonal mortality in 6.2% of the larvae collected. Figure 3 shows that this parasitoid was more frequently reared from the early period collections and accounted for less mortality in the later collections. ANOVA showed no significant differences (*P > 0.05*) in numbers of *R. nolophanae* among the four tillage systems over the entire season or by date.

All other parasitoids together caused mortality in only 3.1% of the GCW larvae. These other parasitoids included at least four species of Hymenoptera and two species of Diptera. The hyperparasitoid *Mesochorus* sp. emerged from four *R. nolophanae* mummies. A large percentage (18.1%) of larvae died from unknown causes during the maintenance period in the laboratory.

1982

A total of 491 GCW larvae was collected from the Nashua tillage plots in 1982. Figure 4 shows the distribution of numbers by tillage system through the season. Peak GCW larval populations occurred in late-July to early-August, but they never reached more than 2.4 larvae/60 cm-row. The
Figure 3. Mortality of GCW larvae caused by *Rogas nolophanae*. Nashua, Iowa, 1981
Figure 4. Mean number of GCW larvae collected from each soybean tillage system. Nashua, Iowa, 1982.
graph lines run close together until 28 July, after which time collections from plow plots were larger than the others until 25 August. Although the numbers of GCW larvae among tillage treatments on 5 August were not significantly different ($P = 0.093$), a comparison between the larval numbers in plow plots versus the other plots combined was significant ($P = 0.0379$). Duncan's multiple-range test for 5 August also detected significantly ($P < 0.05$) greater numbers in plow plots than in till-plant plots with the other tillage systems intermediate in numbers. No significant differences in larval numbers were detected among tillage systems on 12, 18, or 25 August.

ANOVA for the total number of GCW larvae over the entire season failed to detect significant differences among tillage systems ($P > 0.05$). Nevertheless, substantially more larvae were collected from plow plots over the season. The trend in total numbers was 150, 115, 108, and 118 collected in plow, chisel, till-plant, and no-till systems, respectively.

GCW moth flushing data indicated peak flights in late July and again in mid-August (Figure 5). The first peak probably reflected the activity of immigrant moths, initially colonizing and ovipositing in the soybean plots. The second peak was a locally-produced flight from surrounding soybeans and other nearby habitats. The total numbers of moths over the whole season were 126, 125, 112, and 134 for plow, chisel, till-plant, and no-till systems, respectively. ANOVA showed no significant differences among systems over the whole season.

ANOVA by date found significant tillage differences in moth numbers. Plow plots had more ($P = 0.0278$) moths on 14 July than other tillage
Figure 5. Total number of GCW moths flushed from soybean tillage systems. Nashua, Iowa, 1982
systems. On 21 July, significantly (P = 0.0122) more moths were flushed from no-till plots than from the other tillage systems. Also, on 28 July, significantly fewer moths were sampled from till-plant plots than from chisel plots. From these data, it seems that the first flight showed few preferences among tillage systems. From 14 July to 5 August, substantially greater numbers of moths were flushed from no-till plots than from the other systems, but only on 21 July was there statistical significance. No significant differences in moth numbers were found during the second flight.

Biotic mortality factors did not account for the different GCW larval numbers among tillage systems. Of the five biotic mortality factors identified from 1982 collections (Table 2), N. rileyi caused the greatest overall number of deaths (15.8%). The disease was first detected in 28 July collections from plow and till-plant plots (Figure 6). It increased in frequency in all tillage systems throughout the remainder of the season. On 7 September, 100% of the collected larvae from plow and till-plant systems succumbed to the mycosis. ANOVA was unable to detect any tillage differences in N. rileyi incidences over the entire season. ANOVA by date revealed only one significant difference. On 25 August, till-plant plots had fewer (P = 0.0472) N. rileyi deaths than the other tillage systems. In contrast, Sloderbeck (1981) found differences in N. rileyi incidences in his tillage studies. The pathogen was more prevalent in double-crop, no-till soybeans than in tilled soybeans in two of the three years of his study. Roberts et al. (1977) felt that fall or spring plowing of soybean fields inhibited residual pathogens.
Table 2. Fates of GCW larvae collected from Nashua tillage plots, 1982

<table>
<thead>
<tr>
<th>Fate</th>
<th>Tillage systems</th>
<th>Fall moldboard plow</th>
<th>Fall chisel plow</th>
<th>Till-plant</th>
<th>No-till</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diptera: Tachinidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Winthemia sinuata</em> Reinhard</td>
<td></td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><em>Oswaldia assimilis</em> (Townsend)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hymenoptera: Braconidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rogas nolophanae</em> Ashmead</td>
<td></td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td><em>Cotesia marginiventris</em> (Cresson)</td>
<td></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Pathogens:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nomuraea rileyi</em> (Farlow) Samson</td>
<td></td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>69</td>
</tr>
<tr>
<td>Unknown deaths(^a)</td>
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<td>95</td>
<td>68</td>
<td>63</td>
<td>73</td>
<td>299</td>
</tr>
<tr>
<td>Pupated</td>
<td></td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>54</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>150</td>
<td>115</td>
<td>108</td>
<td>118</td>
<td>491</td>
</tr>
</tbody>
</table>

\(^a\)Dead larvae and prepupae.
Figure 6. Mortality of GCW larvae caused by *Nomuraea rileyi*. Nashua, Iowa, 1982
In 1982, peak numbers of living GCW larvae occurred in late-July to early-August, but never exceeded 2.4 larvae/60 cm-row, which was two to three times smaller than larval peaks in 1981. In neither season did the GCW population reach the economic injury level (DeWitt et al., 1980) in the soybean plots. As the GCW larval population collapsed, increasing numbers of *N. rileyi* cadavers were shaken from the soybean canopy. The peak numbers of cadavers did not occur until the last sample date, ca. four weeks after the peak occurrence of living larvae. *Nomuraea rileyi* disease epizootics developed in both 1981 and 1982, but more slowly in the latter year. Fewer GCW larvae may have been one reason.

Only four parasitoid species were reared from 1982 GCW collections. Their combined total mortality was 14.1% of the larvae collected, equal to the mortality effect of *N. rileyi*. No tillage-related differences were detected for occurrence of any of the parasitoids. The most commonly reared parasitoid was *R. nolophanae*, and it accounted for 10.2% of all GCW larvae collected. The peak of *R. nolophanae* incidence occurred in early- to mid-August in 1982, except in no-till plots (Figure 7). ANOVA detected no significant differences among tillage regimes over the entire season or by dates. Other parasitoids were collected in smaller numbers, and no tillage-related differences were noted.

In both years, there was evidence of antagonism between *R. nolophanae* parasitism and *N. rileyi* disease. As the late-season fungal disease epizootic developed, incidences of *R. nolophanae* in the GCW population declined. Barry (1970) found that *N. rileyi* in GCW populations caused a scarcity of suitable hosts for parasitoid development. Burleigh (1975)
Figure 7. Parasitization of GCW larvae by *Rogas nolophanae*. Nashua, Iowa, 1982
and Sloderbeck (1981) found similar incidences of interference between *N. rileyi* and parasitoids of *Heliothis* spp. and GCW larvae.

Sloderbeck (1981) found tillage differences in his collections of two parasitoids, *Protomicroplitis facetosa* (Weed) and *C. marginiventris*. The parasitoids were more prevalent in double-crop, no-till plots in one of the three years of his study. In that single year, the combined mortality caused by parasitoids and pathogens was also significantly greater in the double-crop soybeans than in the tilled early or late soybeans.

A large percentage of larvae died of unidentified causes in 1982; more so than in 1981. Dead larvae were flaccid and black in rearing vials, and later became black and shriveled. Because of their appearance, bacterial septicemia was suspected. Perhaps bacteria gained access to the hemocoel through points of mechanical injury to the cuticle. Isolations from representative dead larvae were plated on nutrient agar, but no known insect pathogens were observed. Also, bioassays of isolates against cabbage looper, *Trichoplusia ni* (Hübner) larvae failed to show pathogenicity.

Some facultative bacterial pathogens may grow readily in artificial media and may flourish in insect gut and invade the hemocoel when insects are under environmental stress (Bucher, 1963; Faust, 1974). Mortality is characterized by flaccid, blackened larvae (Zacharuk, 1973). Also, bacterial septicemia has been noted in larvae previously inoculated with the entomogenous fungi *Metarhizium anisopliae* (Zacharuk, 1973) and *Beauveria bassiana* (Fargues and Vey, 1974). In these cases, normal ecdysis was prevented by penetrant hyphae that bind the exuviae to the new larval cuticle. The wounds also allowed access of bacteria to hemolymph, and these, in turn, caused massive septicemia and death.
The number of predator arthropods shaken from the soybean canopy was analyzed to determine possible tillage preferences. The three major predator groups collected were *Nabis* spp., spiders, and *Orius insidiosus* (Say). *Nabis* spp. nymphs and adults were the most frequent group shaken from soybeans in all tillage systems. Two peaks of *Nabis* spp. abundance occurred, one in late July and the second in late August to early September (Figure 8). ANOVA showed no significant differences among tillage systems in the total number of *Nabis* spp. over the entire collection period. On 14 July, *Nabis* spp. were most numerous in chisel plots, and this was the only significant difference by date detected by ANOVA. Sloderbeck (1981) found smaller populations of *Nabis* spp. nymphs in late and double-crop, no-till soybeans in the last year of his study.

Spiders were the second most frequently collected group of predators from each soybean tillage system. Spiders increased in numbers dramatically during late August and early September (Figure 9). No significant differences in season totals were found among the four tillage regimes. Only on 7 September were there significant differences (*P*=0.0197) among tillage systems, when significantly more spiders were collected from no-till than from the other systems.

There were more *O. insidiosus* collected from plow plots than the other plots over the entire season, but ANOVA comparisons did not show any significant differences (*P*=0.0600). Peaks of *O. insidiosus* abundance in plow plots occurred on August 12 and 25, although ANOVA failed to detect significant differences among tillage systems on those dates (Figure 10). A significant difference (*P*=0.0240) was found in *O. insidiosus* numbers on 14 July, but this was probably due to small sample size.
Figure 8. Number of *Nabis* spp. nymphs and adults in each soybean tillage system. Nashua, Iowa, 1982
Figure 9. Numbers of spiders in each soybean tillage system. Nashua, Iowa, 1982
Figure 10. Number of *Orius insidiosus* in each soybean tillage system. Nashua, Iowa, 1982
Although some soybean producers have been concerned that adoption of soil-conserving tillage practices may aggravate pest insect problems, there is no evidence from this study to support that view. In fact, during the first year of the study, significantly fewer GCW larvae were found in till-plant and no-till plots. In 1982, substantially fewer GCW larvae were collected from reduced-tillage plots, but the numbers were not significantly lower.

Analyses of incidences of natural biotic mortality agents in GCW populations in the four tillage systems could not account for significant tillage differences in GCW numbers. Moth flushing and predator data also did not explain tillage differences. Even though the causes of differences in GCW larval numbers were not revealed in this study, the observations are important. Because GCW numbers were not greater in reduced-tillage plots, soybean producers may be encouraged to implement soil-conserving tillage practices. Better soil stewardship will thereby conserve this rich natural resource for future generations.
PART II. SOYBEAN LEAF CONSUMPTION

BY *Nomuraea rileyi*-INFECTED

GREEN CLOVERWORM LARVAE
INTRODUCTION


Pedigo et al. (1983) developed partial life tables of GCW populations in Iowa encompassing two endemic- and two outbreak-configuration years. The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson, had characteristics of a key mortality factor in the studied GCW populations. Although the disease is less important in GCW-endemic years, *N. rileyi* incidence correlates with the collapse of generation two in outbreak populations. Unfortunately, the disease epizootics do not occur early enough to prevent economic damage to full-bloom (R2) soybeans.

Histopathological studies of several noctuids by Kish and Allen (1978), Mohamed et al. (1978b), and Boucias and Pendland (1982) revealed that *N. rileyi* infection of host tissues causes larvae to become sluggish, to cease feeding, and to die. The mycelia within cadavers propagate the pathogen by producing conidiophores that, in turn, produce conidia. The disease becomes epizootic when a large proportion of a larval population becomes infected.

Few studies have compared consumption patterns of healthy and *N. rileyi*-infected larvae. Mohamed (1982) reported that infected third-instar *Heliothis virescens* (F.) consumed less cotton square tissue than healthy larvae did after five to six days. The feeding reduction was noticeable but not significant three to four days post-inoculation. With
third instar *Heliothis zea* (Boddie), Mohamed et al. (1982) found significant reduction in consumption after two days. *Heliothis virescens* and *H. zea* larval mortality due to *N. rileyi* began ca. six days post-inoculation.

