PROTOZOAN AND FUNGAL DISEASES IN *SOLENOPSIS RICHTERI* AND *S. QUINQUECUSPIS* (HYMENOPTERA: FORMICIDAE) IN BUENOS AIRES PROVINCE, ARGENTINA

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ABSTRACT

The diversity and abundance of protozoa and fungi infecting colonies of the fire ants *Solenopsis richteri* Forel and *S. quinquecuspis* Forel were surveyed in Buenos Aires province, Argentina. A total of 185 roadside sites was selected, and 1,836 colonies were sampled and examined under phase-contrast microscopy. Pathogens were found at 32% of the sites and in 10% of the colonies. The microsporidium *Thelohania solenopsae* Knell, Allen & Hazard was the most common microorganism; it was present at 25% of the sites and 8% of the colonies. In some sites within the surveyed region, the percentage of infected colonies with *T. solenopsae* ranged from 40 to 80%. Other pathogens present were the microsporidium *Vairimorpha invictae* Jouvenaz & Ellis and the fungus *Myrmecomyces annellisae* Jouvenaz & Kimbrough. A field site was selected for future ecological studies.

Key Words: Microsporidia, fungi, natural enemies, biological control, imported fire ants, myrmecology.

RESUMEN

La diversidad y abundancia de protozoarios y hongos infectando colonias de las "hormigas coloradas" *Solenopsis richteri* Forel y *S. quinquecuspis* Forel fueron relevadas en la Provincia de Buenos Aires, Argentina. Un total de 185 sitios en banquina fueron seleccionados y 1.836 colonias fueron muestreadas y examinadas con microscopía de contraste de fase. Se encontraron patógenos en 32% de los sitios y 10% de las colonias. El microsporidio *Thelohania solenopsae* Knell, Allen y Hazard fue el más comun de los microorganismos encontrados, estuvo presente en el 25% de los sitios y en 8% de las colonias. En algunos lugares de la región relevada, el porcentage de colonias infectadas con *T. solenopsae* fue del 40 al 80%. Otros patógenos presentes fueron el microsporidio *Vairimorpha invictae* Jouvenaz y Ellis y el hongo *Myrmecomyces annellisae* Jouvenaz y Kimbrough. Fue seleccionado un sitio de campo para futuros estudios ecológicos.

The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were accidentally introduced into the United States from South America (Lofgren 1986b). *S. richteri* has been displaced by *S. invicta* and now occurs in a relatively small area of northeastern Mississippi and northwestern Alabama. *S. invicta* has spread dramatically and has become one of the most serious medical and economic pests in 11 southeastern States and Puerto Rico (Adams 1986, Lofgren 1986a & 1986b, Mackay et al. 1992, Vinson & Mackay 1990, Vinson & Sorensen 1986).

However, their present status as different species should be investigated because, at least in the United States, these two species cross and produce viable hybrids.

The black and red imported fire ants were introduced into the United States free of their major natural enemies present in their native land (Jouvenaz et al. 1977). Consequently, a classical biological control approach could be possible with a complex of pathogens, parasites, and/or predators being introduced into the North American fire ant populations.

In South America, several pathogens are known from *S. invicta*, *S. richteri* and other members of the *S. saevissima* Smith complex; some of them were surveyed for in Brazil, Uruguay and Argentina (Allen & Buren 1974, Allen & Silveira Guido 1974, Jouvenaz 1983, 1986, Jouvenaz et al. 1980 & 1981, Wojcik et al. 1987).

Since 1988, part of the USDA research on biological control of fire ants has been established at the USDA-ARS South American Biological Control Laboratory in Hurlingham, Buenos Aires Province, Argentina. Because the target species, *S. invicta*, does not occur within that area, the primary goal has been to search, select, and evaluate the potential for natural enemies of native *Solenopsis* spp. as biological agents for control of *S. invicta* in the United States.

The objectives of this work were to survey the diversity and abundance of pathogens in the area of the USDA laboratory and to select a convenient field study site to initiate ecological studies.

