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Long-Term Studies of the Black Imported Fire Ant (Hymenoptera: Formicidae) Infected with a Microsporidium

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ABSTRACT For 4 yr, we studied the host–pathogen relationship of the microsporidium *Thelohania solenopsae* Knell, Allen, and Hazard within field populations of the black imported fire ant, *Solenopsis richteri* Forel, in Argentina. We established and monitored 6 field plots having a high density of fire ant colonies, many infected with the microsporidium. The density of colonies, initially 162 colonies per hectare, decreased to 28 colonies per hectare. The percentage of infected colonies, initially 22.4%, increased to 35.7% and was negatively associated with the density of colonies but positively associated with rainfall. *T. solenopsae* was the only known natural enemy of fire ants present consistently and in high levels within the plots. We suspect it is one of the main factors responsible for decreases in *S. richteri* population densities.

KEY WORDS *Thelohania solenopsae, Solenopsis richteri*, fire ants, microsporidia, natural enemies, biological control

THE BLACK AND red imported fire ants, *Solenopsis richteri* Forel and *S. invicta* Buren, were introduced into the United States from South America in the 1920s and 1930s, respectively. The latter infests ≈10^8 ha in the southeastern United States and is a very serious medical and agricultural pest (Lofgren 1986). Potentially, it can move to the southern and southwestern states and the Pacific coast area (Vinson and Sorensen 1986).

Although many chemicals can control the ants locally, the area infested by fire ants is expanding because there are no natural limiting factors, except for weather. The imported fire ants should be vulnerable to classical biological control because they were introduced practically free of natural enemies present in their native land (Jouvenaz et al. 1977). If specific natural control organisms are limiting fire ant populations in South America, introduction of 1 or a complex of these organisms into the United States could reestablish the equilibrium lost in the new habitat.

Many surveys of diversity and abundance of natural enemies have been made in Brazil, Argentina, Uruguay and Paraguay (Allen and Buren 1974; Allen and Silveira Guido 1974; Williams and Whitcomb 1974; Jouvenaz et al. 1980, 1981; Jouvenaz 1983, 1986; Wojcik et al. 1987; Briano et al. 1995). Several organisms have been found, including pathogens (Protozoa, fungi, and viruses), parasites (nematodes, a parasitic ant, phorid flies, parasitic wasps), and several arthropod predators and inquilines (Whitcomb et al. 1973; Williams et al. 1973; Avery et al. 1977; Jouvenaz 1983, 1990; Nickle and Jouvenaz 1987; Williams and Banks 1987; Wojcik et al. 1987, 1991; Johnson 1988; Wojcik 1990).

However, the effect of none of these natural enemies was ever studied on field populations of fire ants. One of those organisms, the microsporidium *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporida: Thelohaniidae) was found to be the most common pathogen of fire ants in Buenos Aires Province, Argentina (Briano et al. 1995).

Our objective was to study the host–pathogen relationship within field populations of the black imported fire ant in Argentina during 4 yr. Information concerning population trends of healthy and infected colonies will help to determine the potential of *T. solenopsae* for biological control of imported fire ants in the United States.

Materials and Methods

Study Area. A preliminary survey showed that the area of Saladillo, Buenos Aires Province (180 km SW of Buenos Aires), had high densities of fire ant colonies and high prevalence of *T. solenopsae* (Briano et al. 1995); therefore, we selected that area for the field studies. The area is located within a region topographically flat and often with standing water in low areas because of poor drainage. Most of the land is used for livestock grazing in natural or improved pastures.

The only weather information available during the study period was monthly rainfall and number

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of days with frost (frost: 0°C or below for at least 1 h). We obtained this information from the Sociedad Rural of Saladillo, located 1 km from the plots. We considered rainfall between monitoring dates when analyzing the results. We obtained historical weather data for the periods 1951–1960 and 1971–1980 from the Servicio Meteorológico Nacional (1975, 1986).

Establishment of Field Plots. In October 1988, we established 6 circular plots (plots 1–6) ≈2–6 km north of Saladillo in 4 different pastures (7–10 yr old). Unfortunately, 2 plots (plots 1 and 2) were plowed in February and March 1989 and were monitored for only 3 mo; consequently, we deleted them from the study. We replaced the lost plots with 2 new plots (plots 7 and 8). In addition, plots 6 and 8 were plowed at the end of 1990. The size of each plot was 1/8 ha (40 m diameter). They represented an area of ≈4 km^2. Based on cuticular hydrocarbon studies conducted at the USDA–ARS Medical and Veterinary Entomology Research Laboratory, Gainesville, FL, the fire ant species present was identified as *S. richteri*.

