A SURVEY FOR PATHOGENS OF FIRE ANTS, *SOLENOPSIS* SPP.,[1] IN THE SOUTHEASTERN UNITED STATES

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ABSTRACT

In a survey conducted in the Southeastern United States, one colony in a sample of 1,007 colonies of the red imported fire ant, *Solenopsis invicta* Buren, was infected with a microsporidium (Protozoa: Microsporida). The normal host of this parasite appears to be the tropical fire ant, *Solenopsis geminata* (F.). A benign or very mildly pathogenic yeast was associated with 93 (9.24%) of the *S. invicta* colonies, and was most common in areas which have been infested with this ant for the longest periods. No pathogens were associated with 83 colonies of the black imported fire ant, *Solenopsis richteri* Forel. The apparent rarity of bona fide pathogens in imported fire ants in the United States is in marked contrast to the abundance of pathogens in these and other *Solenopsis* spp. in South America.

Four species of microsporidia (possibly new genera) were detected in 22 (7.2%), 12 (3.9%), 6 (2.0%), and 4 (1.3%) of 307 colonies of the tropical fire ant, *S. geminata*. One colony of this species was infected by a neogregarine (Sporozoa: Neogregarinida). No pathogens were found in a small sample (53 colonies) of the Southern fire ant, *Solenopsis xyloni* McCook.

The first observation of a microsporidian infection in ants was made during a taxonomic study of the red imported fire ant, *Solenopsis invicta* Buren (Allen and Buren 1974). While examining alcohol-preserved specimens from Mato Grosso, Brazil, Buren observed subspherical, cyst-like bodies in the gasters of worker ants. Microscopic examination of the cysts showed that they contained spores of a new species of *Thelohania* (Protozoa: Microsporida), recently described by Knell et al. (1977). The discovery of this microsporidium renewed interest in the search for pathogens of fire ants. Earlier, limited surveys for pathogens of imported fire ants had been unsuccessful in northern Florida (B. A. Federici, Div. Biological Control, Dep. of Entomology, Univ. Cal., Riverside, Cal. 92502, personal communication) and in Mississippi (Broome 1974).

Following Buren's observation, Allen and Silveira-Guido (1974) reported microsporidian infections in the black imported fire ant, *Solenopsis richteri* Forel, from Uruguay and Argentina and in an unidentified *Solenopsis* sp. from Uruguay. Recently, Avery et al. (1977) reported virus-like particles in an undescribed *Solenopsis* sp. from Brazil. In addition to the infections in ants from South America, preliminary studies revealed two species of microsporidia and a neogregarine (Sporozoa: Neogregarinida) that infect the tropical fire ant, *Solenopsis geminata* (F.), in Florida (unpublished data). We, therefore, conducted a more extensive survey of *Solenopsis* spp. in the Southeastern United States to determine whether the *S. invicta* there is indeed free of disease and to identify the major microorganisms associated with other *Solenopsis* spp.

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METHODS AND MATERIALS

COLLECTION AND MAINTENANCE OF COLONIES.—Collections were made from a total of 1,007 nests of *S. invicta* from 285 sites in South Carolina, Georgia, Florida, Alabama, Mississippi, and Louisiana. In addition, we examined 83 collections of *S. richteri* from 22 sites in Mississippi and Alabama, 307 collections of *S. geminata* from 74 sites in Florida and Georgia, and 53 collections of the southern fire ant, *Solenopsis xyloni* McCook, from 11 sites in Georgia (Fig. 1).