This study sought to investigate the impact of *N. rileyi* upon leaf consumption by GCW larvae feeding on soybean leaves. The objectives were to compare the consumption patterns and rates of *N. rileyi*-inoculated GCW larvae with those of healthy control larvae.
MATERIALS AND METHODS

Green cloverworm larvae for the study were raised from eggs obtained from field-collected moths. Moths were placed in a 30 x 30 x 30-cm screened cage where females laid eggs nightly on roughened yellow blotter paper taped to the inside of the cage's glass top. The blotter paper was replaced daily, and the egg-bearing pieces were cut into convenient strips to be placed inside 0.5-ℓ ice cream cartons.

Field-collected soybean leaflets were used as a larval food source. Leaflets were surface sterilized in 0.5% NaClO+5H2O plus Tween-80® for ca. three to five minutes and rinsed several times in tap water. Leaflets were placed in Aquapics® of water and inserted through the bottom of the cartons. Blotter papers with eggs were placed alongside the leaflets, a lid added, and the carton was placed in an 18°C growth chamber for egg incubation and eclosion. As neonates emerged and subsequent instars developed, they were utilized in the consumption experiments.

The experimental procedure called for placing larvae individually in 7-dram, plastic, snap-top vials with a small piece of soybean leaflet as a food source. The surface area of each leaflet piece was premeasured with a Li-Cor® model 3000 leaf area meter before use.

At the beginning of each experiment, the head capsule widths of larvae were measured with an ocular micrometer to determine the initial larval instar (Pedigo et al., 1973). One group of larvae was inoculated with N. rileyi conidia by means of a camel's hair brush stroked on their integuments. Control larvae were brushed with a clean brush. Each larva was placed in a labeled vial with a piece of premeasured leaflet. Vials were
maintained in a 27°C growth chamber set for 15-hour photoperiod and 80 to 90% relative humidity.

At ca. 48-hour intervals, each leaflet piece was remeasured to determine the area of leaf tissue consumption during the intervening time period. Fresh, premeasured leaflet pieces replaced the partially consumed pieces as needed. Also, the condition and developmental stage (as determined by head capsule widths) of each larva were recorded. Larvae were maintained until their death or emergence as adults. Head capsule measurements of dead larvae and the sex of emerged moths were recorded.

During the experiment, a total of 542 GCW larvae were reared and separated into several replications based on larval instar at treatment. The numbers of larvae used at instars 1, 2, 3, 4, 5, and 6 were 150, 116, 92, 65, 60, and 59, respectively. Larvae in each replication were apportioned equally between fungus-inoculated and control groups. Only data from control larvae that reached pupation and from inoculated larvae that died of N. rileyi infection were used in the statistical analysis of the consumption experiments.
RESULTS AND DISCUSSION

Consumption Through Time

Analysis of data compared the consumption patterns of inoculated and control larvae. Larvae treated during the same larval instar were compared with others in that group. For example, larvae treated as first instars were either inoculated with *N. rileyi* or were uninoculated. The consumption patterns of all the first instars were recorded as they progressed through time and through their remaining stages.

Measurements of consumption rates (no. cm²/day) reflected the larval responses to treatments. As expected, consumption rates increased as larvae grew larger. Statistical tests compared the consumption patterns of *N. rileyi*-inoculated and control larvae.

Within each age group, analysis of covariance detected no significant differences (*P > 0.05*) in consumption rate or in the increase in consumption rate, at least up to 144 hours post-treatment. After 144 hours, larvae inoculated as first, second, third, or fourth instars showed significant differences from their controls. The responses of treated third instars were illustrative (Figure 1). The consumption patterns of inoculated and control larvae were similar until 144 hours post-treatment. Then, inoculated larvae began dying and control larvae continued to feed until pupation.

In similar experiments, Mohamed (1982) and Mohamed et al. (1982) found differences in cotton square consumption between *N. rileyi*-infected *Heliothis* spp. larvae and healthy larvae. Also, infected *N. zea* larvae fed artificial diet consumed less after 120 hours post-inoculation than did
Figure 1. Mean consumption rate (cm$^2$/day) of GCW larvae treated in stage 3
control larvae. Mohamed et al. (1982) suggested that the reduction in infected H. zea consumption was caused by physiological disturbance resulting from the stress of N. rileyi invasion and colonization that generally occurred between the second and fourth day post-inoculation.

Consumption by Subsequent Larval Stages

Whereas the preceding analyses considered consumption through time, other analyses evaluated consumption by larvae as they progressed through the remaining instars. This was done to eliminate time effects that may have been erroneously attributed to treatment effects. A 2-way analysis of variance (treatment and instar as factors) detected significant differences (P<0.05) in consumption rates for only one group of larvae, those treated as third instars. Third instars inoculated with N. rileyi consumed significantly less soybean leaf tissue as they progressed through the remaining growth stages than did control larvae. As Figure 2 illustrates, the significance was probably mostly attributable to differences in consumption by the sixth instars. During this stadium, healthy larvae consumed large quantities of material, but 87% of the inoculated larvae died, and statistical differences in consumption resulted.

Larvae treated as second instars had significant instar-by-treatment interactions, which indicated that larval stage and treatment effects were not independent of each other. In this case, significant treatment effects were conditional; i.e., treatment effects were dependent upon the instar under consideration. A plot of these data (Figure 3) showed that lines describing, respectively, consumption by inoculated larvae and consumption by control larvae intersected. Analysis of consumption rates for treated
Figure 2. Log mean daily consumption rate, DCR(cm²/day), for GCW larvae treated in stage 3
Figure 3. Log mean daily consumption rate, DCR$^{(cm^2/day)}$ for GCW larvae treated in stage 2
first instars as they progressed through the remaining stages was inconclusive, but differences were probably not significant. Analysis of fourth instar consumption was also inconclusive. The consumption rates of larvae treated during the fifth and sixth instars could not be analyzed because of lack of head capsule measurements for control larvae.

Mean Total Consumption by Treated Larvae

Figure 4 compares the mean total consumption by control and *N. rileyi*-infected larvae of each age-group. The best measurement of total consumption by healthy GCW larvae is 40.0 cm², which represents the mean total consumption by control larvae treated as first instars. This measurement is comparable to one by Hammond et al. (1979). Under similar conditions, they found that GCW larvae consumed ca. 47 cm² of soybean leaf tissue before pupation.

Stone and Pedigo (1972) and Hammond et al. (1979) measured mean soybean leaf consumption by each GCW instar. They concluded that ca. 75% of total leaf consumption is accomplished by the sixth (and occasional seventh) instar. The total consumption by healthy sixth instars in Figure 4 compared to that by first instars agrees with their estimate. Control larvae used as later instars had fewer subsequent stages to complete than did larvae used earlier. An inverse relationship between instar-at-treatment and total consumption by control larvae generally describes the trend illustrated in Figure 4.

GCW larvae inoculated with *N. rileyi* consumed soybean leaf tissue for ca. six days before they died. Figure 4 illustrates a direct relationship in this group between instar-at-treatment and total consumption before
Figure 4. Mean total consumption by control (chk) and *N. rileyi*-infected (N.r.) larvae treated in stages 1 to 6.
death. In fact, larvae inoculated as sixth instars consumed nearly as much soybean tissue before death as did healthy sixth instars before pupation.

*Nomuraea rileyi* has been considered for inundative use as a microbial pathogen in crop protection. Treating an insect population consisting of small larvae would prevent extensive crop damage due to larval feeding. Applications directed at mid- and late-stage larvae would not reduce plant damage as greatly. Nonetheless, the pathogen would be propagated, would infect subsequent larval generations, and would reduce the overwintering insect population in some species.

In addition, *N. rileyi*-caused cadavers would contribute to the overwintering inoculum reservoir. Sprenkel and Brooks (1975) showed the importance of sclerotized cadavers in maintaining *N. rileyi* between seasons in North Carolina. Bechinski and Pedigo (1983) observed that the fungus in GCW pupal cadavers was well-protected from environmental stresses. In Missouri, less than one *N. rileyi*-killed larva per m$^2$ of soil surface in an autumn field was enough to cause mortality the next spring in larvae feeding on soybean seedlings contaminated by soil-borne conidia (Ignoffo et al., 1977b).

**Treatment Effects on Stadial Length**

Treatment effects on stadial length were analyzed by 2-way analysis of variance (ANOVA). First or second instars inoculated with *N. rileyi* had significantly ($P<0.05$) longer subsequent stadia than did control larvae. Respective plots of the data (Figure 5) for first instar inoculated and control larvae are very similar in shape, but the graph of inoculated larvae is higher, indicating more time for each stadium. Larvae treated as
Figure 5. Mean stadal length of GCW larvae treated in stage 1
third instars showed no significant treatment differences in stadial length. Data for larvae treated as fourth instars were inconclusive. Lack of head capsule measurements of surviving control larvae did not allow statistical analysis of larvae treated as fifth and sixth instars.

Overall, infected larvae had interim consumption rates and increases in consumption rates over time that were similar to those of healthy larvae. Significant differences in the consumption patterns occurred only as infected larvae became moribund and died, ca. six days post-inoculation. Even though the following stadia of larvae inoculated as first or second instars were lengthened, i.e., subsequent larval development was slowed by disease, consumption patterns were not directly changed.

Survivorship of *N. rileyi*-Inoculated Larvae

Survivorship of larvae (all instars) ranged from three to 15 days post-inoculation (Figure 6), although greatest mortality occurred between five and 10 days. The ranges of time to mortality compared favorably with observations of other Lepidoptera larvae. Getzin (1961) found that most *N. rileyi*-caused deaths of *Trichoplusia ni* (Hübner) larvae occurred six to seven days post-inoculation. Fifty percent of *Pseudoplusia includens* (Walker) larvae died by the sixth day post-inoculation, and all died within 10 days (Gudauskas and Canerday, 1966). Kish and Allen (1978) determined that most *Anticarsia gemmatalis* (Hübner) larvae died within seven to 10 days. Mohamed et al. (1977) and Mohamed (1982) found that the time to mortality from *N. rileyi* infection ranged from six to 12 days for *H. zea*, and six to 10 days for *H. virescens*. Mohamed et al. (1978a) found that
Figure 6. Percent survivorship curves for GCW larvae inoculated with *N. rileyi* at instars 1 to 6.
mortality in treated first instar *H. zea* occurred nine to 15 days post-inoculation, whereas in subsequent instars the range was six to 12 days.

The survivorship curves of all inoculated GCW age groups were similar. The median number of survival days for inoculated instars one through six were 5.9, 7.6, 7.2, 5.6, 6.7, and 6.1 days, respectively. The average median survivorship was 6.5 days. These observations compare well with data from other researchers. Getzin (1961) found that the LT$_{50}$ of inoculated *T. ni* neonates was 6.2 days. Behnke and Paschke (1966) allowed third and fourth instar *T. ni* larvae to crawl on culture plates of sporulating *N. rileyi*. Eighty percent of the larvae died within 10 days, and the median lethal time was between seven and eight days. Similarly, Gudauskas and Canerday (1966) reported that the LT$_{50}$ for *P. includens* larvae was six days using unquantified *N. rileyi* dosages. The LT$_{50}$ for infected fifth instar *Spodoptera frugiperda* (J. E. Smith) larvae was nine days (Habib and Patel, 1980). Boucias et al. (1982) determined that the LT$_{50}$s for six noctuid species exposed to 7500 *N. rileyi* conidia/mm$^2$ of diet surface ranged from 5.2 to 7.2 days.

**Instar Susceptibility to *N. rileyi***

The mean survivorship time by instar ranged from 6.2 days to 8.4 days post-inoculation (Table 1). The variance by age group ranged from 0.31 to 6.13. Bartlett's test for homogeneity of variances (Snedecor and Cochran, 1967) found significant differences, i.e., heterogeneity. Differences in GCW instar susceptibility to *N. rileyi* have not been previously reported, although researchers (Getzin, 1961; Mohamed et al., 1977; Kish and Allen, 1978) have observed instar susceptibility differences in other noctuid
<table>
<thead>
<tr>
<th>Instar at inoculation</th>
<th>Number ( N. \text{rileyi}-\text{killed larvae} )</th>
<th>Survivorship</th>
<th>Mean no. days</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>7.0</td>
<td>4.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>8.4</td>
<td>6.13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>8.0</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>6.2</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>7.2</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>6.6</td>
<td>2.81</td>
<td></td>
</tr>
</tbody>
</table>
larvae. Also, the heterogeneity of variances may have been caused by small sample sizes, especially in trials involving large instars. For these reasons, the variances were considered homogeneous for purposes of comparing instar susceptibilities.

