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Simultaneous Parasitism of Field-Collected Green Cloverworm, *Hypena Scabra* (Lepidoptera: Noctuidae) Larvae by Endoparasitioids and an Entomopathogenic Fungus

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SIMULTANEOUS PARASITISM OF FIELD-COLLECTED GREEN CLOVERWORM, *HYPENA SCABRA* (LEPIDOPTERA: NOCTUIDAE) LARVAE BY ENDOPARASITOIDS AND AN ENTOMOPATHOGENIC FUNGUS

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ABSTRACT

The impacts of entomopathogens (e.g., fungi, bacteria, protists and viruses) on larval Lepidoptera and their associated insect parasitoids have been examined in laboratory studies but field studies of interaction between these two mortality factors are rare. We present field observations of concurrent insect parasitism and fungal disease infection in larvae of the green cloverworm, *Hypena scabra*, a sporadic pest of soybean (*Glycine max*) in North America. We reared ten parasitoid species from *H. scabra* larvae during our three-month study. Three parasitoid species were dominant and overlapped the period of infection by the entomopathogenic fungus *Nomuraea rileyi*: *Aleiodes nolophanae*, *Cotesia plathypenae* and *Campylochaeta plathypenae*. Two of the three parasitoid species, *Co. plathypenae* and *Ca. plathypenae*, completed development within *H. scabra* larvae infected by *N. rileyi*. Overall incidence of simultaneous parasitism and fungal infection was low, averaging 6.7% of *H. scabra* larvae parasitized by *Ca. plathypenae* and 3.3% of those parasitized by *Co. plathypenae*.

Interactions among insects and their natural enemies, particularly entomopathogens and insect parasitoids, are diverse and complex, and in many cases, not well understood. This complexity is especially apparent when entomopathogen and parasitoid simultaneously utilize a host insect: often both parasitoid and host suffer adversely from pathogen infection (Steinhaus 1954, Jaques and Morris 1981, Brooks 1993). Brooks (1993) noted that entomopathogens can impact parasitoids by making hosts ovipositionally unattractive, by infecting the parasitoid as well as the host, by producing toxic factors that kill parasitoids, by lowering the nutritional quality of the host, or by killing the host before the parasitoid has completed its development. Parasitoids infected by entomopathogens present in the shared host may die as larvae or suffer various sublethal and lethal effects as adults, such as malformations, reduced adult life span, and reduced fecundity (Brooks 1993).

A rich assemblage of insect parasitoids and entomopathogens, including bacteria, fungi, protists and viruses, attack larval Lepidoptera. Laboratory studies have examined the diverse effects of entomopathogens on host larvae and their associated parasitoids in various detail. Especially well studied are the interactions of entomopathogenic fungi with parasitoids. King and Bell (1978) examined the interactions between *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Helicoverpa* (= *Heliothis*) *zea* (Boddie) (commonly known as both the corn earworm and cotton bollworm), and the entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Sampson, in laboratory-reared larvae. *Helicoverpa zea* larvae parasitized by *M. croceipes* were more likely to be infected by *N. rileyi*; however, the fungus impaired development of *M. croceipes* larvae if host larvae were infected within 1 day after being parasitized (King and Bell 1978). In a similar study, Furlong and Pell (2000) investigated

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the effects of *Zoophthora radicans* (Brefeld) (Zygomycetes: Entomophthorales) on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and two of its larval parasitoids, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) and *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae). They showed that the longer the period between parasitization of diamondback moth larvae by the parasitoids and infection by the fungus, the greater the yield of parasitoid cocoons.

Perhaps as a response to potential adverse effects of entomopathogens, some parasitoids possess discriminatory behaviors that enable them to distinguish between infected and noninfected hosts (Versoi and Yendol 1982), while others may avoid pathogens temporally, completing their development when host infection by pathogens is low or nonexistent. Pathogens may also deplete host populations so that parasitoids are unable to locate hosts for oviposition, thereby leading to declines in parasitoid populations on local and regional scales. Parasitoid populations are more directly affected when large-scale epizootics destroy hosts that are parasitized, with the resulting death of the immature parasitoids (Brooks 1993). However, in some cases parasitoids appear to persist even when severe epizootics occur in their host populations (Brooks 1993).