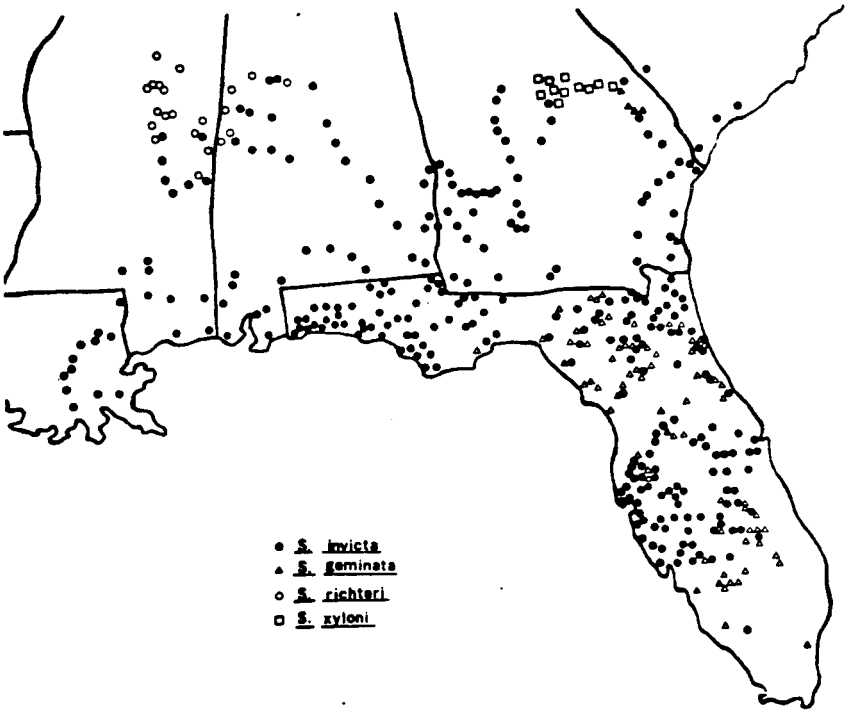

Fig. 1. Sites at which collections of fire ants, *solenopsis* spp., were made for survey for diseases.

Ants were collected by excavating mounds with shovels and transporting the soil and ants to the laboratory in plastic buckets. The inner surfaces of the buckets were dusted with inert talc to prevent escape of the ants. The buckets were left undisturbed overnight to allow the ants to reestablish tunnels and to collect buried immatures. Water was then slowly dripped from medical intravenous fluid tubes into the buckets. This procedure forced the ants to move to the surface of the soil as the water level rose. When the soil was completely submerged, the ants (including immatures which the workers rescued) floated or clung in masses to the sides of the bucket, and were easily transferred with a ladle to new talc-lined plastic tubs. In the new tubs the ants escaped desiccation by moving into moistened nests constructed of 90% plaster of Paris and 10% builder's

cement. All colonies were screened (described below) for pathogens and those suspected of being diseased were maintained for further study in plastic nests similar to those described by Wilson (1962). These colonies were fed our standard ant diet which is composed of macerated laboratory-reared insects, pureed beef, eggs, and vitamins in agar.

A sample of adult workers from each colony was preserved in 70% isopropanol for verification of species and for examination for gross abnormalities.

SCREENING FOR PATHOGENS.—A sample of 1,000-2,000 mixed adult and immature ants from each colony was triturated in a glass tissue homogenizer with water sufficient to just cover the mass of ants. One drop of the crude aqueous extract was placed on a microscope slide, covered with a 22 mm coverslip, and examined by scanning 5 different fields across the preparation at a magnification of 600X using a phase-contrast microscope.

The sensitivity of the procedure for detecting microsporidian spores was estimated by examining an extract prepared by triturating one diseased pupa of *S. geminata* with 499 disease-free pupae of *S. invicta* in 1.0 ml water. Two slides were prepared and each scanned 5 times. Since totals of 286 and 262 spores (57.2. ± 9.4 and 52.4 ± 4.8 per 22 mm scan, respectively) were obtained, microsporidian spores from a single infected specimen in our samples would be detectable with this method. We found this technique useful for detecting other microsporidia or associated yeasts present in low numbers. On occasion, ingested microsporidian spores were found in extracts, and care was taken, through examination of individual specimens, to determine whether infections actually existed within these colonies. Ingested spores are confined to the lumen of the gut and may be seen in intact larvae under low (150-300X) magnification. The presence of vegetative stages of a protozoan indicates infection.

The sensitivity of the procedure for detecting bacteria or occluded viruses is less certain. These organisms would probably not be detached unless they occurred in relatively high numbers. Unfortunately, we know of no rapid, sensitive method of screening large numbers of ants for these organisms. Observations of mortality or morphological abnormalities in individual specimens, particularly immatures, from laboratory and field colonies have been conducted on a continuing basis, and our insectary has been under surveillance for ca. 2 years for colonies having unusually high mortality. (Approximately 175-200 colonies are maintained in culture at all times with irregular turnover.)

Individual adult and immature ants from colonies selected by screening were examined by phase microscopy (whole gasters and smears) and by stained slides. Giemsa stained smears were prepared from the extracts and from squashes of individual ants by air drying the slides, fixing them in methanol for ca. 5 min, staining them with 10% Giemsa in buffered distilled water (pH 7.41) for 10 min, and rinsing with tap water. In addition, living immature ants from these colonies were examined with a dissecting microscope for abnormalities.