T-tests between mean survivorship times of instar groups were compared to Bonferroni's "t" statistic (Bailey, 1977). For 15 simultaneous comparisons, the Bonferroni critical value (P = 0.05) was 2.9951. One significant difference in the ranking of age group survivorship means was detected by comparisons with the critical value. Instar group two inoculants survived significantly longer than did instar group four inoculants (Figure 7).

If the experimental variances were, in fact, heterogeneous, a conservative t' statistic (Snedecor and Cochran, 1967) for independent samples with unequal variances and unequal sample numbers may be calculated. Incorporated into calculations of the t' statistics are Bonferroni's "t" values (Bailey, 1977) for 15 simultaneous comparisons. When t' statistics were compared, significant differences in the ranking of age group mean survivorship times were found (Figure 7).

Both tests indicated significant differences between the mean survivorship times of age group four and age group two inoculants. The t' ranking also detected a significant difference between age group four and age group three inoculants. However, because unquantified dosages of *N. rileyi* conidia were used, it would be unwise to conclude that fourth instar larvae are more susceptible to *N. rileyi* than are second or third instar larvae. *Nomuraea rileyi* mortality was dosage dependent and not instar dependent for instars two to five in *H. virescens* (Mohamed, 1982).
Mohamed et al. (1977) suggested that conidia on rapidly growing *H. zea* larvae may be lost with the exuviae before fungal penetration of the integument takes place. Bell and Hamalle (1980) found that *H. zea* larvae have a "second chance" to become infected with *N. rileyi* conidia, because newly molted larvae quickly consume their exuviae, usually leaving only the head capsules intact. Pedigo et al. (1973) found that GCW larvae also consume their exuviae after molting. "Reinoculation" of larvae, however, would probably not account for the differences in survivorship among GCW age groups in this experiment.

It seems that, until their deaths from *N. rileyi* infection, inoculated GCW larvae had consumption patterns that were similar to those of healthy larvae. There is little evidence of significant differences in instar susceptibility to *N. rileyi*. Therefore, population models may treat *N. rileyi*-infected GCW larvae as healthy consumers until their death. Greater attention should be focused on *N. rileyi* inoculum load in the field, on microclimatic factors influencing *N. rileyi* ontogeny, and on population characteristics of the insect host.

<table>
<thead>
<tr>
<th>Instar group</th>
<th>4</th>
<th>6</th>
<th>1</th>
<th>5</th>
<th>3</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean no. days survivorship</td>
<td>6.2</td>
<td>6.6</td>
<td>7.0</td>
<td>7.2</td>
<td>8.0</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Bonferroni's "t" ranking

Snedecor and Cochran's "t" ranking

Figure 7. Comparison of mean survivorship times of inoculated instar groups
PART III. THE OVERWINTERING POTENTIAL OF

N. RILEYI IN CENTRAL IOWA
INTRODUCTION

The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson, has worldwide distribution (Onions, 1979) and frequently causes epizootics in Lepidoptera larval populations. Early reports from the United States include Watson's (1916) description of "cholera" that decimated *Anticarsia gemmatalis* Hübner larval populations in Florida. *Nomuraea rileyi* epizootics in insect populations have been frequently reported in several states (Hill, 1925; Allen et al., 1971; Pedigo et al., 1973; Carner et al., 1975; Ignoffo et al., 1975b; Dietz et al., 1976, Roberts et al., 1977). *Nomuraea rileyi* has characteristics of a key mortality factor in green cloverworm (GCW), *Plathypena scabra* (F.), populations in Iowa (Pedigo et al., 1983). Epizootics occur late in the growing season, often after the insect pest population has seriously damaged plants (Kish et al., 1976).

Reservoirs of *N. rileyi* inoculum are able to remain viable between growing seasons. The fungus is able to maintain itself at low densities in host populations during the mild winters in southern Florida and the Gulf Coast. Then, adult moths, migrating northward in the spring, carry and disperse conidia (Kish, 1975; Allen and Kish, 1978).

In more northerly climates, the fungus is able to withstand the environmental stresses of winter. Conidia from previous seasons remain viable in the soil and are able to initiate epizootics the following season in Missouri (Ignoffo et al., 1978). Infective conidia have been detected on early-season perennial legumes and on young soybean plants in Missouri (Ignoffo et al., 1975b).
Conidia in vials and on cadavers held at the soil surface in North Carolina were infectious for 138 days and 281 days, respectively (Sprenkel and Brooks, 1977). Conidia from cadavers buried 10 cm in the soil were viable for up to 194 days and the colonized fungus still sporulated through the last sampling date of 281 days. Although infectious conidia are able to survive North Carolina winters, stroma, on the soil surface or buried in the soil, are a more likely overwintering form (Sprenkel and Brooks, 1977).

Pseudosclerotia formed during the host prepupal and pupal stages offer considerable overwintering protection (Sutton et al., 1979). Bechinski and Pedigo (1983) found viable N. rileyi conidia inside GCW pupae stored in the laboratory for two years. Fungal-infected pupae may protect the fungus from ultraviolet light, desiccation, and other environmental stresses.

Three environmentally-resistant N. rileyi structures have been identified in dry cadavers of Anticarsia gemmatalis (Hübner) larvae (Pendland, 1982). Thick-walled intrahyphal hyphae, chlamydospores, and intralarval resting bodies form when conidiogenesis is inhibited by unfavorable environmental conditions. Cadavers stored at room temperatures for 12 months regenerated hyphae and produced conidia when rehydrated. Resistant structures within cadavers lie dormant in soil or plant debris, resume metabolic activity when conditions again become favorable, and produce conidiophores and conidia (Pendland, 1982).

This study of the overwintering potential of N. rileyi was initiated because the fungus is an important natural biotic mortality factor of GCW in Iowa soybeans. The objective of the study was to measure fungal
viability in GCW cadavers and as conidia after exposure to an Iowa winter.
METHODS AND PROCEDURES

During late August, 1981, *N. rileyi*-killed GCW cadavers were field-collected from senescent soybeans. White, nonsporulating cadavers were collected and stored separately from green, sporulating cadavers. Small squares of aluminum-mesh screening were each folded in half and one cadaver was enclosed in each. Edges were stapled together to form an envelope ca. 2 x 5 cm. Enveloped cadavers were tightly tethered to the middle of 20-cm long wooden garden stakes (one envelope per stake) with thin wire ca. 4 to 6 cm long. At a soybean field ca. 5 km south of Ames, 80 random locations within harvested rows were selected on 13 November 1981. A shallow hole was dug ca. 5 to 8 cm deep in the soil at each location. A single enveloped cadaver was dropped into the hole and a wooden stake (with another attached envelope) was placed nearby. The hole was filled and the envelope attached to the stake was laid on the soil surface. Therefore, at each of the 80 locations, one cadaver was exposed to surface soil conditions, and one cadaver was exposed to subsurface soil conditions. Additionally, 80 envelope-enclosed cadavers were stored together in a screw-top glass jar at 2.8°C in the laboratory.

On each of 10 retrieval dates, 16 envelopes (eight from the soil surface and eight from the soil subsurface) were collected from random locations and returned to the laboratory. Envelopes were vigorously washed in tap water to remove adherent soil and then opened to recover the enclosed cadavers. Cadavers were individually placed in separate 7-dr plastic snap-top vials, each with a small strip of distilled-water-moistened paper. Labels on each vial described the field exposure location. Similarly,
eight refrigerated cadavers were washed in water and placed in labeled vials. The 24 vials were maintained at room temperature in a plastic box with moistened paper toweling. The cadavers were inspected daily for evidence of conidiogenesis during ca. two weeks. The presence of conidio­phores and conidia confirmed the viability of the sclerotized fungus.

Conidial pathogenicity was tested by bioassay against third stage cabbage looper, *Trichoplusia ni* (Hübner), larvae. Cadavers from the same exposure location were pooled together in vials and 15 *T. ni* larvae were brushed into each vial. Vials were gently rotated to facilitate larval inoculation. Inoculated larvae were placed in separate plastic cups containing artificial diet. Black cutworm diet (Reese et al., 1972) modified by Cossentine (1982) was used. Also, 15 uninoculated check larvae were maintained in diet cups. The numbers of *N. rileyi*-caused larval deaths and of emerged moths were recorded.

In a second experiment, 240 field-collected, sporulating GCW cadavers were each placed in separate 0.25-dr glass shell vials plugged with cotton dental pack. Two vials were tightly attached ca. 6 to 8 cm apart to each 20-cm wooden garden stake with wire and a few drops of white glue. On 3 December 1981, one stake was placed at each of 80 random locations within harvested soybean rows in a field ca. 5 km south of Ames. At each location, the stake was pushed into the ground so that the upper vial was positioned at the soil surface and the lower vial was 6 to 8 cm beneath the soil surface. Additional vials were stored at 2.8°C in the laboratory.

On each of 8 retrieval dates, eight stakes were removed from random locations and, also, eight vials stored at 2.8°C were brought into the
laboratory. Ten third instar *T. ni* were brushed into each vial and the vials were gently rotated to ensure inoculation of the larvae. Each larva was maintained in a separate diet cup of artificial diet (Reese et al., 1972; Cossentine, 1982). Eighty uninoculated check larvae were also maintained on diet. A total of 320 larvae was processed on each collection date, i.e., 80 for each vial exposure location (surface, subsurface, and 2.8°C) and 80 control larvae. The numbers of *N. rileyi*-killed larvae and of emerged moths were recorded.

Data on air and subsurface soil temperatures were obtained from a weather station located ca. 8 km west of the experimental site. Soil moisture was abundant throughout the experimental period. Some specimens were collected from beneath snow or in standing water.
RESULTS AND DISCUSSION

Fungal Viability of *N. rileyi*-killed Cadavers

The ability of mycelium in cadavers to produce conidiophores and conidia decreased steadily as the length of exposure period increased (Table 1). Mycelium in cadavers stored at a constant 2.8°C was best able to sporulate, producing conidia even after 171 days of storage. Mycelium in cadavers exposed to surface and subsurface soil conditions produced conidia after 93 and 105 days of exposure, respectively, but mycelia exposed longer failed to produce conidia. No significant differences ($P_X = 0.05$) were detected between the numbers of sporulating infected cadavers from each treatment environment. Small sample sizes on each collection date probably contributed to the insignificance. In general, bioassays confirmed the pathogenicity of conidia that were produced (Table 2).

During the cadaver exposure periods, the daily average subsurface temperature at 5.1 cm fluctuated between -4.7°C and 23.1°C. The range of daily average air temperatures at 1.5 m above the soil surface was between -24.4°C and 23.6°C.

In this experiment, conidiogenesis by mycelium in surface and subsurface cadavers ceased after 14 and 26 February, respectively. Therefore, viability did not extend through the winter and into the next growing season. However, conidiogenesis by mycelium from cadavers maintained at 2.8°C occurred until 3 May, which suggests that mycelium may retain viability under moderate, nonfluctuating environmental temperatures and conditions and, perhaps, in sheltered field locations. *Nomuraea rileyi*-colonized hosts were able to overwinter in North Carolina where air
Table 1. Impact of environmental exposure on viability of *N. rileyi* (in screen envelopes)

<table>
<thead>
<tr>
<th>Treatment environment</th>
<th>Collection date</th>
<th>Number of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Subsurface</td>
<td>21</td>
<td>40</td>
</tr>
</tbody>
</table>

(Number of cadavers\(^a\) that sporulated after exposure)\(^b\)

<table>
<thead>
<tr>
<th>Treatment environment</th>
<th>Number of days that cadaver was exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

\(^a\)Number out of 8 tested.

\(^b\)No significant differences at the 5\% level of chi-square were found.

\(^c\)Number out of 7 tested.

Table 2. Pathogenicity of conidia produced by mycelium in cadavers exposed to various environments (in screen envelopes)

<table>
<thead>
<tr>
<th>Treatment environment</th>
<th>Number of days that cadaver was exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

(Survivorship of *Trichoplusia ni* larvae\(^a\) inoculated with conidia)

<table>
<thead>
<tr>
<th>Treatment environment</th>
<th>Number of days that cadaver was exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

\(^a\)Out of 15 larvae.

