Relationship Between Colony Size of *Solenopsis richteri* (Hymenoptera: Formicidae) and Infection with *Thelohania solenopsae* (Microsporida: Thelohaniidae) in Argentina

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ABSTRACT We studied the association between the size and composition of field colonies of the black imported fire ant, *Solenopsis richteri* Forel, and the presence of the microsporidian disease *Thelohania solenopsae* Knell, Allen, & Hazard in Buenos Aires Province, Argentina. We compared volumes of infected colonies with those of healthy colonies within an area where infected colonies were common and with those of healthy colonies from an area free of the infection. We also compared presence of polygyny, numbers of queens, presence and abundance of sexuals, broods, and myrmecophiles. Mound volume of infected colonies was substantially smaller than that of healthy colonies from the same area and from the disease-free area (4.9 versus 14.7 and 18.7 liters, respectively). Polygyny, number of queens per colony, presence of worker broods, and presence of winged females and males did not differ significantly in infected and healthy colonies. Sexual broods were less frequent in infected than in healthy colonies (17 versus 37%, respectively). Presence of myrmecophiles was as frequent in infected as in healthy colonies, but the bug *Blissus parasigaster* (Bergroth) was more numerous in infected colonies. *T. solenopsae* may be a good candidate for the biological control of imported fire ants in the United States.

KEY WORDS *Solenopsis richteri*, fire ants, *Thelohania solenopsae*, microsporidium, colony size, biological control

THE BLACK IMPORTED fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were introduced into the United States from South America in the 1930s. Since then, *S. invicta* has become a serious economic pest in the southeastern states (Lofgren 1986) and its range will probably expand into the southwestern and Pacific coast states. Many effective toxic compounds have been evaluated and developed as fire ant baits. Application of those products over large areas is generally expensive, and long-lasting relief is difficult to obtain unless baits are applied several times a year.

According to Porter et al. (1992), fire ants are much more abundant in the United States than in South American and this is consistent with the hypothesis that *S. invicta* and *S. richteri* were introduced without any major natural enemies (Jouvenaz et al. 1977). This suggests that North American fire ant populations should be susceptible to classical biological control techniques. The microsporidian disease *Thelohania solenopsae* Knell, Allen, & Hazard (1977), an obligate intracellular pathogen, was discovered by Allen and Buren (1974) in

alcohol-preserved specimens of *S. invicta* collected in Mato Grosso, Brazil, in 1973. Later, this microsporidium was found to be the most common known pathogen in fire ants in Brazil and Argentina (Jouvenaz et al. 1980, Jouvenaz 1983, Wojcik et al. 1987; Briano et al., unpublished data). A preliminary field study on populations of *S. richteri* in Argentina indicated that decreasing densities of fire ants were associated with increasing presence of this pathogen (Briano 1993, Briano et al. 1995). Based on these population studies, we concluded that *T. solenopsae* might be an important weakening factor for fire ant colonies. The objective of the work described here was to compare size and mound colony composition of infected and healthy colonies of *S. richteri*.

Materials and Methods

Study Area. We conducted the study over 9 mo from April (fall) to December (late spring) 1992 in improved pastures (8–10 yr old) near Saladillo (180 km SW of Buenos Aires) and Las Flores (90 km SE of Saladillo and 190 km SW of Buenos Aires). Our preliminary work showed that a high percentage of the fire ant colonies within the area of Saladillo were heavily infected with *T. solenopsae*. In contrast, the fire ant population in the area of Las Flores was free of infection. Both areas are similar

in habitat, climate, and land use. They are flat and often have standing water over low spots because of poor drainage. Most of the land is used for livestock grazing in natural or improved pastures. Chemical taxonomy studies (gas chromatography of cuticular hydrocarbons from worker ants) done at the USDA–ARS Medical and Veterinary Entomology Research Laboratory at Gainesville, FL, showed that the fire ant species present in both areas was *S. richteri*.

Colony Attributes. We compared 3 basic colony attributes between infected and healthy colonies: (1) the volume of the mound (the aboveground portion of the fire ant nest); (2) the presence of polygyny and number of queens per colony, and the presence and abundance of broods and sexuals; and (3) the presence and abundance of myrmecophiles (parasitic or commensal arthropods associated with the fire ant colonies).

Mound Volumes. We measured the height, length, and width of the mounds of the 1st 96 colonies (in Saladillo) and 103 colonies (in Las Flores) found within random transects established in the pastures. We calculated the volumes with the formula of the hemispheroid $V = 2/3\pi ABC$ (V, volume; A, height; B, 1/2 length; C, 1/2 width [Porter et al. 1992]). We compared the volumes of mounds from Saladillo between infected and healthy colonies and between polygyne and probable monogyne colonies. We also compared the volumes of all mounds (infected and healthy) from Saladillo with the volumes of mounds from Las Flores.

