SOME PROTOZOA INFECTING FIRE ANTS, *Solenopsis* SPP

Donald P. Jouvenaz

Insects Affecting Man and Animals Research Laboratory
Agricultural Research
Science and Education Administration
U.S. Department of Agriculture
Gainesville, Florida

I. INTRODUCTION

The black and the red imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*, are medically and agriculturally important insects that infest ca. 8×10^7 ha (2×10^8 acres) in the southeastern United States. Both species were introduced into the United States from South America at Mobile, Alabama; the black imported fire ant about 1918 and the red imported fire ant about 1940. *Solenopsis richteri* is now restricted to a relatively small area in northeastern Mississippi and northwestern Alabama; *S. invicta* infests Florida and Louisiana, and parts of North Carolina, South Carolina, Georgia, Alabama, Mississippi, Texas, and Arkansas. The tropical fire ant, *Solenopsis geminata*, may also be an introduced species; however, it has been a resident of this country for so long it is generally regarded as native.

The southern and desert fire ants, Solenopsis xyloni and Solenopsis aurea, are truly native to the United States. None of these latter three species are important as pests, except in Hawaii where S. geminata has been introduced.

II. RECENT EFFORTS TO IDENTIFY POTENTIAL PATHOGENS OF FIRE ANTS

Efforts to control the imported fire ants by chemical means have been the subject of serious controversy for more than 15 years (Lofgren et al., 1975). Consequently, surveys for pathogens of S. richteri were conducted in Mississippi (Broome, 1974) and of S. invicta in Florida (B. A. Federici, pers. commun.). These surveys failed to identify pathogens other than ubiquitous, non-specific, facultative organisms such as the bacterium Serratia marcescens. Recently, Jouvenaz et al. (1977) made an extensive survey in six states, the results of which confirmed the rarity of disease in these ants in the United States.

In a sample of 1007 S. invicta colonies from 285 collection sites in six states, only one colony was infected with a bona fide pathogen, a microsporidium. Since this parasite was found infecting four colonies of S. geminata (in a sample of 307) from collection sites in three Florida counties, it seems probable that S. geminata is the normal host. (We have transmitted one parasite of S. geminata, the microsporidium Burenella dimorpha, to S. invicta in the laboratory; however, the infection does not persist in S. invicta colonies. Thus, the naturally occurring infection also may have been abortive.) Since the conclusion of the survey, several hundred additional colonies of S. invicta (primarily from central Florida) have been examined; all were devoid of pathogens.

Similarly, no potential pathogens were found in 83 colonies of S. richteri. (Although this sample was small it actually represents a more intensive sampling of this species because S. richteri is restricted to a very small area in northeastern Mississippi and northwestern Alabama.)

An unidentified endozoic yeast was associated with 93 (9.25%) of the colonies of S. invicta. These yeast cells occur free in the hemolymph of immatures and adults. With proper care, laboratory colonies harboring this organism can live quite well; however, with neglect or stress they appear to have elevated mortality rates. The yeast grows poorly on standard mycological media, but grows well in insect tissue culture media. Yeasts grown in vitro can be transmitted per os to healthy colonies of S. invicta. Taxonomic studies of the yeast and an evaluation of its pathogenicity and host range are in progress at this laboratory. (Endozoic yeasts have not yet been detected in fire ants in South America.)

Virus-like particles have been detected in S. geminata and in an undescribed Solenopsis sp. from Brazil (Avery et al., 1977). The pathogenicity of these viruses is as yet undetermined. Ultimately, we seek to establish a complex of natural enemies (including enemies othern than pathogens) that will exert continuing stress or control of the fire ants. To be of value, pathogens and other natural enemies of fire ants need not be rapidly fatal to colonies or to large numbers of individual ants. Debilitating diseases may enable our native ants which compete with fire ants to do so more successfully. Surveys for natural enemies and extensive ecological studies over a period of several years in South America are planned.

The role of pathogens in fire ant population dynamics in South America is unknown. Indeed, little is known even about fire ant population densities in various habitats.

The incidence of disease among colonies in fire ants in South America may be limited, at least in part, by the dry season, which presumably is a period of stress. Reasonably, then, one would expect higher mortality among diseased colonies than among healthy colonies during this time. Thus, the overall incidence of disease may be lowest at the end of the dry season. During the wet season, the environment presumably is much more favorable so the numbers of colonies and, after a lag period, the incidence of disease, may increase. Recurrence of the dry season would prevent disease from reaching epizootic proportions.