\(^b\)Numbers followed by the same letter (vertical columns) are not significantly different by chi-square analysis at the 5\% level.
temperatures ranged between -1.3°C and 32.4°C (Sprenkel and Brooks, 1977).

Infectivity of Exposed Conidia

The design of this portion of the experiment was a compromise between fidelity to environmental conditions and ease of conidial recovery. Glass shell vials provided little insulation against the effects of temperature, either in the field or in the 2.8°C refrigerator. Unless covered by snow or plant debris, vials of conidia on the soil surface were exposed to solar radiation. The daily average temperature at 5.1 cm below the soil surface ranged between -4.7°C and 23.1°C during the conidial exposure period. Average daily air temperatures during the exposure periods ranged between -24.4°C and 23.3°C.

Conidia held at 2.8°C were infective and caused mycoses in T. ni larvae throughout the 183-day testing period (Table 3). Although not statistically different from the control, some mortality caused by mycelium from conidia from surface and subsurface vials was detected after 168 and 183 days of exposure (20 May and 4 June), respectively. These dates are usually within the soybean planting period in Iowa. Conidia remained viable under field conditions, even though they were often collected from very moist soil conditions. Some reduction in conidial pathogenicity under moist conditions may occur (Kish, 1975), but, nonetheless, pathogenicity was detected in this study. Winter survival of conidia through a central Iowa winter is supported by data from Missouri. Ignoffo et al. (1978) determined that ca. 99% of the original infectivity of N. rileyi conidia was lost in buried and surface samples after ca. 350 and 450 days, respectively.
Nonetheless, viable conidia remain after winter in sufficient numbers to initiate epizootics the following season in Missouri.

Mycelial establishment from *N. rieyi* conidia exposed to field conditions was detected throughout the winter, even though it was rarely at statistically significant levels. This study was unable to detect conidio-ogenesis by field-exposed cadavers through the winter, perhaps because of small sample size. Nonetheless, survival of *N. rieyi* in the stromal form is likely because of reports of resistant structures (Pendland, 1982) and of overwintering viability (Sprenkel and Brooks, 1977) from other locales. *Nomuraea rileyi* inoculum reservoirs are probably able to overwinter and initiate mycoses in host larvae the next season in Iowa.

### Table 3. Pathogenicity of conidia after exposure to various environments (in plugged vials)

<table>
<thead>
<tr>
<th>Treatment environment</th>
<th>Collection date</th>
<th>Number of days in environment</th>
<th>(Survivorship out of 80 inoculated <em>Trichoplusia ni</em> larvae)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feb.</td>
<td>March</td>
<td>April</td>
</tr>
<tr>
<td>Surface</td>
<td>26</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Subsurface</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory 2.8°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^aNumbers followed by same letter (vertical columns) are not significantly different by chi-square analysis at the 5% level.
PART IV. GREEN CLOVERWORM, ITS PARASITOIDS, PREDATORS, AND *N. RILEYI* INCIDENCES IN CENTRAL IOWA ALFALFA AND SOYBEANS -- A COMPARISON
The life cycle of the green cloverworm (GCW), *Plathypena scabra* (F.), includes spring migration to central Iowa and annual reestablishment on various legumes, most notably on alfalfa in the early season. Colonization of soybeans occurs later as immigrant moths oviposit on developing soybean plants. Although not considered an important pest on alfalfa, the GCW is occasionally a pest defoliator on soybeans (Pedigo et al., 1973).

The relative abundance of immigrant GCW moths in alfalfa and soybean fields in Iowa is measurable with black-light traps. Wellik (1977) found six times more moths in alfalfa than in soybean fields during a nonoutbreak season. By contrast, Buntin and Pedigo (1983) collected more GCW moths from black-light traps in soybean than in alfalfa fields during an endemic and an outbreak GCW season. Although the preceding studies disagree on the relative seasonal abundance of GCW moths in the two crops, the alfalfa habitat is not an important source of GCW moths that colonize soybean fields in Iowa (Buntin and Pedigo, 1983). Frequent harvesting of alfalfa may not allow large numbers of GCW larvae to complete development and emerge as adults. Therefore, alfalfa fields act as "population sinks" for immigrating adults in the spring and indigenous adults that disperse from nearby soybean fields in the summer.

Lentz and Pedigo (1975) studied GCW population dynamics and the population ecology of GCW parasitoids in Iowa alfalfa and soybean ecosystems. They found that the total seasonal parasitization by all parasitoids was 28.4% and 33.3% in alfalfa and soybeans, respectively. Increased parasitization during midseason in soybeans may have resulted from movement of
adult parasitoids from cut alfalfa to soybeans. The most abundantly reared GCW parasitoid was *Rogas nolophanae* Ashmead, with a total seasonal parasitization of 22.8% and 18.1% in alfalfa and soybeans, respectively. *Rogas nolophanae* activity was greatest early in the season, dropped by mid-season, and gradually increased for the remainder of the season.

In central Illinois, alfalfa is a "natural nursery" for many predators and parasitoids that eventually migrate to soybeans (Roberts et al., 1977). A greater variety of parasitoids occurs in soybeans than in alfalfa, but the percent parasitization is less. Also, more pathogens are associated with lepidopterous larvae in alfalfa than with those in soybeans, perhaps because of greater foliage density. Compared with the heavy tillage prescribed for soybean fields, the uncultivated condition of alfalfa fields may protect residual pathogens.

The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson, frequently causes epizootics among Lepidoptera larvae (Watson, 1916; Hill, 1925; Allen et al., 1971; Carner et al., 1975; Ignoffo et al., 1975b; Dietz et al., 1976; Roberts et al., 1977). The disease occurs in GCW populations in Iowa (Pedigo et al., 1973), and it has characteristics of a key mortality factor in Iowa soybeans (Pedigo et al., 1983).

In central Iowa, perennial alfalfa fields offer early season habitats for immigrant GCW moths and their subsequent larval offspring. This study sought to determine if alfalfa fields are early season nurturing centers for biotic mortality agents that later attack GCW populations in soybeans. Data on the comparative incidences of *N. rileyi*-caused disease in alfalfa and soybean GCW populations are especially lacking, and these comparisons were the primary objective of this study. Also, the comparative numbers of
predatory arthropods in alfalfa and soybean collections were recorded. An additional objective was to compare the incidences of parasitoids in GCW larvae collected from the two crops.
METHODS AND PROCEDURES

Since the alfalfa agroecosystem is disrupted periodically by harvesting, collections were made on a rotational basis using the same three alfalfa fields each year. All sampled fields were within a 5-km radius of Ames, and samples were taken only in tall, standing alfalfa.

1981 Alfalfa

On each of 16 sample dates between 10 June and 10 September, the first 25 GCW larvae swept from the foliage with a 36-cm-diam net were each placed in individual 7-dr plastic snap-top vials with a sprig of fresh alfalfa. The vials were labeled as to collection date, site, and estimated larval stage at collection.

1982 Alfalfa

Thirty sample dates between 1 June and 10 September were used in 1982. On some dates, samples were taken in more than one field, and, hence, a total of 42 samples were taken through the season. The same three alfalfa fields sampled in 1981 were used in 1982. On each sample date, 100 sweeps with a 36-cm-diam net were taken along a "U-shaped" course through the field. Each GCW larva collected was placed in an individual 7-dr plastic vial with a sprig of fresh alfalfa. Each vial received an identification label. In 1982, predator arthropods were field-identified and their numbers recorded as they emerged from the sweep net.
1981 Soybeans

Two soybean fields near Ames were sampled; field A was located ca. 5 km south and field B ca. 5 km west of Ames. Eight sample dates between 15 July and 11 September were used at field A. Field B was sampled eight times at ca. 10-day intervals between 30 July and 11 September. On each sample date, 10 randomly located shake-cloth samples were taken.

The shake-cloth sampling technique is an efficient method for establishing GCW larval population estimates in soybeans (Hammond and Pedigo, 1976). The procedure was to place a ground cloth between two soybean rows and shake 30-cm sections from each adjacent row vigorously for ca. 25 seconds over the cloth. Dislodged insects that fell on the cloth were field-identified, counted, and the data were recorded. GCW larvae were each placed in individually labeled 7-dr plastic vials with a piece of soybean leaflet as a food source.

1982 Soybeans

Two fields were sampled; field C was ca. 5 km south of Ames and field D was ca. 5 km west of Ames. Field C was sampled 11 times at one- to 10-day intervals between 8 July and 3 September. Field D was sampled nine times between 8 July and 19 August at one- to eight-day intervals.

On each sampling date in 1982, flush samples of GCW adults also were made (Pedigo et al., 1982). In this procedure, one sampler walked between two soybean rows and vigorously brushed the canopy on either side with two 91-cm aluminum rods. A recorder-observer followed behind by ca. five to six paces. Ten passes were made through each field with at least six rows separating each pass. The total area flush-sampled in each field was ca.
0.1 ha. As GCW moths flew up from the sampled rows, sightings were called out, and the number was recorded. Sampling began on the downwind side of each plot so that flushed moths would fly into previously sampled areas and not be counted again.

Laboratory Procedure

The GCW larvae in vials were returned to the laboratory and maintained at room temperature. The progress of each larva was followed until death or pupation, and determinations of mortality agents were made. Parasitized hosts and parasitoid cocoons and pupae were placed in gelatin capsules for later parasitoid emergence and identification. Dead and moribund larvae were retained for ca. two weeks to check for pathogenic fungus development. In 1982, the emergence date and sex of each GCW moth also were recorded.
RESULTS AND DISCUSSION

Nomuraea rileyi

1981

The major entomopathogen identified from GCW larvae in all 1981 and 1982 collections was the fungus *N. rileyi*. Isolated incidences of *N. rileyi* in alfalfa-collected GCW larvae occurred in the laboratory on 10 June, and again on 11 July. The two isolated incidences may be evidence of a low level *N. rileyi* infection rate in the GCW field population that was poorly detected by the sweep-net sampling program. The disease became epizootic in GCW populations in alfalfa during mid-August to early September, so that, in the 10 September collection, 68.0% of the larvae succumbed to *N. rileyi*.

*Nomuraea rileyi* was the most frequently identified mortality agent from 1981 soybean-collected GCW larvae. The pathogen was first detected in 10 August larval collections, about the same time as the disease began to expand in alfalfa GCW populations. The GCW larval densities on 10 August were 4.0 and 2.6 larvae/60 cm-row at soybean field A and field B, respectively. Both of these densities occurred near the beginning of larval population increases that peaked ca. 21 or 22 August (Figure 1). The first fungus-killed cadaver directly shaken from soybean foliage was collected on 30 August, 20 days after initial disease detection in laboratory-maintained larvae and during a drop in population numbers of living GCW larvae in soybeans. By the last 1981 sampling date, the number of cadavers collected from shake samples exceeded the number of living larvae collected.
Figure 1. Mean numbers of GCW larvae (± S.E.) and *N. rileyi*-caused cadavers in samples from soybean near Ames, Iowa, 1981
Mortalities caused by *N. rileyi* on 11 September at field A and field B were 94.3% and 98.4%, respectively.

A post-harvest collection was made in field A on 18 September, 1981. Within hours of combine harvesting, a 1-m² wooden frame was placed on the soil surface at random locations. The surface litter within the frame was searched by hand for ca. 10 minutes for each sample, and the numbers of GCW pupae and *N. rileyi*-caused cadavers were recorded. Eight GCW pupae and 25 cadavers were found in 20 samples, for an average of 1.25 cadavers for m². This "inoculum load" entering an overwintering period might be considered sizeable, because it reflects a late-season *N. rileyi* epizootic among GCW larvae of the 1981 season. If *N. rileyi* were able to survive the rigors of the winter season, inoculum would be available for infection of larvae in the 1982 season.

Follow-up attempts were made in 1982 to find evidence of *N. rileyi* activity at the field A location. Sweep-net sampling was used to collect Lepidoptera larvae ca. weekly between 10 June and 31 July in oats planted at the site. Each sample date provided ca. 500 sweeps accumulated from standing oats and from field edges. Although few Lepidoptera larvae were collected, the most abundant were armyworm, *Pseudaelia unipuncta* (Haworth), larvae. This species is susceptible to *N. rileyi* infection (Charles, 1941; Thorvilson, 1980, Dept. of Entomology, Iowa State University, Ames, Iowa, unpublished data). None of the 24 armyworm larvae collected and maintained in 7-dr plastic vials in the laboratory died of *N. rileyi* infections. Therefore, there was no evidence of carry-over of infective *N. rileyi* inoculum from the previous season's epizootic in soybeans.
1982

The first 1982 collection of a *N. rileyi*-infected GCW larva from alfalfa was on 18 June, but the fungus was not detected again until 7 July. During August, the disease caused increasing mortality of alfalfa-collected GCW larvae. Disease incidence in alfalfa preceded detection of the disease in 1982 soybeans by two to four weeks.