Examination for Infection. In Saladillo, we dug up the 1st 76 colonies within the transect beginning in April (9 colonies), and then in June (9 colonies), July (4 colonies), August (14 colonies), September (11 colonies), October (19 colonies), and December (10 colonies). We put them in 10-liter buckets dusted with talc to prevent the escape of the ants and brought them to the laboratory; then we separated the ants from the soil with the flotation technique described by Banks et al. (1981). We took a random sample of ≈1,000 workers from each colony to detect the presence or absence of the infection. We killed the ants from these samples by freezing, then ground them in a glass tissue grinder with 2–4 ml of water. We examined the ants by placing 1 drop of the aqueous extract on a glass slide under a phase-contrast microscope (400×) to search for the presence of spores of *T. solenopsae*. We recorded the percentage of infection to detect possible seasonal differences in infection rates. We placed each infected and healthy colony separately in plastic trays (40 by 30 by 15 cm) dusted with talc to prevent the ants from escaping. We examined the colonies as soon as we separated them from the soil. Because the examination of each colony took 2–3 d, we held the other colonies at 5°C with food (house fly [*Musca domestica* L.] adults) and water until examination.

In December 1992 at Las Flores, we excavated 28 colonies selected systematically within the tran-

sect. We examined the ants as described above to confirm the absence of *T. solenopsae* in that area, and we also recorded the presence of multiple queens, winged females, and males.

Presence of Queens, Broods, Sexuals, and Myrmecophiles. In the laboratory, we examined all colonies from Saladillo to detect the presence and abundance of inseminated queens, worker broods, sexual broods, winged females, males, and myrmecophiles. We scored the abundance by using 4 categories: 0, none; 1, scarce; 2, common; and 3, abundant.

We separated the true queens from wingless females by dissecting and examining them under the microscope (6.4×) for a spermatheca full of sperm. We considered colonies with only 1 inseminated queen as *probable monogyne* and colonies with >1 inseminated queen as *polygyne*.

Statistical Analysis. Statistical tests were done with Minitab Statistical Software (1991). We used 2-sample *t*-test to analyze the number of queens per colony; 2-sample *t*-test and 1-way analysis of variance (ANOVA) to analyze the volumes of mounds (after normalization of the volumes using the square-root transformation); Chi-square test to analyze the proportion of polygyny, presence of sexuals, broods, myrmecophiles, and seasonal differences in infection rates; and Mann–Whitney *U* test to compare the abundance of sexuals, broods, and myrmecophiles. We report the means ± 1 SEM.

Results and Discussion

Infection Rates of *T. solenopsae*. In Saladillo, we found infected 43 (45%) of the 96 measured colonies and 41 (54%) of the 76 excavated colonies. We did not observe large seasonal differences in the infection rates. In fall, we found 67% of the colonies infected, in winter 59%, and in spring 48% ($\chi^2 = 1.009$, df = 2, $P > 0.5$). As expected, we did not find any infected colonies in the 103 colonies sampled in Las Flores.

Mound Volumes. The mounds of infected colonies were substantially smaller than mounds of healthy colonies from both Saladillo and Las Flores. The mean volume of mounds of infected colonies in Saladillo was 4.9 ± 1.0 liters (range, 0.3–31.4), whereas the volume of mounds of healthy colonies was 14.7 ± 1.8 liters (range, 0.1–54); the volume of mounds of colonies from Las Flores (all healthy) was 18.7 ± 1.7 liters (range, 0.4–65) ($F = 17.36$; df = 2, 196; $P < 0.0001$).

In Saladillo, all mound dimensions were smaller in infected compared with healthy colonies: length, 25.2 ± 1.7 cm (range, 10–60) versus 37.7 ± 2.0 cm (range, 10–75) ($t = -4.80$, df = 94, $P < 0.0001$); width, 22.0 ± 1.3 cm (range, 10–50) versus 32.0 ± 1.7 cm (range, 10–60) ($t = -4.71$, df = 94, $P < 0.0001$); and height, 12.1 ± 0.6 cm (range, 5–20) versus 17.8 ± 0.8 cm (range, 2–35) ($t = -4.88$, df = 94, $P < 0.0001$).