Materials and Methods

The northeastern region of Buenos Aires province was surveyed from March to September 1988. The area covered approximately 40,000 km^2. A total of 185 collecting sites was selected systematically every 10 to 50 km (depending on time available) along the roadsides of the major highways of the region. At each site, usually the first 10 colonies found on the roadside were sampled, which resulted in a total of 1,836 fire ant colonies being sampled. According to Trager (1991), the only fire ant species in that area are *S. richteri* and *S. quinquecuspis*. Although not all the samples were identified, we estimate that at least 80% of them were *S. richteri*.

The colonies were sampled by inserting a 7-ml vial into the mounds. The inner walls of the vials were dusted with talc to prevent escape of the ants. When several dozen ants had fallen into the vials, they were removed, capped and placed on ice in a cooler for transportation to the laboratory. Once in the laboratory, the ants were killed by freezing. Then each sample was placed in a glass tissue grinder with about 2-4 ml of water and ground up for about 30 s. One drop of the aqueous extract was examined under phase-contrast microscopy (400×) for the presence of spores of protozoa and fungi. The sensitivity of this procedure for detecting spores in low numbers has been successfully tested in previous research (Jouvenaz et al. 1977).

The main criteria in the selection of the field site for future ecological studies were: the incidence of diseases in the local fire ant populations, the type of habitat, and the proximity to the USDA laboratory. Arbitrarily, the limits of the region surveyed were established at 300 km from the USDA laboratory.

Results and Discussion

The region surveyed and the approximate location of most of the pathogen-positive sampling sites are shown in Fig. 1. Of the total of 185 sites, protozoa and fungi were found at 59 (32%) of the sites (Table 1). The most common pathogen was the protozoan *Thelohania solenopsae* Knell, Allen, & Hazard (1977) (Microsporida: Thelohaniidae),