*Nomuraea rileyi* was first observed in larvae collected on 22 July from soybean field D. The first *N. rileyi*-caused death from field C occurred in larvae collected on 30 July. The GCW larval densities on the dates of first *N. rileyi* incidence were 1.9 and 3.0 larvae/60 cm-row at field C and field D, respectively. Both of these densities occurred during GCW larval population increases that peaked later, i.e., 11 August at field C and 30 July at field D (Figure 2). The first cadavers directly shaken from soybean foliage were collected eight to 12 days after the first detection of the disease in laboratory-maintained larvae. The rate of disease continued to increase throughout the remainder of the season.

Moths flushed from field D peaked in number on 27 July, but they represented a flight that did not contribute to the 30 July larval peak. The larval population collapsed during mid-August as *N. rileyi* incidence increased. Moth activity at field C peaked on 27 July, and it probably contributed to the larval population that peaked nearly two weeks later. The downturn in larval numbers in late August and early September was accelerated by *N. rileyi*-caused mortality.

In 1981 and 1982, low levels of *N. rileyi* infection among alfalfa GCW populations were detected in June and early July. These isolated
Figure 2. Mean numbers of GCW larvae (± S.E.), adults, and \( n. \) rileyi-caused cadavers in samples from soybean near Ames, Iowa, 1982
incidences preceded the appearance of disease among soybean GCW larvae by as much as eight weeks. Ignoffo et al. (1975b) were able to detect the first seasonal incidences of *N. rileyi* on legumes in Missouri by feeding field-collected plants to laboratory-reared *Trichoplusia ni* (Hübner) larvae. By this method, they detected the presence of *N. rileyi* inocula on perennial red clover and alfalfa on the same date that inoculum was detected on young soybean leaves. However, field GCW mortality in soybeans did not occur until nearly three more weeks had passed.

When *N. rileyi* was first detected in soybean GCW larvae in the current study, the host density ranged from 1.9 to 4.0 larvae/60 cm-row. Ignoffo et al. (1976b) estimated a host density of 0.8 to 1.6 larvae/60 cm-row would be needed to initiate and sustain a *N. rileyi* epizootic. Johnson et al. (1976) found that epizootics developed normally with a host density of 3.4 larvae/60 cm-row.

By late July to early August, the disease appeared consistently, and nearly simultaneous subsequent epizootics developed in both habitats (Figure 3). By that time of season, the soybean canopy was well-developed. The importance of canopy in providing a favorable microclimate for *N. rileyi* survival and establishment was emphasized by Burleigh (1975), Dietz et al. (1976), Roberts et al. (1977), Jaques (1978), and Mayse (1978).

**Predators**

The three most abundantly collected predatory arthropods in both alfalfa and soybeans in 1982 were *Nabis* spp., *Orius insidiosus* (Say), and spiders. These three groups together account for ca. 90% of the predator fauna in Iowa soybeans (Bechinski and Pedigo, 1981). Greater numbers of
Figure 3. Percent mortality of GCW larvae in alfalfa and soybean caused by *Nomuraea rileyi* near Ames, Iowa, 1981 and 1982.
predators were collected from alfalfa than from soybeans, but the different sampling methods make comparisons of limited value.

*Nabis* spp. were the most frequently collected predators in both crops. They were especially abundant in early-season alfalfa, but, as the season progressed, their numbers generally declined in alfalfa collections (Figure 4). Peak numbers in soybeans occurred in mid-August collections. *Nabis* spp. complete at least one generation in alfalfa before they colonize soybeans in Kentucky (Yeargan and Braman, Dept. of Entomology, University of Kentucky, personal communication). It is very likely that similar emigration to soybeans occurs in Iowa, probably after canopy closure (Price, 1976).

*Orius insidiosus* was the second most abundantly collected predator in soybeans, and peaked in numbers during mid-August (Figure 4). Collections from alfalfa yielded very high numbers of the predator early in the season but less than 25% as many during July and August. The mean number of spiders throughout the season ranked them as the second most frequently collected group in alfalfa. The numbers of spiders shake-sampled from 1982 soybeans were low (Figure 4).

**Parasitoids**

Four species of primary parasitoids were reared from 1981 and 1982 alfalfa and soybean GCW collections. They were, in descending order of frequency, *R. nolophanae, Cotesia marginiventris* (Cresson), *Winthemia sinuata* Reinhard, and *Oswaldia assimilis* (Townsend). There were also four unknown specimens: three Diptera in 1981 and one Hymenoptera in 1982. Equal numbers of parasitoid species attacked GCW in alfalfa and in soybeans,
Figure 4. Numbers of three predatory arthropod groups collected in alfalfa and soybean near Ames, Iowa, 1982

(-----) = Mean number of predators/100 sweep net samples in alfalfa

(-----) = Mean number of predators/10 shake samples in soybean
supporting the findings of Lentz and Pedigo (1975) in Iowa. In contrast, Roberts et al. (1977) found more parasitoid species in central Illinois soybean GCW than in alfalfa GCW populations.

The percentages of total GCW larvae parasitized in 1981 alfalfa and soybeans, and 1982 alfalfa and soybeans were 27.3%, 7.9%, 26.2%, and 13.0%, respectively. If comparisons of parasitization include only the time periods of simultaneous collections in alfalfa and soybeans, the respective percentages are 11.6%, 6.5%, 12.9%, and 11.8%. Clearly, parasitoid activity was greater in alfalfa than in soybeans during the two seasons. Roberts et al. (1977) also found greater parasitization in GCW larvae collected from alfalfa than in those from soybeans. Lentz and Pedigo (1975) intensively sampled GCW parasitism in soybean and alfalfa for one growing season. They observed a midseason increase in total parasitization in soybeans that was possibly associated with immigration of adult parasitoids from cut alfalfa. They found total seasonal parasitization rates of 28.4% and 33.3% in alfalfa and soybeans, respectively, but the results were confounded by suspension of collecting in alfalfa for ca. one month during midseason.

*Rogas noiophanae* was the parasitoid most frequently reared in both years of the present study from both alfalfa and soybean GCW larvae, and it previously was reported as the most prevalent parasitoid of GCW larvae in Tennessee (Hill, 1925), Delaware (Whiteside et al., 1967), Missouri (Barry, 1970), Iowa (Lentz and Pedigo, 1975), and Kentucky (Sloderbeck, 1981). In 1981 and 1982, parasitization by *R. noiophanae* for the entire season ranged in three alfalfa fields from 16.2% to 33.9%. This compares favorably with the 22.8% *R. noiophanae* attack rate observed earlier in Iowa alfalfa (Lentz and Pedigo, 1975), although the earlier reported seasonal
attack rate in soybeans was 18.1% (Lentz and Pedigo, 1975), considerably higher than the 3.8% to 9.0% found in 1981 and 1982.

Lentz and Pedigo (1975) reported that the activity of *R. noilophanae* was greatest in early season alfalfa, but it declined for the remainder of the season. Data from 1981 and 1982 support their findings (Figure 5). The greatest incidence of parasitization is in June and early July in alfalfa. At that time, soybean plants were too small for shake-sampling. *Rogas noilophanae* collections from soybean GCW larvae reflect the similar late-season downward trend in parasitization seen in alfalfa.

In both seasons, there is evidence of interference between *R. noilophanae* parasitism and *N. rileyi* infection. As the late-season disease epizootics developed (Figures 1, 2, and 3), incidences of *R. noilophanae* parasitization declined. Barry (1970) found that *N. rileyi*-caused disease in Missouri GCW populations caused a scarcity of suitable hosts for parasitoid development. Similar interference between the disease and parasitoids occurred in *Heliothis* spp. larvae (Burleigh, 1975) and Kentucky GCW larvae (Sloderbeck, 1981).

During 1981 and 1982, sporadic, early-season incidence of *N. rileyi*-caused disease reflected low level infection rates in alfalfa GCW populations. As inoculum levels increased, alfalfa fields acted as "foci of infection" from which the pathogen dispersed to surrounding areas, especially by means of the wind. Conidia can be dispersed on the leg scales of moths as well (Kish, 1975). By late July or early August, *N. rileyi* epizootics occurred nearly simultaneously in alfalfa and soybean habitats (Figure 3).
Figure 5. Percent *Rogas nolophanae* parasitization of GCW larvae in alfalfa and soybean near Ames, Iowa.
Predatory arthropods and parasitoids were more active in alfalfa than in soybeans during the two seasons of the study. Massive emigration of these organisms from harvested alfalfa fields to nearby soybean fields seems likely, as hypothesized by Lentz and Pedigo (1975).

Iowa observations during 1981 and 1982 suggest that early season activity of important biotic mortality agents of the GCW in alfalfa may lead to later establishment of these agents in the soybean habitat. This supports the hypothesis from Illinois (Roberts et al., 1977) that alfalfa fields may act as early season "nurseries" for biotic mortality agents.
PART V. NOMURAEEA RILEYI AND THE POPULATION DYNAMICS OF GREEN CLOVERWORM IN IOWA SOYBEANS
INTRODUCTION

The green cloverworm (GCW), *Plathypena scabra* (F.), is an occasional pest of Iowa soybeans. GCW moths immigrate into Iowa in late May and early June (Myers and Pedigo, 1978), lay eggs primarily on alfalfa in the early season, and later oviposit on soybeans as the plants develop (Pedigo et al., 1981). Widespread larval population outbreaks causing economic damage in soybeans occurred in 1966, 1968, and 1973 in Iowa (Pedigo, 1974). More recent localized outbreaks were identified in 1975 in northern Iowa [Insect, Weed and Plant Disease Newsletter, IC-421 (20 and 21), 1975], 1977 in southeastern Iowa [Insect, Weed, and Plant Disease Newsletter, IC-434 (14 and 15), 1977], 1978 and 1979 (Pedigo et al., 1983). Outbreaks are caused primarily by large numbers of immigrating moths that oviposit in soybeans in June and early July. The larval generation that is produced can cause economic damage to full-bloom soybeans. If small numbers of moths immigrate, the generation of larvae is less dense and an endemic population configuration exists. Life-table studies of the GCW revealed two larval generations each year in central Iowa soybeans, the second of which does not cause economic damage to soybeans (Pedigo et al., 1983). Buntin and Pedigo (1983) expanded the hypothesis of GCW population dynamics in Iowa by proposing that four adult flights and three larval generations typically occur in central Iowa, with distinct outbreak or endemic population configurations occurring in soybeans.

Pedigo et al. 1983) identified the entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson, as having characteristics of a key biological mortality factor in GCW populations in Iowa. During GCW outbreak years,
epizootics of the disease develop late in soybean GCW generation one and continue throughout generation two, causing late-season collapse of the population. The disease is enzootic during endemic GCW population years, causing some second GCW generation mortality late in the season. Buntin and Pedigo (1983) noted that larval densities in some endemic years were large enough for \textit{N. rileyi} epizootics to develop in late season.

A long-term study of GCW population phenology and the biological mortality factors affecting larvae was initiated in 1971 by the soybean insect research group at Iowa State University. Collections of GCW larvae from soybeans were made each year through 1980. Ten primary parasitoids were most commonly reared from GCW larvae and \textit{N. rileyi} was the most commonly collected pathogen [Iowa Legume Insect Research Summary, 1980. Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, Project 2248]. Because \textit{N. rileyi} has been characterized as a key biotic mortality factor in GCW populations in Iowa, the present report focuses on the disease and on GCW larval phenology. The major objective of the analysis was to associate \textit{N. rileyi} disease incidence and epizootic development with GCW larval population configurations during the 10 years of accumulated observation.
METHODS AND PROCEDURES

GCW larval population levels in soybean fields were determined each year by sweep-net sampling of three fields within an 8-km radius of Ames, Iowa. Following procedures outlined by Lentz and Pedigo (1975) and Pedigo et al. (1983), weekly samples were taken in each field from June to September. Samples consisted of six "box sweeps" with 38 cm-diam sweep nets (Pedigo et al., 1972b) in each of four blocks within each field. GCW larvae from the weekly 24 sweeps from each field were individually isolated in separate plastic zipper cases with soybean leaflets as a food source. The stage of each larva at collection was recorded. Larvae were maintained at room temperature in the laboratory until death or adult emergence. The date and cause of death were recorded for each larva. Larval deaths of unknown origin were recorded under a separate category.
RESULTS AND DISCUSSION

GCW Population Types, 1971 to 1980

GCW populations in Iowa soybeans may be characterized by a large first larval generation followed by a smaller second generation and have an "outbreak configuration" (Pedigo et al., 1983). Conversely, populations with a small first generation followed by a larger second generation have an "endemic configuration". In Iowa, larval generation one usually occurs from mid-June to mid-July, and generation two occurs from early August to late September. There is more overlap of generations during outbreak configuration years than during endemic years (Pedigo et al. 1983).