The mound volumes of polygyne and probable monogyne colonies were similar. The mean volume of mounds of polygyne colonies (infected and healthy) was 5.8 ± 1.5 liters, and the mean volume of mounds of probable monogyne colonies (infected and healthy) was 6.2 ± 0.8 liters ($t = -0.27$, df = 74, $P = 0.79$). These results contrast with results reported by Porter (1992), who observed smaller volumes for polygyne colonies of *S. invicta* in Florida. However, we are not certain whether we were dealing with a mixed population of monogyne and polygyne colonies or whether it was a polygyne population in which some queens were lost during excavation of the colonies. The mean volume of all mounds from Saladillo was significantly smaller than the volume of mounds from Las Flores (10.3 ± 1.2 versus 18.7 ± 1.7 liters; $t = -3.90$, df = 179, $P < 0.0001$).

Because mound volumes vary with (among other things) different soil types and weather conditions (Lofgren et al. 1975), a comparison of mound sizes among distant areas and different habitats is difficult. The volumes we report here for healthy colonies of *S. richteri* (14.7 liters) are similar to the volumes found by Porter et al. (1992) for *Solenopsis* spp. in Brazil (13.8 liters). For *S. invicta* in the United States, Porter et al. (1992) reported larger volumes (27.0 liters) than the ones we report here. Porter (1992) found that, in Florida, Louisiana, Georgia, and Texas, *S. invicta* has similar mound diameters (32–36 cm), a measurement that is comparable with the data we report for healthy colonies of *S. richteri*.

Knell et al. (1977) indicated that progressive destruction of the fat body could cause debilitation of the fire ant workers. This conclusion is supported by the general statement made by Issi (1986), who reported that microsporidian infections in insects can reduce field populations by the combination of a reduction of the insect functional energy, hormonal disbalance, and general decrease of activity of the host organism. In our study, *T. solenopsae* may affect the vigor of the fire ant colonies by reducing the number of workers or their activity (or both), as suggested by mound size. This biotic agent could be compounded by additional stress produced by other biotic factors such as predators, parasites, or other pathogens. Abiotic factors such as extreme temperatures or severe droughts, could debilitate the fire ant colonies or put them at a disadvantage in competition with other species of ants.

Number of Queens per Colony. Polygyny was common in both infected and healthy colonies from Saladillo and in the healthy fire ant population in Las Flores. In Saladillo, the percentage of polygyny was 41.5% in infected colonies and 25.7% in healthy colonies, but the difference was not significant ($x^2 = 2.323$, df = 1, $P > 0.1$). The mean number of queens per polygyne colony was 4.6 ± 1.2 in infected colonies and 7.2 ± 3.6 in healthy colonies. However, the difference was not significant ($t = -0.84$, df = 23, $P = 0.41$). In Las Flores, the percentage of polygyny was 46%, and the mean number of queens per polygyne colony was 4.5 ± 1.1.

We underestimated the presence of multiple-queen colonies because of our sampling methods. Because we were unable to excavate the complete colony, detection of no queens or just 1 queen might mean that other queens were lost during excavation. Therefore, some, if not all, colonies considered probable monogyne may have in fact been polygyne. Ours is the 1st report of high incidence of polygyny in fire ant populations in South America. Jouvenaz et al. (1989) found multiple-queen colonies in only 5% of the *S. richteri* and *S. quinquecuspis* Forel colonies sampled in central and northern Argentina. They did not find polygyny in *S. invicta* colonies from Mato Grosso, Brazil. Wojcik (1986) and Porter et al. (1992) found no polygyne colonies in Brazil. The presence of multiple-queen colonies in central Argentina is comparable with the incidence of the polygyne form of *S. invicta* in the United States. Porter (1992) and Porter et al. (1991, 1992) found multiple-queen colonies in 15–17% of the sites infested with *S. invicta* in Florida and 54% of the sites in Texas.

Worker Broods. The presence of *T. solenopsae* did not seem to affect worker brood production greatly. The percentages of infected and healthy colonies with worker brood were 56 and 63%, respectively ($x^2 = 0.357$, df = 1, $P > 0.5$). Small differences, although not detected, could be biologically important and would be magnified over time so that growth of infected and healthy colonies could be considerably different as population development progressed. When present, worker brood was abundant in both infected and healthy colonies ($U = 508.0$, $n_1 = 22$, $n_2 = 23$, $P = 0.968$). If there are fewer adult workers in infected colonies than in healthy colonies (based on the differences in mound size) but worker brood is not reduced, fewer workers may emerge in infected colonies. This deserves further investigation.

In both infected and healthy colonies, we found worker broods when the temperature was warm; we found them in April (fall), September, October, and December (spring). Worker broods were not found in June, July, and August (late fall and winter).