Monitoring the Plots. We examined the plots every 30–50 d from October 1988 to July 1990 and every 60–90 d from April 1992 to January 1993. We monitored plots 3, 4, and 5 during the complete study period; plot 6 from October 1988 to July 1990; plot 7 from May 1989 to January 1993, and plot 8 from May 1989 to July 1990. The monitoring included measuring fire ant colony density and sampling each active colony for the presence of *T. solenopsae* infection (see below).

Density of Colonies. We identified, numbered, and sampled 1,348 active colonies during the study period within all the plots. We report the densities of colonies per plot and, to be more meaningful, per hectare.

Infection with T. solenopsae. We sampled 1,000–2,000 ants from each active colony by collecting them in a vial dusted with talc to prevent escape of the ants. We kept the vials on ice in a cooler for transportation to the laboratory, where we killed the ants by freezing. Then we placed them in a glass tissue grinder with 2–4 ml of water and ground them for ≈30 s. We examined 1 drop of the aqueous extract using phase-contrast microscopy (400×) and recorded the number of healthy and infected colonies for each plot. We also recorded the prevalence of other known natural enemies of fire ants.

Statistical Analysis. Data were analyzed with Minitab Statistical Software (1991). We used linear regression analysis to relate the number (and percentage) of infected colonies to the density of total colonies and rainfall and also to determine the relationship between density of colonies and rainfall. We report means with ±1 SD.

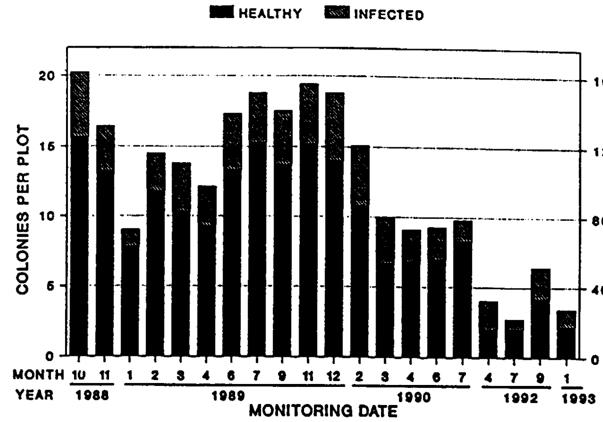

Fig. 1. Mean density of colonies of *S. richteri* within Saladillo plots for each monitoring date.

Results

Density of Colonies. The mean density of colonies for each monitoring date decreased from 162 colonies per ha in October 1988 to 28 colonies per ha in January 1993 (Fig. 1). However, the density showed cyclic variations; the highest densities (120–162 colonies per ha) occurred in October through November 1988 (spring) and June 1989 through February 1990 (winter–spring–summer). Medium densities (72–118 colonies per ha) were present in January–April 1989 (summer–fall) and March–July 1990 (fall–winter). The lowest densities (22–52 colonies per ha) occurred during the last period of study from April 1992 to January 1993 (fall–winter–summer). These cyclic variations were not seasonal. For example, the density of colonies was medium in January 1989 (summer), high in December 1989–February 1990 (summer), and low in January 1993, whereas it was high in October–November 1988 (spring) and July–August 1989 (winter) (Fig. 1). For individual plots, the mean densities during the study period ranged from 55±37 colonies per ha (range, 8–104) in plot 7 to 193±74 colonies per ha (range, 104–350) in plot 6 (Table 1).

There was no linear relationship between density of colonies and rainfall between monitoring dates ($r^2 = 0.10$; $F = 1.27$; df = 1, 11; $P = 0.28$). Annual rainfall was stable and very similar to historical data for the periods 1951–1960 and 1971–1980. The mean number of d with frost during 1988–1993 was lower than in the historical data (Table 2).

Infection with *T. solenopsae*. The number of infected colonies in each monitoring date is presented in Fig. 1. The mean percentage of infection was 22.4% at the beginning of the study, increased to a maximum of 50% in April 1992, and finally, was, 35.7% at the end of the study. The mean percentage of infection during the complete study ranged from 9.1±10.5% (range, 0–38%) in plot 3 to 34.9±19.0% (range, 17–75%) in plot 4 (Table 1). The density of infected colonies within each individual plot was variable. In plots 3, 5, and 7, *T. solenopsae* was not detected on some monitoring dates, whereas in the other plots it was always present.