To determine the configuration of populations sampled from 1971 to 1980, a practical "split date" between first and second GCW larval generations for each year was utilized. The numbers of small (1st and 2nd instars), medium (3rd and 4th instars), and large (5th and 6th instars) larvae collected on each date were plotted against yearly Julian dates. The split date was chosen at the midpoint of generation overlap. The overlap period was estimated from decreasing totals of large larvae and increasing numbers of small larvae in late July or early August. Split dates ranged between Julian date 205 and 223 (24 July and 11 August), with a mean of 214 (2 August) over the 10 years of observations (Figure 1).

The larval populations of 1971, 1973, 1978 and 1979 showed outbreak characteristics in central Iowa (Figure 1). In each outbreak year, the second generation peak numbers were ca. one-half those of the first generation. The 1973 first generation peak was the highest recorded in the 10-year study (Table 1). The mean generation split date in outbreak years was
Figure 1. GCW larvae populations and numbers of *N. rileyi*-caused cadavers in three soybean fields near Ames, Iowa, 1972-1980 (Split dates between larval generations are given in bold face for each year)
Table 1. Comparison of Julian dates and numbers of larvae at generation peaks and at first *N. rileyi* incidence in outbreak and endemic GCW years

<table>
<thead>
<tr>
<th>Year</th>
<th>Date of first generation peak</th>
<th>Peak no. of larvae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Generation split date</th>
<th>Date of second generation peak</th>
<th>Peak no. of larvae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Date of first <em>N. rileyi</em> incidence</th>
<th>Larval generation at first <em>N. rileyi</em> incidence</th>
<th>No. of larvae on date of first <em>N. rileyi</em> incidence&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Outbreak</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>1971</td>
<td>215</td>
<td>106</td>
<td>222</td>
<td>229</td>
<td>59</td>
<td>201, then 222</td>
<td>1,2</td>
<td>44.5, then 52.5</td>
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<tr>
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<td>206</td>
<td>132</td>
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<td>1</td>
<td>132</td>
</tr>
<tr>
<td>1978</td>
<td>187</td>
<td>122</td>
<td>215</td>
<td>222</td>
<td>44</td>
<td>187</td>
<td>1</td>
<td>122</td>
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<tr>
<td>1979</td>
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<td>1972</td>
<td>209</td>
<td>56.3</td>
<td>223</td>
<td>252</td>
<td>116</td>
<td>209, then 230</td>
<td>1,2</td>
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<td>205</td>
<td>18.7</td>
<td>220</td>
<td>238</td>
<td>82</td>
<td>231</td>
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<td>195</td>
<td>22.0</td>
<td>210</td>
<td>220</td>
<td>114</td>
<td>233</td>
<td>2</td>
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</tr>
<tr>
<td>1976</td>
<td>195</td>
<td>7.7</td>
<td>210</td>
<td>225</td>
<td>18</td>
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<td>81.7</td>
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<tr>
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<td>17.3</td>
<td>205</td>
<td>225</td>
<td>28</td>
<td>214</td>
<td>2</td>
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<sup>a</sup>Number of larvae/24 box sweeps.
216 (4 August), and there was considerable generation overlap in each year. In three of the outbreak years, the second generation peak occurred between 13 and 18 days after the first generation peak. In 1978, the first generation peak was early and preceded the second generation peak by 35 days. The differences in the spans between peaks (13 to 35 days) might be caused by differences in severity of N. rileyi epizootics and subsequent degrees of truncation in developing second generations. The early collapse of the second generation prevents the development of large numbers, which, if unhindered, would peak somewhat later in the season. The biotic potential of outbreak moth populations is, thus, not realized because of N. rileyi truncation of second generation larval populations.

Larval populations in 1972, 1974, 1975, 1976, 1977, and 1980 were endemic in central Iowa (Figure 1). Second generation peak numbers were between 1.6 and 7.7 times larger than first generation peak numbers (Table 1). In general, endemic first generation peak numbers occurred earlier than those in outbreak years (mean dates 196 and 202, respectively). The mean generation split date was 212 (31 July), also slightly earlier than in outbreak years. Endemic generation curves were more distinct and had less overlap than those of outbreak populations. This was probably because low numbers of immigrant moths displayed a relatively shorter egg laying period, and, therefore, a first larval generation of more uniform age-structure was produced. Because the age-spread of larvae was narrow, subsequent pupation and oviposition by emerged moths also occurred in relatively narrow time-frames. Together, these events created more distinct population split dates. Endemic second generation peak dates occurred between 25 and 48 days after the first generation peak dates. Since endemic second larval
generations were not truncated by *N. rileyi* epizootics, greater differences between peak larval dates occurred.

**Occurrence of *N. rileyi***

*Nomuraea rileyi*-infected larvae occurred in collections each year of the study (Figure 1), with the exception of 1976. The absence of *N. rileyi*-caused mortality during 1976 may have resulted from very low GCW population levels and below-normal precipitation during the season. In 1976, precipitation totals for May, June, July, August, and September were below normal by -3.86, -0.08, -5.92, -8.59, and -7.47 cm, respectively. Temperatures during the same months, however, were about normal.

The first *N. rileyi* incidence was synchronous with the first generation peak numbers in the outbreak years 1973, 1978 and 1979 (Table 1). After initial collections, the disease was continuously observed for the remainder of the season. In 1971, one aberrant, fungal-infected larva was collected on date 201 and represented a low-level population infection. Infected larvae were again detected on date 222 (the generation split date) as the disease expanded. In outbreak years, the first infected larvae were collected from the first generation when mean population densities ranged between 44.5 and 132.0 larvae per 24 box sweeps (Table 1). This range of larval densities is equivalent to between 1.85 and 5.57 larvae/30 cm-row (Pedigo et al., 1972b). These larval densities occurred on or before Julian date 206 (25 July) in this study.

The dates of first *N. rileyi* incidence in endemic years were consistently later than in outbreak years (Table 1). With the exception of 1972, the disease first occurred during the second generation when larval numbers
ranged between 11.3 and 81.7 larvae/24 box sweeps (=0.44 and 3.66 larvae/30 cm-row, respectively). The critical densities occurred between 11 days before and 13 days after the second-generation peak numbers. One infected larva was collected from the 1972 first generation (date 209), but the disease was not detected again until date 230. In 1972, an epizootic developed in second generation larvae and reached 54.2% mortality by date 252. No infected larvae were collected from the very low host population in 1976. The first incidence of the disease in 1980 was at a larval density of 11.3 larvae/24 sweeps (=0.44 larva/30 cm-row) and preceded the second generation peak of only 28 larvae/24 box sweeps (1.15 larva/30 cm-row). Because 1980 followed two GCW outbreak seasons (1978 and 1979), in which epizootics developed in the second generation, abundant *N. rileyi* inoculum was probably available. The early dates of first *N. rileyi* incidence in endemic years 1972 and 1980 support a hypothesis of inoculum "carryover" from epizootic seasons to the next seasons.

### Host-Density Relationship with *N. rileyi*

*Nomuraea rileyi* occurrence in GCW populations is hypothesized to be host density-dependent (Pedigo et al., 1983). To investigate the relationship, the number of larvae under population curves for each year (Figure 1) was calculated using the trapezoid method. Then, a regression analysis determined the dependency of the date of first *N. rileyi* incidence on the accumulated number of larva-days up to that date. The regression coefficient (slope) of the equation describing the relationship ($y = 199.7 + 0.0140x$; $R^2 = 0.42$) was not significantly different ($P = 0.058$) from zero.
and, therefore, was of questionable value in predicting the date of first incidence.

Larvae that died of colonization by *N. rileyi* during the maintenance period in the laboratory had been inoculated naturally in the field. Because GCW larvae succumb to the pathogen ca. six days after inoculation, mortality figures may reflect the population condition that was present several days before the collection date. Therefore, additional regression analyses determined the dependency of the date of first *N. rileyi* incidence on the accumulated number of larva-days up to three days and six days before collection. The three-day "setback" analysis described the population condition mid-way between the six-day extreme and the date of first incidence. The equations \(y = 201.6 + 0.0151x, R^2 = 0.49\), and \(y = 202.9 + 0.0169x, R^2 = 0.53\) significantly \((P < 0.05)\) describe the relationships for three-day and six-day setbacks, respectively. In one-half of the years, first *N. rileyi* incidence occurred when between 270 and 450 larva-days or between 75 and 365 larva-days had accumulated under the population curve up to three-days or six-days before collection, respectively.

After the date that finds at least 2% *N. rileyi*-caused mortality in collected GCW, infected larvae are usually present in every collection for the remainder of the season. For this reason, the date of the first 2% mortality rate may be considered the beginning of substantial disease impact on larval populations or "prominent mortality." Regression analyses of the dependency of date of prominent *N. rileyi*-caused mortality on accumulated larva-days up to that date produced a significant \((P < 0.05)\) equation of \(y = 203.7 + 0.0112x (R^2 = 0.54)\). Similar significant equations
based on accumulated larva-days up to three days before the date, and six
days before the date of prominent mortality were \( y = 205.95 + 0.0111x \) \((R^2 =
0.55)\), and \( y = 207.5 + 0.115x \) \((R^2 = 0.57)\), respectively.

A plot of the six-day setback data (Figure 2) illustrates a grouping
of five years within a small range of accumulated larva-days. In three
outbreak and two endemic years, the date of first prominent mortality oc­
curred six days after accumulated larva-days were between ca. 1000 and
1450.

Two outlying "groups" of years are also evident. The initial dates of
prominent mortality in 1973 and 1980 were earlier than in other years (Fig­
ure 2) and occurred at lower accumulated larva-days. Both of these years
were preceded by seasons in which \( N. rileyi \) epizootics developed during the
second GCW generation. The initial dates of prominent mortality in 1975
and 1977 occurred late and at much higher larva-day accumulations than the
other years. \( Nomuraea rileyi \) development may have been retarded because of
a dry July in 1975 and the hot and dry June and July in 1977. In addition,
each year followed at least one season of endemic GCW populations with low
second-generation \( N. rileyi \) levels.

No \( N. rileyi \)-caused deaths were observed in 1976 collections. The
year was characterized by very low GCW populations and very dry July and
August months. In addition, 1976 followed two endemic population years
with low second generation \( N. rileyi \) levels.

Although the given equations might be used to predict the first inci­
dences of \( N. rileyi \) in box-sweep collections of GCW larvae in soybeans,
the \( R^2 \)-values are small. With this in mind, another predictive index was
tested.
Figure 2. Relationship between the dates of first 2% *N. rileyi*-caused mortality and the accumulated number of larva-days up to six days before those dates.

The equation for the line shown in the diagram is:

\[ Y = 207.5 + 0.0115X \]

with a correlation coefficient of \( R^2 = 0.57 \).

- **O** = Endemic
- **Δ** = Outbreak
The Relationship Between the Size of the First GCW Generation and the Second GCW Generation
Mortality Rate Caused by *N. rileyi*

Figure 1 suggests that the size of the second GCW larval generation in any year is inversely proportional to the size of the first generation. Also, *N. rileyi*-caused mortality in the second generation is directly proportional to first generation size in most years. A ratio of the accumulated number of *N. rileyi*-killed second generation GCW larva-days over the accumulated number of second generation larva-days was constructed and was calculated for each year. Regression analysis tested the dependence of the ratio on the accumulated number of first generation larva-days. The resulting slope was significantly different from zero; i.e., the second generation mortality rate from *N. rileyi* infection is significantly dependent on the size of the first generation (Figure 3). Generally, outbreak years are characterized by large first generations followed by large *N. rileyi* mortality in the second generation. However, *N. rileyi*-caused mortality in 1971 was not as great as expected. Five endemic years are grouped near the origin of the plot and illustrate that a small first generation is followed by low *N. rileyi* mortality in the second generation.