Sexual Broods. We found this stage of sexuals less frequently in infected than in healthy colonies. The percentage of colonies with sexual broods was 17% for infected colonies and 37% for healthy colonies ($x^2 = 3.922$, df = 1, $P < 0.05$). According to Markin et al. (1973), the smaller the colony, the fewer sexuals produced. Thus, fewer sexual broods might be produced because of the smaller size of the colonies and not because of the direct effect of the infection. When present, sexual broods were abundant in infected and healthy colonies ($U = 56.0$, $n_1 = 7$, $n_2 = 13$, $P = 0.135$). In both infected

and healthy colonies, we found this stage only in October and December (spring).

Winged Females. In Saladillo, we detected similar frequencies of infected and healthy colonies with winged females. Winged females ocurred in 29% of the infected colonies and in 23% of the healthy colonies ($\chi^2 = 0.4$, df = 1, $P > 0.5$). When present, winged females were abundant in infected colonies and significantly more scarce in healthy colonies ($U = 149.0$, $n_1 = 12$, $n_2 = 8$, $P = 0.046$). In infected colonies, we found females in June, August, September, October, and December (late fall, winter, and spring); in healthy colonies we found them in July, August, September, and October (winter and spring). However, we found winged females more frequently in Las Flores, where large colonies were more abundant. The percentage of colonies with winged females was 67%, significantly higher than in Saladillo ($\chi^2 = 20.3$, df = 1, $P < 0.005$).

Adult Males. In Saladillo, adult males were uncommon in both infected and healthy colonies. The percentage of colonies with adult males was only 2% for infected colonies and 9% for healthy colonies ($\chi^2 = 1.424$, df = 1, $P > 0.1$). Despite the lack of significance (because of the small sample size), this difference could be biologically important if it were magnified over time.

In healthy colonies, we found males in August, September, and October (winter and spring); whereas in infected colonies, we found a few males (only in 1 colony) in December (late spring). This possible delay in the emergence of males in infected colonies deserves further investigation.

Males were much more common in Las Flores, where large colonies were more abundant. The percentage of colonies with adult males was 54%, significantly higher than in Saladillo ($\chi^2 = 31.9$, df = 1, $P < 0.005$).

Myrmecophiles. All insects and mites that we found were as common in infected as in healthy colonies. The most common arthropods associated with the fire ants were the bug *Blissus parasigaster* (Bergroth) (Hemiptera: Lygaeidae), in 100% of both infected and healthy colonies; an unidentified mite (Acari) in 90% of the infected colonies and 89% of the healthy colonies; a beetle, *Myrmecaphodius* sp. (Coleoptera: Scarabaeidae), in 39% of the infected colonies and in 49% of the healthy colonies ($\chi^2 = 0.701$, df = 1, $P > 0.5$); another beetle, *Myrmecosaurus* sp. (Coleoptera: Staphylinidae), in 20% of the infected colonies and in 31% of the healthy colonies ($\chi^2 = 1.43$, df = 1, $P > 0.1$); and the social parasite *Solenopsis daguerrei* Santschi (Hymenoptera: Formicidae), in 7% of the infected colonies and in 6% of the healthy colonies. The bug *B. parasigaster* was the most common and the only myrmecophile present in all the colonies (infected and healthy). However, this species was more numerous in infected colonies ($U = 1,793.5$, $n_1 = 41$, $n_2 = 35$, $P = 0.016$). This myr-

mecophile might have some role in the life cycle of the pathogen.

We conclude, based on evidence described here, that there was a strong negative association between colony size and infection with *T. solenopsae* in field colonies of *S. richteri* in Argentina. This pathogen could cause substantial inhibition in the growth of young colonies but, further studies are necessary to establish a causal link. The presence of smaller and weaker colonies would make imported fire ant populations in the United States less of a problem, and possibly make these populations more susceptible to the action of other biotic or abiotic control factors. Another negative association, indirectly related with the presence of the pathogen, was the less-frequent presence of sexual brood in infected colonies. The genetic fitness of infected colonies might be affected by the reduction of their reproductive capacity.

We detected a contrast in colony size and composition between the fire ant populations in Saladillo and Las Flores. In the Saladillo area, the colonies were predominantly small and, consequently, immature; in the area of Las Flores, larger and more mature colonies were predominant. This survey should be replicated and confirmed for other areas.

The microsporidium *T. solenopsae* may be a good candidate for biological control of imported fire ants in North America. Studies on pathobiology, taxonomy, and specificity are essential and are currently under way so that this microsporidium can be considered for introduction into the United States.

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