RESULTS AND DISCUSSION

Bona fide disease appears to be rare in imported fire ants in the United States. Only 1 microsporidian infection (possibly a new genus) was found in

1,007 colonies of *S. invicta* that were examined. Since this species of parasite infected 4 *S. geminata* colonies (in a sample of 307) from collection sites in 3 Florida counties, it is probable that *S. geminata* is the normal host. (We have transmitted 1 of the other microsporidian parasites of *S. geminata* to *S. invicta* in laboratory tests; it does not persist in *S. invicta* colonies, however.) Although they are of doubtful pathogenicity, gregarines were associated with only 5 colonies of *S. invicta*. The occurrence of a few fungal mycelia in a mass extract on one occasion is not significant since individual ants in thriving colonies may occasionally harbor nonspecific entomogenous fungi.

A benign or very mildly pathogenic unidentified yeast was associated with 93 (9.23%) of the *S. invicta* colonies. With proper care, laboratory colonies harboring this organism thrive; however, with neglect or stress such as pesticide screening, some colonies appear to have slightly increased mortality rates. The yeast cannot be cultured on standard mycological media, but some growth has been obtained in insect tissue culture media. In living ants, yeast cells are free in the hemolymph.

A relationship may exist between the incidence and geographic distribution of the yeast and the pattern of spread of the ant. In Mobile Co., Alabama, the introduction site of *S. invicta*, the yeast was present in 34 (60.7%) of 56 colonies. In 3 counties in northern Alabama, 6 (30%) of 20 colonies harbored this organism. In 9 northwest Florida counties and one adjacent Georgia county, it was present in 23 (26.7%) of 86 colonies. The incidence declined to 16 (13.7%) of 117 colonies from 6 northeast Florida counties; in 7 counties in southern Florida, 11 (8.9%) of 123 colonies were positive. These 2 areas in Florida probably were infested by disjunct ant populations which might have been introduced in nursery stock from Mobile. All 93 colonies which were positive for the yeast were found in 30 counties from which 424 colonies were collected. The remaining 583 colonies were collected in 140 counties or parishes. This suggests that the yeast may have been associated the longest with *S. invicta* in Mobile Co. and has spread less efficiently than the ants. We have not yet observed yeasts in fire ant colonies from South America.

Five colonies of *S. invicta* harbored yeasts other than the above type. These apparently benign microorganisms were observed in the guts of intact gasters also.

All colonies of *S. xyloni* and *S. richteri* that were examined were free of potential pathogens; however, the number of *S. xyloni* (53) samples was small The examination of 83 colonies of *S. richteri* actually represents more intensive sampling of this species than of *S. invicta*, since *S. richteri* is restricted to a very small area in northeastern Mississippi and northwestern Alabama. Our essentially negative results with the imported species agree with those obtained in Florida by Federici and in Mississippi by Broome.

Four species of microsporidia, probably in new genera, infected (respectively) 22 (7.2%), 12 (3.9%), 6 (2.0%), and 4 (1.3%) of the 307 colonies of *S. geminata* which were examined. A neogregarine (possibly a new species) which causes pupae to blacken and die infected 1 colony of this species. (In preliminary studies, we repeatedly collected the neogregarine in 1 locality, and 2 of the microsporidia in other localities. In the survey, we avoided areas in which we already knew diseases occurred in *S. geminata* in

order to better estimate the prevalence of disease in this species.) One microsporidium, *Burenella dimorpha*, which causes distinct symptoms and death to pupae has been described by Jouvenaz and Hazard (1977); the other microsporidia (as yet undescribed) cause increased mortality in adults in laboratory colonies and are under study.

The rarity of bona fide disease in imported fire ants in the United States is in striking contrast to the prevalence of disease in these ants in South America. In several trips to South America, we found 20-25% or more of the colonies infected with microsporidia, and other pathogens also occurred. Diseases have been found in 22 described and undescribed species of the *Solenopsis* complex in Brazil, Paraguay, Uruguay, and Argentina. (Due to uncertainties concerning the taxonomy of the ants and the pathogens, a report of the results obtained in exploratory trips to date would be premature.) Our present populations of imported fire ants are evidently descended from 1 or a few healthy colonies of each species. The introduction of pathogens from South America, the possible use of the pathogens of our native species, and perhaps the discovery and use of other types of natural enemies of *Solenopsis*, may reduce the pest status of imported fire ants to that of our native species.

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