One endemic year (1972) is exceptional and may, in fact, represent a "borderline" GCW population configuration between endemic and outbreak types. Although 1972 had the endemic characteristic of a smaller first generation than second generation, the first generation was the largest of the endemic years. In addition, a *N. rileyi*-infected larva was collected from the 1972 first generation, and a *N. rileyi* epizootic developed.
Figure 3. The relationship between the ratio
\[
\frac{\text{accumulated number of } N. \text{ rileyi-killed second generation GCW larva-days}}{\text{accumulated number of second generation larva-days}}
\]
and the size of the first generation.
in the 1972 second generation. These are characteristics of an outbreak population configuration.

In general, the analysis confirms the intuitive relationship between the size of the first generation and the impact of *N. rileyi* on the second generation in outbreak and endemic years. However, 1972 may represent a season wherein the GCW population had characteristics of both configurations.

**Percentage *N. rileyi*-caused Mortality in Each GCW Generation**

The percentage mortality caused by *N. rileyi* within each generation was calculated for each year (Table 2). Small percentages of first generation larvae died from infection by *N. rileyi* during outbreak years and in the exceptional endemic year, 1972. During these years, second generation mortalities were seven to more than 100 times greater than those in the first generation. With the exception of 1972, GCW populations in endemic years had no *N. rileyi*-caused mortality until the second generation. Although mortality rates were not as large as in outbreak years, many GCW larvae were killed by *N. rileyi* by season's end. Pedigo et al. (1983) suggested a causal effect between GCW population configuration and *N. rileyi* impact.

Figure 4 illustrates the percentages of GCW larvae dying from *N. rileyi* by sampling date in three representative years (outbreak 1978, endemic 1975, and an aberrant endemic year, 1972). During 1978, disease incidence began early and continued to expand rapidly through the remainder of the season. In another outbreak year (1979), 100% of the collected
Table 2. Percentage mortality caused by *N. rileyi*

<table>
<thead>
<tr>
<th>Year</th>
<th>Split date between generations</th>
<th>Generation 1</th>
<th>Generation 2</th>
<th>Combined generations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1971</td>
<td>222</td>
<td>0.8</td>
<td>6.1</td>
<td>3.2</td>
</tr>
<tr>
<td>1972</td>
<td>225</td>
<td>0.2</td>
<td>22.5</td>
<td>14.9</td>
</tr>
<tr>
<td>1973</td>
<td>218</td>
<td>2.7</td>
<td>26.1</td>
<td>8.6</td>
</tr>
<tr>
<td>1974</td>
<td>220</td>
<td>0.0</td>
<td>5.8</td>
<td>5.1</td>
</tr>
<tr>
<td>1975</td>
<td>210</td>
<td>0.0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>1976</td>
<td>210</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1977</td>
<td>205</td>
<td>0.0</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>1978</td>
<td>215</td>
<td>4.3</td>
<td>30.7</td>
<td>9.6</td>
</tr>
<tr>
<td>1979</td>
<td>207</td>
<td>0.2</td>
<td>19.6</td>
<td>4.6</td>
</tr>
<tr>
<td>1980</td>
<td>205</td>
<td>0.0</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Combined years</td>
<td>1.7</td>
<td>8.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Figure 4. Incidence (%) by sampling date of GCW larval deaths by *N. rileyi* in an outbreak year (1978), an endemic year (1975), and an aberrant endemic year (1972); generation split dates were 223, 210, and 215 for 1972, 1975, and 1978, respectively.
larvae were infected with *N. rileyi* on the last sampling date. Great second generation mortality caused by the pathogen seems characteristic of outbreak configurations. In fact, *N. rileyi* is the key mortality factor in the late-season collapse of outbreak GCW populations (Pedigo et al., 1983).

The first incidence of *N. rileyi* in the 1972 first generation was followed by a *N. rileyi* epizootic in the second generation (Figure 4). If epizootic development is characteristic of outbreak population types, then this disease outbreak adds credence to the hypothesis that 1972 has a "borderline" configuration. In comparison, a typical endemic year (1975) has higher *N. rileyi* mortality near season's end, but mortality does not reach epizootic proportions.

*Nomuraea rileyi* Disease Progression
after First Incidence

After it was detected in GCW populations, the disease tended to increase in frequency throughout the remainder of the season. Analyses compared the rates of increase for *N. rileyi*-caused mortality in endemic and outbreak GCW populations. First, the dates of first *N. rileyi* incidences in all years were transformed to a common Julian date (000). Then, the logarithms of *N. rileyi*-caused mortality rates for collections made on individual sampling dates after initial disease incidence were plotted against transformed Julian dates of collection for all outbreak years. To avoid negative values, 1.00 was added to each *N. rileyi*-caused mortality rate before transformation to logarithms. The regression line significantly (P < 0.05) described the relationship ($y = 0.948 + 0.0675x$, $R^2 = 0.625$).
Figure 5. Comparisons of increases in *N. rileyi* mortality in endemic and outbreak GCW populations after dates of first incidence and of first 2% mortality rate.
The similarly calculated regression line describing the relationship in endemic years \(y = 0.883 + 0.0159x, R^2 = 0.059\) accounted for very little of the variability among years, and the slope was not significantly different \((P > 0.05)\) from zero (Figure 5).

In most years, mortalities caused by *N. rileyi* did not increase greatly in GCW populations until after ca. 2% mortality per collection was reached, i.e., prominent mortality. Therefore, a second series of regressions were calculated using the date of the first prominent mortality as the starting date (Figure 5). The significant \((P < 0.05)\) regression line for outbreak years was \(y = 1.856 + 0.0538x\). The lower \(R^2\)-value \((R^2 = 0.462)\) indicated a poorer fit than the outbreak equation from the previous analysis. The \(R\)-value of the line describing endemic years \((R = 0.158)\) was higher than the previous endemic equation. However, the slope of the equation \((y = 1.359 + 0.525x)\) did not contribute significantly \((P > 0.05)\) to the model.

The significant equations for outbreak years may be expressed in exponential form (Figure 6). The curve describing the increase in *N. rileyi* infection rates after the first incidence of prominent (2%) mortality achieves 50% mortality in ca. 38 days. The curve commencing at the date of first *N. rileyi* incidence starts at a lower level and does not reach 50% mortality until ca. 43 days later. The considerable variability in percentage mortality among outbreak years may be caused by several population and environmental factors. For example, epizootics in 1971 and 1978 may have been unable to sustain their development because of reduced larval numbers at the end of each generation.
Figure 6. Increases in *N. rileyi*-caused mortality in outbreak GCW populations after dates of first incidence and of first prominent mortality rate (solid line). Data from several outbreak GCW years (dotted line)
The Effect of Precipitation on

*N. rileyi* Incidence

Kish (1975) and Kish and Allen (1978) recognized the necessity of high relative humidity for *N. rileyi* conidial production and infectivity. Rain may have a detrimental effect on field inoculum load by washing conidia from cadavers and plants to the ground. Considering these observations, the effects of both larval numbers and precipitation on the date of first prominent *N. rileyi*-caused mortality during the 10-year study were addressed. Stepwise multiple regression analysis was used to test the dependence of the date of first incidence of 2% mortality on three independent variables: (1) LD, the accumulated number of larva-days up to the incidence date, (2) RF, the precipitation accumulated from 1 June to the incidence date, and (3) PNR, the rate of *N. rileyi*-caused deaths during the second GCW generation of the previous year. The equation that was accepted from the analyses was $y = 202.03 - 0.017\text{LD} + 0.367\text{RF}^2 (P < 0.05, R^2 = 0.79)$. Independent variables that were tested but that did not contribute substantially to the model were LD$^2$, RF, and PNR.

Using the same independent variables as in the preceding analysis, the dependency of NR, the number of second generation *N. rileyi*-caused deaths in any given year, was tested. Unfortunately, great variability among years prevented the determination of any significant influences on NR by the independent variables. However, from previous analysis, we know that NR is directly proportional to LD, the accumulated number of larva-days up to the incidence date (Figure 3).
Analysis of Population Parameters Between Seasons

Regression analyses were completed to test for interseasonal dependence among various population parameters. Analyses tested the dependency of:

1. The number of *N. rileyi*-caused deaths in the second GCW generation on the number of second generation *N. rileyi*-caused deaths the previous season;

2. The date of first prominent *N. rileyi*-caused mortality on the number of second generation *N. rileyi*-caused deaths in the previous season;

3. The ratio of second generation *N. rileyi*-caused deaths/number of collected generation larvae on the same ratio of the previous year; and

4. The date of first prominent mortality on the ratio in the previous year.

These analyses showed no significant (P > 0.05) relationships. Great variability existed among data in the 10-year study. However, these nonsignificant regression analyses do not preclude the possibility of interseasonal relationships. For example, large amounts of *N. rileyi* inoculum produced during late-season epizootics probably overwinter and provide a reservoir for disease initiation during the next season (Ignoffo et al., 1978; Thorvilson, Dept. of Entomology, Iowa State Univ., unpublished.)
Contributions to the Hypothesis of GCW

Population Dynamics in Iowa Soybean

The results of this study support the hypothesis explaining GCW population dynamics in Iowa soybean (Pedigo et al., 1983; Buntin and Pedigo, 1983), viz., that populations may be classified as having endemic or outbreak configurations. In the study of GCW populations from 1971 to 1980, typical outbreak configurations existed when first generation larval numbers exceeded ca. 90 larvae/24 box sweeps (ca. 3.8 larvae/30 cm-row). *Nomuraea rileyi*-infected larvae were initially collected from the first generation, and the disease became epizootic in the second generation.

The first generation peak in endemic-configuration years did not exceed ca. 22 larvae/24 box sweeps (ca. 0.9 larva/30 cm-row), and *N. rileyi*-infected larvae were not collected until the second generation. However, *N. rileyi* epizootics occasionally develop in second generations of endemic populations. In the present study, the 1972 first GCW generation was atypically large (peaking at ca. 2.3 larvae/30 cm-row), and *N. rileyi*-infected larvae were collected during the first generation. Later, an epizootic developed in the second generation. This configuration was termed "borderline" to emphasize its combined characteristics of endemic and outbreak configurations. Buntin and Pedigo (1983) noted similar events in 1981, an endemic year. In one endemic year (1976), neither generation peak exceeded ca. 19 larvae/24 box sweeps (ca. 0.8 larva/30 cm-row), and *N. rileyi* infections were not detected. Unusually dry weather in 1976 also may have retarded *N. rileyi* development, however.

This study has supplemented the hypothesis of GCW population dynamics in soybean by providing quantitative expressions of GCW and *N. rileyi*
relationships. Since \textit{N. rileyi} has characteristics of a key mortality factor, the date of first occurrence of \textit{N. rileyi}-infected larvae is important and might be predicted using the accumulated number of larva-days as an independent variable in regression equations. The R²-values of such equations may be improved by adding another independent variable, the square of the accumulated precipitation from 1 June, to the expression. Once infected larvae are detected, the increase in mortality caused by \textit{N. rileyi} is significantly described in outbreak GCW populations by other equations presented in this study that relate the mortality rate to the number of days after initial disease incidence (the independent variable).

In summary, this study supports the hypothesis that \textit{N. rileyi} is a key mortality agent of GCW populations in Iowa soybean and it provides quantitative prediction tools for future work. Further studies should be directed toward the interactions of \textit{N. rileyi} with other biotic mortality agents, such as parasitoids, in GCW population regulation.
PART VI. THE HISTOPATHOLOGY OF N. RILEYI IN

PLATHYPENA SCABRA LARVAE
INTRODUCTION

The entomopathogenic fungus, Nomuraea rileyi (Farlow) Samson, causes natural epizootics among populations of Lepidoptera larvae (Watson, 1916; Hill, 1925; Getzin, 1961; Allen et al., 1971; Pedigo et al., 1973; Carner et al., 1975; Ignoffo et al., 1975b; Dietz et al., 1976; Roberts et al., 1977). As a natural biological mortality agent, N. rileyi shares ontogenetic characteristics of other insect pathogenic Deuteromycetes (Madelin, 1966). Cuticular penetration by hyphae from germinating N. rileyi conidia was noted in Trichoplusia ni (Hübner) larvae (Getzin, 1961). The ontogeny of N. rileyi in Pseudoplusia includens (Walker) larvae was followed by Kish (1975) and detailed by Kish and Allen (1978). Mohamed et al., (1978b) described the histopathology of the fungus in Heliothis zea (Boddie) larvae. The progress of N. rileyi in Anticarsia gemmatalis Hübner larvae was examined by Boucias and Pendland (1982) and Pendland and Boucias (1982).