Table 1. Density of colonies of *S. richteri* and infection with *T. solenopsae* during the study period within Saladillo plots

Density of colonies, % infection	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Per plot	7.5 ± 2.9 (0–14)	11.2 ± 5.8 (2–26)	15.0 ± 9.0 (1–30)	24.2 ± 9.6 (13–44)	6.9 ± 4.8 (1–13)	12.8 ± 4.4 (5–20)
Per hectare	60 ± 22 (24–111)	90 ± 45 (16–207)	122 ± 71 (8–239)	193 ± 74 (104–350)	55 ± 37 (8–104)	102 ± 35 (40–159)
% infection	9.1 ± 10.5 (0–38)	34.9 ± 19.0 (17–75)	30.8 ± 17.9 (0–67)	19.1 ± 6.8 (7–28)	18.6 ± 23.0 (0–67)	32.6 ± 12.3 (14–50)

All values are mean ± SD (range).

The percentage of infection showed a weak negative association with the density of colonies (Fig. 2) ($y = 35.3 - 0.099x$; $r^2 = 0.21$; $F = 4.80$; df = 1, 18; $P = 0.04$). This means that the higher the density of colonies, the lower the proportion of infected colonies.

The proportion of infected colonies showed a positive linear relationship with rainfall (Fig. 3) ($y = 8.49 + 0.15x$; $r^2 = 0.60$; $F = 16.13$; df = 1, 11; $P = 0.002$).

We found other natural enemies of fire ants within the plots during the study period. The microsporidium *Vairimorpha invictae* Jouvenaz and Ellis (Microsporida: Burenellidae) was present in 1.8% of the colonies; the parasitic ant *Solenopsis daguerrei* Santschi (Hymenoptera: Formicidae) was found in 1.4% of the colonies, and the fungus *Myrmecomyces annellisae* Jouvenaz and Kimbrough (Deuteromycotina: Hyphomycetes) was detected in 0.7% of the colonies.

Discussion

Density of Colonies. The decrease in the density of colonies was substantial (83%) during the study period. This reduction in an area heavily infected with *T. solenopsae* is consistent with the hypothesis that natural enemies limit fire ant populations in South America (Porter et al. 1992).

We believe that the reduction in fire ant density was not caused by colony movement. Although fire ants move their mounds very frequently (Horton 1973, Wojcik 1986, Briano et al. 1995a), we presume that movement of colonies outward from the plots was balanced with inward movements from outside the plots. In other words, fire ant colony movement should not have had any effect on colony density.

It seems unlikely that the reduction in the fire ant density observed within the Saladillo plots was caused to the lack of energy sources. Tennant and Porter (1991) showed that the red imported fire ant, *S. invicta*, and the fire ant, *S. geminata* Forel, feed on several food sources such as insects, homopteran honeydew, plant sap, plant nectars, and seeds. Although studies on feeding habits of *S. richteri* in Argentina are lacking, it is unlikely that there are major differences between *S. richteri* and *S. invicta*; both species hybridize naturally in the United States producing fertile progeny, so their taxonomic status as separate species is under discussion. More research is needed in this area.

Preliminary bait trap studies on ant fauna in the area of Saladillo showed that *S. richteri* was the predominant ant species in the area (J. B., unpublished data). This would show that some degree of interspecific competition was present but probably was not important. This deserves further investigation.

The reduction in the density of fire ant colonies observed during the study did not correspond to any important change in the rainfall for that period. In addition, winters during the study period were mild and more favorable for fire ant reproduction because the imported fire ants cannot withstand freezing conditions for long periods (Vinson and Sorensen 1986). Unfortunately, other useful climatic information such as temperature and humidity was not available for the area of Saladillo. This limits our ability to make conclusions about the potential effects of climatic conditions on colony density during the study period.

Natural reductions in fire ant densities are not reported for the United States. Comparison of fire ant densities reported by other authors for other countries is very difficult because of the differ-

Table 2. Weather information for the area of Saladillo during the study period compared with historical data

	Study period[a]						Historical data[b]	
	1988	1989	1990	1991	1992	Mean 1988–1992	Mean 1951–1960	Mean 1971–1980
Annual rainfall, mm	996	723	1,135	950	976	956	934	959
No. days with frost	18	7	19	11	6	12	17	26

[a] Source: Sociedad Rural de Saladillo (unpublished data).
[b] Source: Servicio Meteorologico Nacional (1975 and 1986).