It is important to note that not all parasitoid-entomopathogen interactions result in adverse effects on parasitoids. Synergism between *Cotesia* (= *Apanteles*) *melanoscelus* (Ratzeburg), a parasitoid of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), and the entomopathogen *Bacillus thuringiensis* Berliner, has been demonstrated in both the field and laboratory (Weseloh and Andreadis 1982, Weseloh et al. 1983). In this instance, infection of *L. dispar* larvae resulted in depressed larval growth rates, which in turn provided the parasitoids with hosts of a suitable size (i.e., 2nd and 3rd instars) for a longer period of time. The prolonged larval growth period produced increases in percent parasitism of caterpillars by *C. melanoscelus* and corresponding greater mortality of gypsy moth larvae (Weseloh and Andreadis 1982, Weseloh et al. 1983).

Much of the work on parasitoid-pathogen-host interactions has been laboratory-based and field observations are few. From the perspective of biological control of herbivorous pest insects, it is essential to identify actual and potential antagonisms between entomopathogens and insect parasitoids in order to maximize the effectiveness of biological control efforts.

We present the first observations of simultaneous insect parasitism and fungal disease infection in field-collected larvae of the green cloverworm, *Hypena scabra* (F.). The larval stage of *H. scabra* is a sporadic pest of soybean (*Glycine max* [L.]) in North America. This species does not appear to over-winter north of 41°N latitude but it migrates annually into this region each spring (Pedigo et al. 1973). In warm southeastern regions, breeding is essentially continuous year-round; typically only one or two generations of *H. scabra* occur in northern soybean-growing regions (Pedigo et al. 1973). *Hypena scabra* supports a parasitoid-entomopathogen complex consisting of over 10 species of parasitoids and the fungus *N. rileyi* (Pavuk and Barrett 1993, Williams et al. 1995). *Nomuraea rileyi*, sometimes referred to as the green mustardine fungus, is an entomopathogenic fungus that utilizes the larvae of lepidopteran species as hosts, including those species that are pests of soybean (Carruthers and Soper 1987). Caterpillars infected with *N. rileyi* become mummified and covered by a white mycelial mat. Conidiophores are produced on the mycelia and chains of ovoid conidia form; these vary from a yellow-green to blue-green in color (Boucias and Pendland 1998). When these conidia contact susceptible host caterpillar cuticle, attachment occurs, the conidia germinate, and germ tubes are produced, and hyphae penetrate the cuticle via proteases and chitanases (Boucias and Pendland 1998). Hyphal bodies of *N. rileyi* replicate within the hemocoel by budding and septation, and death due to infection of larvae by this fungus usually occurs 5-7 days after contact with infectious conidia. Only young larvae are infected by *N. rileyi;* last instar larvae, eggs, pupae, and adults are not usually infected by this fungus (Boucias and Pendland 1998).