Larval populations of the green cloverworm (GCW), Plathypena scabra (F.), occasionally reach economic levels in Iowa soybeans (DeWitt et al., 1980). Pedigo et al. (1983) showed that N. rileyi had characteristics of a key mortality factor in GCW larval populations, especially in generation two of outbreak configuration years in central Iowa. Because the pathogen is an important biotic factor in GCW regulation, disease development within larvae is of interest. The objective of this study is to histologically examine the development of N. rileyi in infected GCW larvae.
METHODS AND PROCEDURES

GCW larvae were reared from eggs obtained from field-collected moths. Moths were placed in a screened oviposition cage (30 x 30 x 30-cm) where females nightly laid eggs on roughened yellow blotter paper taped inside the glass top of the cage. Each morning, blotter papers with eggs were removed, cut into strips, and placed inside 0.5-1 paper ice cream cartons. Surface-sterilized soybean leaflets in Aquapics® were inserted through the carton bottoms to be used as larval food. A lid was placed over each carton, and the cartons were then maintained in a growth chamber at 18°C with 15-hour photophase and 80% to 90% relative humidity.

Third-stage larvae were brushed into a plastic cup containing N. rileyi conidia. As the cup was gently rotated, larvae tumbled inside and were inoculated with conidia. Larvae were individually brushed into separate 7-dr plastic, snap-top vials, each containing a piece of surface-sterilized soybean leaflet. The vials were maintained at room temperature.

At successive 12-hour intervals, two inoculated larvae were removed from their vials and fixed in 60°C alcoholic Bouin's fixative for one hour and then at room temperature for an additional 24 hours. Fixed specimens were stored in 70% ethyl alcohol. Dehydration and clearing were accomplished by passing the specimens through an alcoholic series and then through a series of methyl benzoate-benzene solutions. The initial passage through methyl benzoate was done under vacuum to aid in the removal of trapped air from specimens. Specimens were infiltrated with a 1:1 mixture of benzene and Paraplast®, evacuated, and embedded in Paraplast. Six μm
sections were cut on a rotary microtome and affixed with albumen fixative to slides by floating sections on 1.5% formalin.

Sections were stained by the periodic acid-Schiff reaction, aqueous technique (Humason, 1979). Following Mohamed et al. (1978b), two counter stains were used: Groat's hematoxylin for nuclei and indigo carmine in saturated aqueous picric acid as a general background stain. Stained sections were mounted in Kleermount® xylene solution under no. 1½, 24 x 50-mm cover glasses.
RESULTS AND DISCUSSION

The first evidence of conidial germination on GCW integument occurred in specimens fixed 12 hours post-inoculation. Similarly, conidia in contact with *P. includens* integument germinated in less than eight hours (Kish and Allen, 1978), and at between six and 18 hours on *A. gemmatalis* (Boucias and Pendland, 1982). Germination occurred two days post-treatment in experiments with *H. zea* (Mohamed et al., 1978b) and with the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Sutton et al., 1981).

Germ tubes directly penetrated the cuticle by 36 hours post-inoculation (Figure 1), but the extensive epicuticular and exocuticular lysis reported by Mohamed et al. (1978b) was not observed. Boucias and Pendland (1982) also did not find extensive lysis of the cuticle when hyphae penetrated the epithelium 24 to 48 hours post-treatment.

Laterally-branched hyphae grow just above the epidermis and parallel to the endocuticular laminae of the GCW by three and one-half days, post-inoculation (Figure 2). This phenomenon suggests some epidermal resistance to penetration by hyphal branches (Mohamed et al., 1978b). In specimens fixed four and one-half days after inoculation, hyphae had penetrated the epidermis (Figure 3), but hyphal bodies were not yet present in the hemocoel. No aggregations of hemocytes occurred at points of entry to the hemocoel, such as those observed by Sutton et al. (1981) in the fall armyworm where the insect immune system attempted to encapsulate or phagocytose invading hyphae.

One-to-four-celled hyphal bodies and hemocytes occurred together in the hemocoel by day five (Figure 4). Hyphal bodies are capable of
Figures 1-4. Penetration of *Plathypena scabra* cuticle by hyphae from germinated *Nomuraea rileyi* conidia and colonization of hemocoel

Figure 1. Germ tube (gt) from germinated conidium (gc) has penetrated cuticle (cu) 36 hours after inoculation (400x)

Figure 2. Laterally-branched hyphae (hp) grew parallel to endocuticular laminae (en) between epicuticle (ec) and epidermis (epd). Three and one-half days post-inoculation. he = hemocoel (1000x)

Figure 3. Four and one-half days post-inoculation, hyphae (h) penetrated the epidermis (epd) and grew into the hemocoel (he) (1000x)

Figure 4. Hyphal bodies (hb) and hemocytes (blood cells, bc) cohabited the hemocoel (he) by day five. There was no evidence of invasion of the gut epithelium (ge) nor muscle (m) tissues (250x)
digesting water-soluble blood proteins (Sutton et al., 1981), and, as their numbers increase, they cause extensive lysis of the hemocytes (Boucias and Pendland, 1982). Suspended in the hemolymph, hyphal bodies multiply by both a budding process and through septum formation (Boucias and Pendland, 1982) and circulate throughout the insect hemocoel. By this time, \textit{N. rileyi} hyphal colonization in \textit{Heliothis zea} (Boddie) and \textit{Heliothis virescens} (F.) brain tissue (Mohamed et al., 1978b; Mohamed, 1982, respectively) may have caused effects on feeding and digestion. However, there was no evidence of invasion of brain tissue in GCW larvae by day 5. Further, no evidence of neural or hormonal disruption of feeding and digestion was indicated in the results of Part II that compared soybean leaf consumption by \textit{N. rileyi}-infected GCW larvae with that by healthy larvae. In fact, until their deaths, infected larvae had consumption patterns similar to those of healthy larvae. By five and one-half days after inoculation, hyphal bodies were very abundant in the GCW hemocoel, and they became even more numerous by day six and one-half. Nevertheless, fat body, gut epithelium, striated muscle tissues (Figure 5), and brain tissue (Figure 6) were uncolonized by \textit{N. rileyi} hyphae. By this time, Mohamed et al. (1978b) had observed complete invasion of fat lobes, Malphigian tubules, muscles, mesenteron, and head capsule in \textit{H. zea}.

By seven days after inoculation, mycelium had completely ramified throughout all GCW larval tissues, including neural tissues, and had begun to emerge through the mummified cadaver's cuticle (Figure 7). As noted in previous observations (Mohamed et al., 1978b), the diameter of filamentous hyphae invading muscle tissue was less than that of hyphae found in
Figures 5-6. Colonization of hemocoel by *N. rileyi* six and one-half days post-inoculation

Figure 5. Hyphal bodies (hb) were abundant in the hemocoel. Gut epithelial (ge), fat body (fb), and muscle (m) tissues were, as yet, not invaded. (250x)

Figure 6. Hyphal bodies (hb) were abundant in the head capsule but there was no involvement of the brain (b) nor muscle (m) tissues after six and one-half days post-inoculation. (100x)

Figures 7-8. Invasion of larval tissues by *N. rileyi*

Figure 7. By seven days after inoculation, hyphae (h) invaded muscle (m) and gut epithelial (ge) tissues as well as the larval cuticle (cu). The contents of the gut (gct) are visible. (250x)

Figure 8. Seven and one-half days after inoculation, newly-formed conidia (c) were found on conidiophores. (250x)
the hemocoel. The mass of emerged mycelium gave the dead larva a completely white appearance. Often the larva died with the anterior portion of its body in an elevated position, a typical posture assumed by *N. rileyi*-killed cadavers. Seven and one-half days post-inoculation, newly formed conidia were visible on conidiophores (Figure 8). At this time, the GCW cadavers became completely covered by a velvety-green blanket of conidia (Figure 9). The complete developmental cycle of the pathogen in these GCW larvae took ca. seven and one-half days and closely followed the course of the pathogenic cycle observed in other noctuid larvae.
Figure 9. A scanning electron microscope portrait of a green cloverworm cadaver completely covered with conidiophores and conidia. (12x)
OVERALL CONCLUSION

The studies in this dissertation support the hypothesis explaining GCW population dynamics in Iowa (Pedigo et al., 1983; Buntin and Pedigo, 1983), viz., that GCW larval populations may be classified as having endemic or outbreak configurations. Studies reported herein also suggest that fungal epizootics interact predictably with these GCW populations. If large numbers of immigrant moths oviposit in soybeans in June and early July, the resulting large first larval generation has an outbreak configuration that may cause economic damage to full-bloom soybeans. The combined activity of natural biotic mortality agents is unlikely to keep this larval population below the economic injury level; however, the second GCW generation collapses under the mortality pressures of *N. rileyi* epizootics. Endemic GCW populations rarely cause economic injury to soybeans. The second generation in endemic years occasionally may be large enough to trigger a *N. rileyi* epizootic.

Analyses of 10 years of GCW collections in soybean provide several quantitative expressions that supplement the GCW hypothesis. The date of first *N. rileyi* occurrence may be predicted by regression equations that utilize the accumulated number of larva-days during the season and the square of the accumulated precipitation from 1 June as independent variables. Also, the seasonal increase in percentage *N. rileyi*-caused mortality in outbreak populations may be described as a function of the number of days after the date of initial detection of infected larvae. These equations should be useful in the construction of systems models of GCW population dynamics in Iowa.
Comparisons of the incidences of biotic mortality agents and GCW larval populations in alfalfa and soybean habitats support the hypothesis that alfalfa fields may act as "nurseries" for biotic mortality agents (Lentz and Pedigo, 1975; Roberts et al., 1977). Biotic mortality agents increase in numbers in alfalfa during the early season, and then migrate to habitats, such as soybean, where hosts may be more abundant. In the present report, early-season incidences of *N. rileyi*-infected larvae demonstrate a low-level infection of alfalfa GCW populations. As inoculum levels increase, alfalfa fields may act as "foci of infection" from which the pathogen is dispersed, thereby contributing to the inoculum load in soybean fields. GCW larvae in nearby soybean fields may thus be exposed to additional inoculum.

The seasonal reoccurrence of *N. rileyi*-infected larvae and data from this study support a hypothesis that *N. rileyi* conidia and sclerotia are likely to survive Iowa winters. Then, such reservoirs of *N. rileyi* inoculum can initiate mycoses in host larvae the next season. Studies in a bordering state, Missouri (Ignoffo et al., 1975b, 1978), support the hypothesis.

The effects of soybean tillage systems on GCW populations and on their biotic mortality agents was unknown in Iowa. Results of this study showed that concerns about increased GCW problems in reduced-tillage soybeans were unfounded. Significantly fewer GCW larvae were collected in till-plant and no-till soybean plots than in fall moldboard plow and fall chisel plow plots during the first year of the study. And, substantially more larvae were collected from plots that were fall moldboard plowed than from
reduced-tillage plots in the second year of the study. The reasons for the differences in tillage plot GCW numbers were not explained by analysis of GCW moth activity nor by the occurrence of natural biotic mortality agents, including *N. rileyi*. Nonetheless, because GCW numbers were not greater in reduced-tillage plots, soybean producers may be encouraged to adopt these systems to reduce soil erosion.

Because *N. rileyi* is a key mortality agent in GCW population dynamics in Iowa (Pedigo et al., 1983), the pathogenic cycle within infected larvae was followed under controlled laboratory conditions. Also, the effects of *N. rileyi* infection upon larval consumption of soybean leaf tissue were measured. Results showed that *N. rileyi* mycoses develop quickly within inoculated GCW larvae and cause death in ca. six to seven days. Histopathological examination of infected larvae revealed that colonization of the hemocoel occurs soon after penetration of the cuticle by invasive hyphae. Hyphal bodies, transported in hemolymph throughout the host's body, cause lysis of hemocytes and later form mycelium that invades all internal tissues. Death of larvae is followed by formation of conidiophores and conidia on the outside surface of the cadaver. Studies found little evidence of significant differences in GCW instar susceptibility to *N. rileyi*. However, during the infection period, inoculated larvae had consumption patterns that were very similar to those of healthy larvae, right up to the time of death. Therefore, population models may treat *N. rileyi*-infected larvae as healthy consumers until their deaths. Greater attention may be focused in the future on microclimatic factors affecting *N. rileyi* dispersal and infection rate.


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