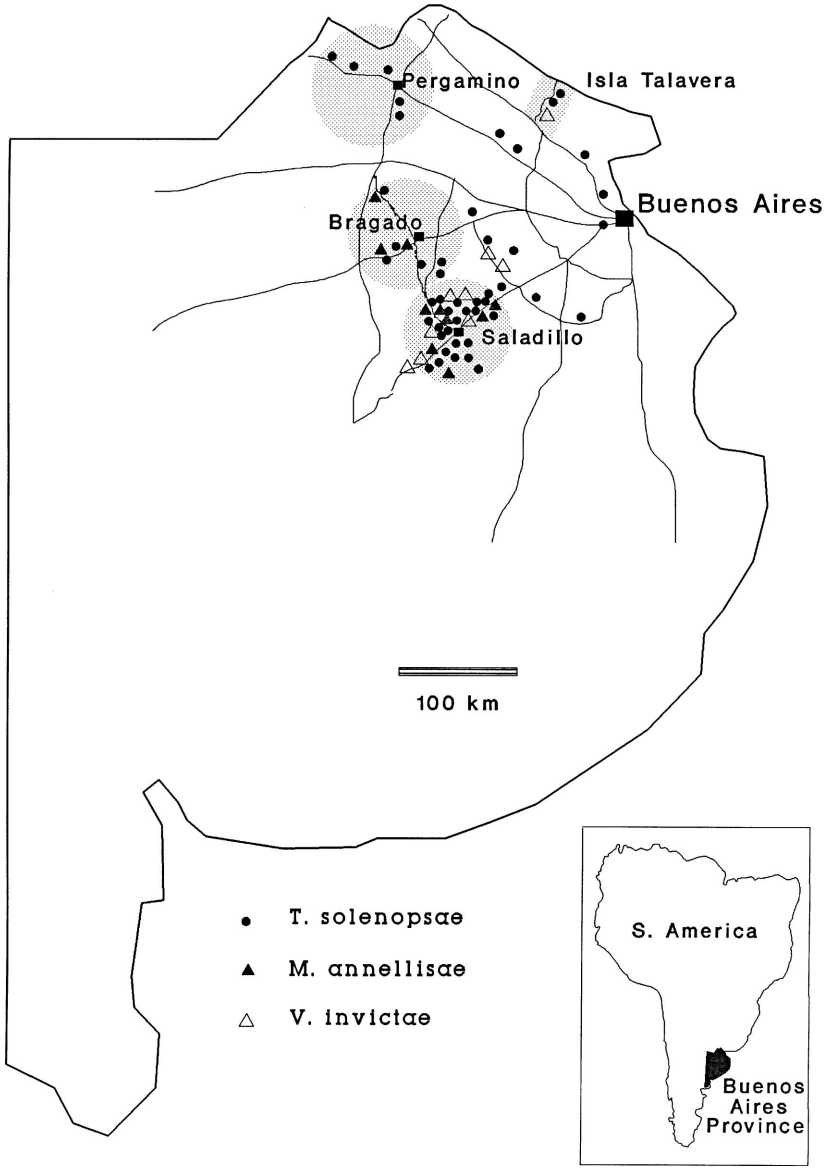

Figure 1. Region surveyed in Buenos Aires province and location of most of the pathogen-positive sites. Shaded areas show the main sampling areas.

it was present at 46 (25%) of the sites. The fungus *Myrmecomyces annellisae* Jouvenaz & Kimbrough (1991) (Deuteromycotina: Hyphomycetes) was found at 14 (8%) of the sites and the protozoan *Vairimorpha invictae* Jouvenaz & Ellis (1986) (Microsporida:

TABLE 1. PATHOGEN-POSITIVE SAMPLING SITES WITHIN AREA SURVEYED IN BUENOS AIRES PROVINCE.

Area	Number of Sampling Sites	Number (%) of Sampling Sites with Pathogens			
		T. solenopsae	*V. invicta*	*M. annellisae*	Total[1]
Saladillo	49	23 (47)	6 (12)	7 (14)	32 (65)
Isla Talavera	6	2 (33)	1 (20)	0	3 (50)
Bragado	27	5 (18)	0	3 (11)	6 (22)
Pergamino	24	5 (21)	0	0	5 (21)
Others	79	11 (14)	2 (<3)	0	13 (16)
Total	185	46 (25)	9 (5)	14 (8)	59 (32)

[1]Sites had >1 pathogen, so totals are lower than the sum of 3 columns.

Burenellidae) was found at 9 (5%) of the sites. At some sites, more than one pathogen was found infecting different colonies, but never the same colony. However, dual infections with *T. solenopsae* and *V. invictae* were observed within extremely stressed laboratory colonies (J. B., unpublished data).

According to the incidence of diseases, the region surveyed was arbitrarily divided into 4 main areas (Fig. 1): Saladillo (livestock grazing area), Isla Talavera (swampy area), Pergamino (annual crops area), and Bragado (mixture of annual crops and livestock grazing area). The area of Saladillo (180 km SW of Buenos Aires) had the highest incidence of pathogens with 65% of the sites infected (Table 1). The most common pathogen was *T. solenopsae*; it was present at 47% of the sites. The fungus *M. annellisae* was found at 14% of the sites, and *V. invictae* at 12%. The area of Isla Talavera (100 km NW of Buenos Aires) showed the second highest incidence of diseases with 50% of the sites infected; *T. solenopsae* was found at 33% and *V. invictae* at 20%. In the area of Bragado (224 km W of Buenos Aires) 22% of the sites were infected, 18% with *T. solenopsae* and 11% with *M. annellisae*. The area of Pergamino (250 km NW of Buenos Aires) had 21% of the sites infected with *T. solenopsae*. No other disease was found in this area. In other areas of the province, only 16% of the sites had diseases; *T. solenopsae* was present in 14% and *V. invictae* in less than 3%. (Table 1).