MATERIALS AND METHODS

Our observations were made during a larger study of intercropping effects on *H. scabra* natural enemies conducted at the Miami University Ecology Research Center (Butler County, southwestern Ohio) from 27 July to 14 September 1990 (Williams et al. 1995). The objective of the study was to evaluate the effect of the larval endoparasitoid-*Nomuraea rileyi* complex on larval *H. scabra* populations in soybean monocultures and in soybean-sorghum strip cropping systems which varied by sorghum plant height and row pattern. Study plots, each 0.45 ha in area, were used to address this objective. Twelve experimental plots were established; four of these were soybean monocultures assigned randomly. The remaining eight plots had four different treatment combinations assigned randomly, with each treatment being replicated twice. The four treatments consisted of two different sorghum varieties (dwarf and tall) and two row patterns of sorghum and soybean (2 rows of sorghum alternating with 10 rows of soybean, and 6 rows of sorghum alternating with 6 rows of soybean). The possible combinations of these two factors were dwarf sorghum planted in two- and six-row combinations, and tall sorghum planted in two- and six-row patterns, for a total of four treatments. The treatments provided different levels of structural and taxonomic diversity; and allowed the testing of two major hypotheses. Soybean monocultures would be expected to be found and colonized more rapidly by *H. scabra* than cropping systems that included sorghum, or natural enemies would not be as effective in the monoculture, or both, resulting in this cropping system having the largest numbers of *H. scabra* larvae (Hypothesis 1). The monoculture treatment would exhibit the greatest plant apparency and also may lack some important requirements for the occurrence and effectiveness of natural enemies. Conversely, the tall sorghum planted in the six row pattern would be predicted to have the smallest *H. scabra* populations because of the greatest structural and taxonomic diversity; these characteristics would make the soybean plants more difficult to find and colonize, or enhance natural enemy activity, or both (Hypothesis 2).

Soybeans (variety 'Williams 82') and tall and dwarf sorghum varieties were planted using no-till methods on 1-3 June 1990. Row-spacing for both crops was 76 cm (30 in). Fertilizer (390 kg [AI]/ha urate of potash and diammonium phosphate) was applied to the plots immediately after planting. Herbicides were also applied to control weed populations. Basagran (BASF, Parsippany, NJ) was applied postemergence to all plots on 18 June at a rate of 0.95 kg [AI]/ha to control broadleaf weeds and sedges; Poast (BASF) was applied at a rate of 0.18 kg [AI]/ha on 25 June to soybean monocultures and soybean strips to control grass species; and Blazer (BASF) was applied at a rate of 0.24 kg [AI]/ha to soybean monocultures and soybean strips to control populations of broadleaf weeds, particularly ragweed species. Hand-pulling and cultivation were also employed to remove weeds from sorghum strips on a regular basis.

Larvae of *H. scabra* were randomly sampled from soybean plants using a ground cloth (Rudd and Jensen 1977) each week in the experimental plots from 27 July through 14 September for a total of eight samples. The ground cloth, 1-m in length, was positioned between two soybean rows, and the plants from both rows were shaken onto the cloth for ≈ 30 s. All *H. scabra* dislodged from the plants were counted and placed in plastic storage bags for transport to the laboratory. Five randomly selected soybean strips in each strip cropped treatment were chosen for sampling, and five randomly selected locations within each of these strips were sampled using the ground cloth. This provided a total of 25 samples per strip crop replicate on each sampling date. A random walk technique was used to choose sampling locations within soybean monocultures; 15 samples were taken from each monoculture on each sampling date.

Collected *H. scabra* larvae were maintained in individual, resealable plastic bags and fed surface-sterilized (0.5% sodium hypochlorite) leaflets from field-grown soybeans. Surface sterilization eliminated fungal spores, especially

those of *N. rileyi*, that could have caused erroneous conclusions (Daigle et al. 1988, Pavuk and Barrett 1993). Rearing bags were cleaned and soybean leaflets were replaced every 2 d. All larvae were maintained in controlled temperature chambers at 27ºC and a photoperiod of 14L:10D. Larvae were examined every 2 d for emergence of parasitoids, evidence of infection by *N. rileyi*, or pupation. Larval mortality rates were calculated by dividing the number of larvae dying from a specific cause by the number of larvae collected (Van Driesche 1983). Parasitoids emerging from larvae were sent to the appropriate specialists at the U.S. Department of Agriculture, Systematic Entomology Laboratory, Taxonomic Services Unit, ARS, Beltsville, MD., for identification to species. Larvae that succumbed to infection by *N. rileyi* were diagnosed by the appearance of yellow-green or blue-green spores on the surface of larval cadavers (Boucias and Pendland, 1998).