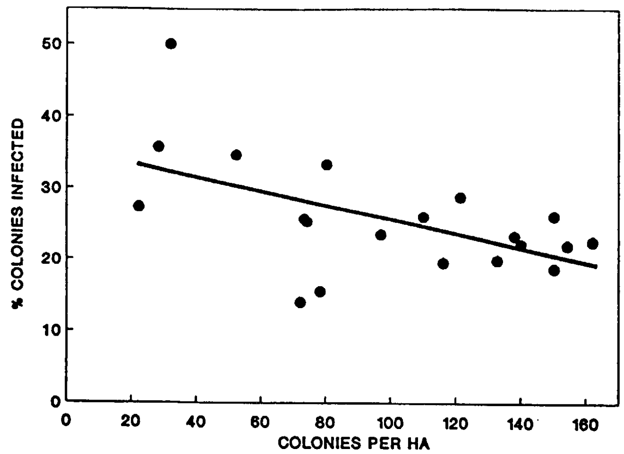

Fig. 2. Association between the density of colonies of *S. richteri* and the percentage of infection with *T. solenopsae* within Saladillo plots.

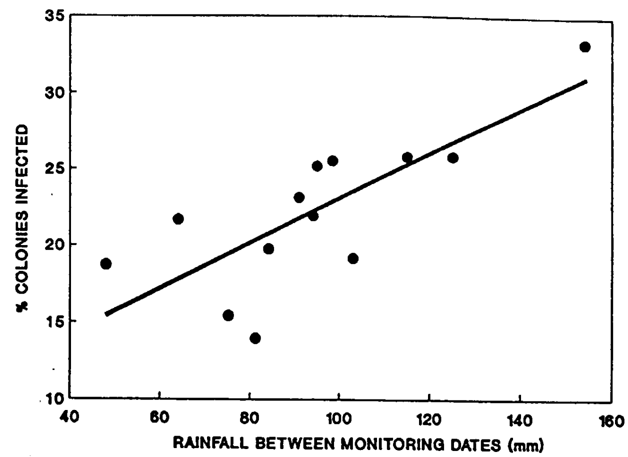

Fig. 3. Association between rainfall and the percentage of colonies of *S. richteri* infected with *T. solenopsae* within Saladillo plots.

ences in ecology, development, use of the land, and sampling methodology. In this particular case, comparison is even more difficult because no similar long-term study on fire ant population trends has been conducted in the United States. However, the low densities of fire ants reported here for the end of the study agreed with those of Porter et al. (1992) who, in the only work based on uniform sampling procedures across countries, reported that fire ant densities were much higher in the United States than in Brazil. They concluded that one of the reasons for this was the abundance of natural enemies in South America.

Infection with *T. solenopsae*. The percentage of infection of the black imported fire ant in Saladillo was very high and increased during the study period (Table 1; Fig. 1). The consequence that the weak negative association between the percentage of infection and the density of colonies (Fig. 2) has on the growth of the fire ant population remains unclear. If in a given place under natural conditions fire ant densities increase, it would be desirable that the percentage of infection with *T. solenopsae* also increases. The capacity of this microsporidium to spread within fire ant populations in expansion should be investigated.

The positive relationship between the proportion of infected colonies and rainfall (Fig. 3) is consistent with the findings of Franz (1971) and Tanada and Kaya (1993), who reported that rainfall (and probably humidity) favors infection with protozoan parasites by preventing spore desiccation and by increasing the persistence and dispersal of the spores in the environment.

The percentages of infection with *T. solenopsae* found within the Saladillo plots are one of the highest ever reported for this pathogen in South America. We probably underestimated the presence of this pathogen because we could have missed light infection rates during our examination. Levels of infection were much higher than the ones reported by Jouvenaz et al. (1980), Jouvenaz (1986), Wojcik (1986), and Wojcik et al. (1987) for *S. invicta* in several surveys made in Mato Grosso and Mato Grosso do Sul, Brazil, from 1976 to 1986. In sampled colonies, they reported percentages of infection with this pathogen of 2.2–11.4%. It must be confirmed if the microsporidia of *S. invicta* from Brazil and *S. richteri* from Argentina are the same species. The possible presence of a complex of 2 or more sibling species of microsporidia on different fire ant hosts is under investigation.

The *T. solenopsae* infection was the only fire ant natural enemy we found consistently and in high levels within the plots. The prevalence of other known potential control agents (*S. daguerrei* and *M. annellisae*) was occasional and insignificant.

Our results, combined with additional evidence we have reported (substantially smaller size of colonies infected with *T. solenopsae* compared to healthy colonies, fewer number of sexual broods in infected colonies [Briano et al. 1995b], and a higher mortality rate of infected colonies compared with healthy colonies under laboratory conditions [J. B., unpublished data]), suggest that the infection with *T. solenopsae* has a detrimental effect on populations of *S. richteri* in Argentina. Although additional studies on pathobiology, taxonomy, and specificity are essential to confirm this microsporidium as a good biocontrol organism, its potential for biological control of the black and red imported fire ants in the United States is promising.

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