Of the 1,836 fire ant colonies sampled, 182 (10%) were infected, 8% with *T. solenopsae*, 1% with *V. invictae* and 1% with *M. annellisae* (Table 2). The highest percentage of infected colonies (33%) was found in the area of Isla Talavera, then in Pergamino (17%), Saladillo (12%), and Bragado (9%). In all these areas, *T. solenopsae* was the most common pathogen (Table 2). In the other areas of the region, only 4% of the colonies were infected.

At several collecting sites, *T. solenopsae* was found infecting a high proportion of colonies. For example: 80% at Rt. 8, km 254 (Pergamino); 66% at Rt. 8, km 247 (Pergamino); 50% at Rt. 205, km 180 (Saladillo); 47% at Rt. 12, km 104 (Isla Talavera); and 40% at Rt. 5, km 224 (Bragado). At one collecting site, *V. invictae* infected 60% of the colonies (Rt. 12, km 91, Isla Talavera). The prevalence of *T. solenopsae* and *V. invictae* in those sites represents the highest infection rates with these pathogens in fire ants ever reported for South America (See references below). However, the total percentages of colonies infected with these two microsporidia in the region surveyed (8 and 1%, respectively) were similar to those found in *S. invicta* in Mato Grosso and Mato Grosso do Sul, Brazil (2.2 to 11.4% and 2 to 4% of the colonies respectively) (Jou-

TABLE 2. PATHOGEN-POSITIVE COLONIES WITHIN AREA SURVEYED IN BUENOS AIRES PROVINCE.

Area	Number of Sampled Colonies	Number (%) of Colonies with Pathogens			
		T. solenopsae	*V. invicta*	*M. annellisae*	Total
Isla Talavera	90	24 (27)	6 (7)	0	30 (33)
Pergamino	175	29 (17)	0	0	29 (17)
Saladillo	613	49 (8)	12 (2)	14 (2)	75 (12)
Bragado	205	16 (8)	0	3 (<2)	19 (9)
Others	753	27 (<4)	2 (<1)	0	29 (4)
Total	1,836	145 (8)	20 (1)	17 (1)	182 (10)

venaz et al. 1980, Jouvenaz 1986, Jouvenaz & Ellis 1986, Wojcik 1986, Wojcik et al. 1987). In two surveys conducted in 1987 in the provinces of Buenos Aires, Santa Fe, and Entre Rios, Argentina, 10.5% of the fire ant colonies were infected with *T. solenopsae* (D. J. & D. W., unpublished data).

The relatively high prevalence of fire ant pathogens in Argentina and Brazil contrasts with the lack of specific pathogens in the North American populations of the black and red imported fire ants (Jouvenaz et al. 1977). Surveys in areas infested with both species of imported fire ants in the United States revealed few colonies infected with pathogens (Lofgren et al. 1975). Jouvenaz et al. (1977) reported considerable variation in the rate of infection of *S. invicta* with *M. annellisae* in Florida, Alabama and Georgia. This is probably the only (specific?) microorganism imported into the United States in association with the imported fire ants.

The prevalence of pathogens reported in this paper was probably underestimated because the sampling of the fire ant colonies included only adult workers. Possibly early infective stages were present in immature fire ants and missed. In addition, other pathogens known to occur in fire ants in South America, such as bacteria and viruses (Avery et al. 1977, Jouvenaz 1983) were not detected with the light microscope. The prevalence of these other pathogens deserves further investigation.

We conclude that, within the region surveyed, the microsporidium *T. solenopsae* was the most common pathogen of indigenous fire ant populations. In some sites, it infected a high proportion of the colonies. Therefore, it should be evaluated as a potential biological control agent for the imported fire ants in the United States. Although the areas of Isla Talavera and Pergamino showed higher number of colonies infected with *T. solenopsae,* the area of Saladillo was selected as the field study site because: (1) it showed the highest number of sites infected with *T. solenopsae,* (2) it was the only area where the three pathogens were present, (3) it represented the most appropriate habitat for future long-term ecological studies, and (4) it was near the USDA laboratory and frequent field work would be simplified.

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