RESULTS AND DISCUSSION

We reared a total of ten parasitoid species from *H. scabra* larvae during the study (Table 1). Three species were dominant and overlapped the period of infection by *N. rileyi*: *A. nolophanae*, *Co. plathypenae* and *Ca. plathypenae* (Fig. 1). The data in Figure 1 represent a pooling of the results from Williams et al. (1995); the total numbers of *H. scabra* larvae parasitized by larval endoparasitoids and the fungus are depicted in the figure without indication of the different experimental treatments, i.e., soybean monoculture and soybean-sorghum strip treatment plots. Appearance of fungal-infected larvae did not take place until late July or early August in the experimental plots (Williams et al. 1995). All three species declined in abundance when incidence of *N. rileyi* infection rose in *H. scabra* larvae, but the decline was more abrupt and occurred earlier for *A. nolophanae* and *Co. plathypenae* than for *Ca. plathypena* (Williams et al. 1995). Of the three parasitoids, *Ca. plathypenae* exhibited the greatest overlap in occurrence with *N. rileyi*. All parasitoids that did emerge from *N. rileyi*-infected larvae did so within 3-4 days of host death.

Two of the three parasitoid species, *Co. plathypenae* and *Ca. plathypenae*, completed larval development within field-collected *H. scabra* larvae infected by *N. rileyi*. All parasitoids that did emerge from *N. rileyi*-infected larvae did so within 3-4 days of host death. Cocoons of *Co. plathypenae* were recovered from infected *H. scabra* larvae on two occasions (10 and 31 August sample dates) but no viable adult parasitoids emerged from them. Puparia of *Ca. plathypenae* were recovered from nine *N. rileyi* infected *H. scabra* larvae collected on three sample dates (24 August, 7 and 14 September) with five puparia (55.6%) producing live adults. The overall incidence of simultaneous parasitism and fungal infection was low, averaging 6.7% of *H. scabra* larvae parasitized by *Ca. plathypenae* (N = 135 larvae) and 3.3% of those parasitized by *Co. plathypenae* (N = 60 larvae). No instance of simultaneous fungal infection and parasitism by *A. nolophanae* was observed in field-collected *H. scabra* larvae. We recognize that some parasitoids may have succumbed to *N. rileyi* and would not have emerged from reared larvae and so our observations should be viewed as a conservative estimate of the degree of simultaneous fungal infection and insect parasitism in *H. scabra*. However, Boucias and& Pendland (1998) point out in their description of the biology of *N. rileyi* that this entomopathogenic fungus, unlike *Beauveria bassiana*, does not produce appreciable amounts of *in vivo* toxic metabolites during the time the fungus is developing vegetatively as hyphal bodies within the host hemolymph. Evidence for this was demonstrated by injecting cell-free hemolymph from infected *Spodoptera* exigua (Hubner) (Lepidoptera: Noctuidae) larvae into naïve larvae of the same species. The injected larvae did not suffer severe toxic effects from the injected hemolymph (Boucias and Pendland 1998). Therefore, perhaps at least some endoparasitoid larvae are able to complete development in *H. scabra* larvae infected by *N.* rileyi because this fungus does not produce appreciable amounts of toxins during the hyphal body stage of the life cycle (Boucias and Pendland 1998).

Table 1. Endoparasitoids Reared from *Hypena scabra* Larvae.