In the United States, extended dry seasons comparable to those of Mato Grosso do not occur, though our winters may exert similar control of epizootics. Nevertheless, severe, extended winters do not occur in much of the area infested by fire ants in the United States. Other (unknown) abiotic or biotic factors that limit the incidence of disease in South America may also not be operative in this country. We plant to conduct studies of population dynamics of fire ants and their pathogens and natural enemies in South America. However, we are very cautious in extrapolating the findings to our different environment.

The imported fire ants in the United States present the classic situation for imported pests that have been freed of natural enemies. The identification, importation, and establishment of a complex of natural enemies may ameliorate this problems.

III. PROTOZOAN PATHOGENS

The first observation of a protozoan infection in fire ants was made by W. F. Buren during a taxonomic study of S. invicta

from Mato Grosso, Brazil (Allen and Buren, 1974). While he was examining alcohol-preserved specimens, Buren observed subspherical, cyst-like bodies in the partially cleared gasters of worker ants. These bodies were found to contain spores of a microsporidium that was subsequently named Thelohania solenopsae by Knell et al. (1977) who described the parasite from fresh material. Soon after this initial observation, Allen and Silveira-Guido (1974) reported similar microsporidia infecting S. richteri in Uruguay and Argentina and an unidentified Solenopsis sp. In Uruguay.

T. solenopsae infects fat body cells of workers and sexuals and the ovaries of queens. Infected cells hypertrophy to form the cysts observed by (Allen and Buren, 1974). The disease is not rapidly fatal, but the destruction of the fat body that occurs results in premature death of adult ants. Consequently, colonies are debilitated.

T. solenopsae is the only member of its genus that is dimorphic (Knell et al., 1977). Two types of spores develop simultaneously in the same tissues: uninucleate, nonmembrane-bounded (MB) spores in octets, and binucleate, membrane-bounded (NMB) spores. Of the remaining 7 genera of the family Thelohaniidae, at least two, Parathelohania and Amblyospora, are dimorphic; however, their NMB spores are uninucleate (Hazard and Oldacre, 1974).

Atempts to transmit T. solenopsae per os failed (unpubl.).

T. solenopsae has been detected in 22 described and undescribed species of fire ants in South America (Jouvenaz et al., 1977). In many areas, it infects 25% or more of the colonies; other pathogens, including protozoa, also occur in these ants. However, there are no data concerning the effects of diseases on fire ant populations in South America.

Four species of microsporidia and one neogregarine are known to infect S. geminata in the southeastern United States (Jouvenaz et al., 1977). One microsporidium, Burenella dimorpha, was recently described by Jouvenaz and Hazard (1978) was the type species of a new genus that represent a new family (Burenellidae). This family is characterized by two sequences of sporogony that produce morphologically different sporonts and spores. One sequence has disporous sporonts that sporulate in the hypodermis, producing binucleate NMB spores. The second sequence has multi-nucleate sporonts that produce 8 uninucleate MB spores in fat cells. The species of Burenellidae are distinguished from the dimorphic species of Thelohaniidae by the mode of formation of NMB spores. The NMB spores of Thelohaniidae arise from disporous sporonts, while in Thelohaniidae a variable number (6-40) of NMB spores arise from

plasmodia. Also, the MB sporonts of species of Burenellidae do not secrete granules in the lumen of the pansporoblast as do those of Thelohaniidae. In B. dimorpha, the pansporoblastic membrane enclosing the spores in the second sequence is fragile and sub-persistent, and consequently MB spores are not seen in octets by light microscopy. NMB spores begin development earlier in the course of infection than do MB spores and always predominate in number. Typically, they constitute ca. 60-70% of the total spores in advanced infections, but occasionally may constitute more than 99% of the spores in individual ants.

The cycle of B. dimorpha infection within an ant colony is as follows: NMB spores develop in the hypoderm, which is destroyed, producing clear areas in the heads, petioles, and gasters of pupae (Fig. 1). As the infection progresses, the cuticle becomes very fragile and eventually ruptures. The adult ants cannibalize these ruptured pupae but do not ingest the spores. Instead, the spores, together with other particulate matter, are diverted to the infra-buccal cavity and formed into an infrabuccal pellet. This pellet is expelled and placed on a specialized anteroventral area, the praesaepium of fourth-instar larvae. The praesaepium, which bears spines specialized for holding solid food while the larva feeds, is not present on earlier instars, which feed only on liquids. Because of this method of feeding, the fourth-instar larva is the only stage which is vulnerable to infection. Both spore types are ingested, but only the NPMB spore is infective. The PMB spores are expelled unextruded in the meconium upon pupation.