Species	Number of Parasitoids Reared	Percent of Larvae Parasitized by Each Species (n = 1522)
Hymenoptera: Braconidae		
Aleiodes nolophanae (Ashmead)	100	6.6
Cotesia plathypenae (Muesebeck)	58	3.8
Cotesia marginiventris (Cresson)	26	1.7
Diolcogaster facestosa (Weed)	15	1.0
Austrozele uniformis (Provancher)	2	0.13
Hymenoptera: Ichneumonidae		
Sinophorus teratis (Weed)	53	3.5
Charops annulipes Ashmead	2	0.13
Venturia nigriscapus (Viereck)	2	0.13
Diptera: Tachinidae		
Campylochaeta plathypenae (Sabrosky)	134	8.8
Winthemia sp. nr. *Sinuata*	3	0.20
Unknown Tachinidae	27	1.8

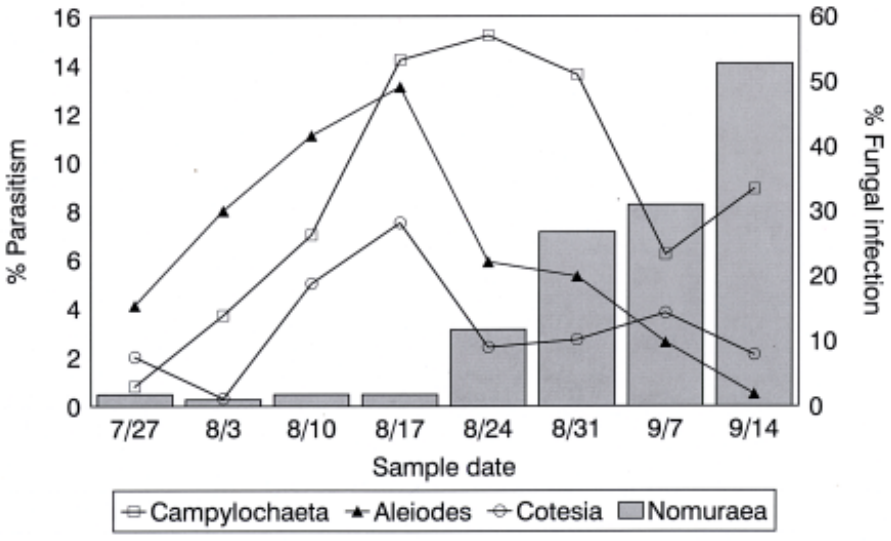

Figure 1. Incidence of insect parasitism and infection by the fungal pathogen *Nomuraea rileyi* in field-collected larvae of *Hypena scabra* from July to September 1990.

Thorvilson et al. (1985) noted decreased incidence of *A. nolophanae* during epizootics of *N. rileyi* in *H. scabra* populations, and speculated that reduced parasitism may be due to lowered host numbers, discrimination by the parasitoid, or antagonistic development between the pathogen and parasitoid. In our study, abundance of *H. scabra* larvae peaked from 3 to 10 August and declined gradually thereafter (Williams et al. 1995). Prevalence of *A. nolophanae* and *Co. plathypenae* parasitism during the early sample dates suggests that these parasitoids may restrict their activity to periods of greater host abundance, and in so doing, may inadvertently avoid overlap with *N. rileyi*. In contrast, *Ca. plathypenae* was most abundant later in the season when *H. scabra* populations were in decline and the incidence of *N. rileyi* infection was rising. Thus, the probability of encountering a host infected by *N. rileyi* would be greater for *Ca. plathypenae* than for *A. nolophanae* and *Co. plathypenae*, explaining in part the more frequent co-occurrence of *N. rileyi* and *Ca. plathypenae* in *H. scabra* larvae.

Results of this study indicate that there are at least a small number of parasitoids that are able to complete development within *H. scabra* larvae that are also infected by *N. rileyi*. Whether or not the proportion of larvae simultaneously parasitized by endoparasitoids and infected by an entomopathogenic fungus that also have parasitoids surviving successfully to the adult stage is large enough to have a significant impact on the ultimate numbers of adult parasitoids, and consequently the number of *H. scabra* larvae attacked by these parasitoids, remains to be determined. Detailed studies are needed to assess how discrimination of infected hosts by parasitoids, differential development of *N. rileyi* and parasitoids within the host, and (or) other factors in conjunction with phenology, may affect the balance of the parasitoid-entomopathogen complex of *H. scabra* and the population dynamics of this host.

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