The function of the PMB spore remains unknown. A most attractive hypothesis is that it either infects an alternate host or is primed in the gut of a mechanical vector for extrusion upon subsequent ingestion by ant larvae. Either would explain the mode of intercolonial transmission of the infection (fire ants are territorial and aggressive toward conspecific ants) (Jouvenaz et al., 1981). Many candidate species exist for the role of vector; a large and varied arthropod fauna is associated with fire ants. Collins and Markin (1971) listed 52 species of insects that have been collected from fire ant nests; other invertebrates also occur. At least some of these organisms have symbiotic relation-ships with fire ants and are known to travel between fire ant nests (Wojcik, 1975).

B. dimorpha has been transmitted per os to S. xyloni, S. invicta, and S. richteri as well as to its normal host, S. geminata. All four species are readily infected by feeding them boiled egg yolk wetted with suspension of spores; however, the infection does not persist in colonies of S. xyloni, S. invicta, or S. richteri. Apparently the cuticle does not rupture as readily in these species, and the infection does not spread as

Fig. 1. Pathologic changes in pupae (left and right) infected with *Burenella dimorpha*. Note the clear areas in the occipital region of the head and petiole. The pupa in the center is uninfected.

efficiently to larvae. In other respects, the course of infection appears similar in all four species of ants.

The remaining microsporidia of *S. geminata* infect oenocytes and/or fat cells, causing hypertrophy but not formation of cysts as occurs in *T. solenopsae* infection. At least one of the three undescribed species of microsporidia of *S. geminata* is dimorphic, with the spore types developing in different fat body cells. Ants infected by these microsporidia survive to the adult stage. Similar microsporidia occur in fire ants in South America.

The neogregarine of *S. geminata* is a new species of *Mattesia* (Neogregarinida: Ophrocystidae). It develops in the oenocytes of the hypodermis, causing destruction of the hypodermis, melanization, and eye malformation of pupae (Jouvenaz and Anthony, 1979). The disease appears to be invariably fatal in the pupal stage of development. Attempts to transmit the infection *per os* (using fresh, aged, and variously treated spores) or by placing infected pupae in healthy colonies (conspecific pupae are adopted) have failed thus far. A similar or identical neogregarine also occurs in fire ants from South America.

The protozoan pathogens of *S. geminata* do not appear to offer promise as biological control agents for the imported fire ants, since *S. invicta* and *S. richteri* have been residents of the United States for ca. 40 and 60 years, respectively, and have not permanently acquired any of the diseases of *S. geminata*. Experimental laboratory and field transmission, where successful, have not produced infections that persisted and spread in the *S. invicta* population. Adaptation of a parasite of *S. geminata* to *S. invicta* through strain selection may, of course, be possible.

Our primary purpose in studying the diseases of *S. geminata* is to gain basic knowledge of the taxonomy and biology of fire ant pathogens, and to develop techniques for the propagation, study, and possible eventual introduction of exotic pathogens, especially those of *S. invicta* and the *S. invicta* complex.

IV. CONCLUSIONS

The red and black imported fire ants, *S. invicta* and *S. richteri*, are medical and agricultural pests that infest ca. 8×10^7 ha in nine southeastern states. In their native South America, these ants are parasitized by various protozoa and other pathogens; in the United States they appear to be essentially free of natural enemies. Thus, they present the classic situation with regard to imported pests, and the problem may be ameliorated by the introduction of a complex of natural enemies.

In South America, the most common pathogen of fire ants is *Thelohania solenopsae*. This microsporidium infects 20-25% or more of the colonies in many areas. The sites of infection are in the fat body cells of workers and sexuals and in the ovaries of the queens. The infected cells hypertrophy and form cysts. The disease is not rapidly fatal, but destruction of the fat body occurs, which results in premature death of adult ants. Consequently, colonies are debilitated.

The tropical fire ant, *S. geminata*, a species native to the United States, is the host of our microsporidia, of a neogregarine, and of a virus. These organisms lack potential as biological control agents for imported fire ants, but they are being studied as models.

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