EFFECT OF THE MICROSPORIDIUM \textit{THELOHANIA SOLENOPSAE} (MICROSPORIDA: THELOHANIIDAE) ON THE LONGEVITY AND SURVIVAL OF \textit{SOLENOPsis RICHTERI} (HYMENOPTERA: FORMICIDAE) IN THE LABORATORY

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\textbf{ABSTRACT}

The longevity of colonies of the black imported fire ant, \textit{Solenopsis richteri} Forel, and the survival of starved workers and sexual females was compared between healthy colonies and colonies infected with the microsporidium \textit{Thelohania solenopsa}e Knell, Allen, & Hazard. The colonies were collected in the field and reared for approximately four mo. Individual workers and sexuals were held without food until death. The body weight of infected and healthy workers was compared. After 3 mo of laboratory rearing, longevity of infected colonies was significantly shorter than that of healthy ones; mortality of infected colonies was 92\% and mortality of healthy colonies was 49\%. At 27°C, mortality rate of workers from infected colonies was higher than in healthy workers. Workers from infected colonies lived between 8.8 and 29.2\% less than healthy workers. At 22°C, no statistical significance was observed. At 21°C, only the initial mortality of sexual females was higher in infected than in healthy individuals. The weight of infected workers was very similar to that of healthy workers. \textit{T. solenopsea} should be considered for the biological control of the imported fire ants in the United States.

Key Words: \textit{Solenopsis invicta}, imported fire ants, microsporidium, ant longevity
RESUMEN

La longevidad de colonias de la “hormiga colorada” (u “hormiga brava”) Solenopsis richteri Forel y la supervivencia de obreras y hembras sexuadas en inanición fueron comparadas entre colonias sanas y colonias infectadas con el microsporidio Thelohania solenopsae Knell, Allen y Hazard. Las colonias fueron colectadas en el campo y criadas durante aproximadamente cuatro meses. Las obreras y sexuadas fueron mantenidas sin alimento hasta su muerte. Se comparó el peso corporal de obreras enfermas y sanas. Después de 3 meses de cría en laboratorio, la longevidad de las colonias infectadas fue significativamente menor que la de las colonias sanas; la mortalidad de las colonias infectadas fue del 92% y la mortalidad de las sanas fue 49%. A 27°C, la tasa de mortalidad de colonias de obreras enfermas fue mayor que la de obreras sanas. En las colonias enfermas sobrevivieron entre 8.8 y 29.2% menos que las obreras sanas. A 22°C, no se observó significancia estadística. A 21°C, sólo la mortalidad inicial de las hembras sexuadas fue mayor en los individuos enfermos que en los sanos. El peso de las obreras enfermas fue muy similar al de las obreras sanas. T. solenopsae debería ser considerado para el control biológico de la “hormiga colorada” en los Estados Unidos.

The presence of a microsporidian pathogen in the red imported fire ant, Solenopsis invicta Buren, was first reported by Allen & Buren (1974) from Brazil, and was later described as Thelohania solenopsae Knell, Allen, & Hazard (1977) (Microspora: Thelohanidae). A similar microsporidium was discovered in the black imported fire ant, Solenopsis richteri Forel, and other Solenopsis species in Argentina and Uruguay (Allen & Silveira Guido 1974). The presence of this microsporidium was later confirmed in surveys of fire ant natural enemies conducted in South America (Jovenaz 1983, 1986; Jovenaz et al. 1980, 1981; Wojcik et al. 1987; Briano et al. 1995). A comparative study conducted by Moser (1996) confirmed that these microsporidia were conspecific. Thelohania solenopsae is the most common microorganism of fire ants in Buenos Aires Province, Argentina (Briano et al. 1995). Recently, it was discovered infecting colonies of S. invicta in the United States (Williams et al. 1997). Briano et al. (1995a, 1995b, 1996) reported for Argentina a high intracolonial prevalence of the infection and a detrimental effect on native fire ant field colonies and populations. They suggested that T. solenopsae may be a suitable candidate for the biological control of the red and black imported fire ant in the United States.

Although Knell et al. (1977) reported that field-collected colonies of S. invicta infected with this microsporidium cannot be maintained under laboratory conditions as long as healthy colonies, this detrimental effect was never quantified. We speculated that a similar effect of T. solenopsae could be expected in S. richteri. Our primary objective was to compare the longevity of field-collected healthy fire ant colonies, and the survival of individual workers and female sexuals, with those infected with T. solenopsae. This work reports the results of laboratory tests conducted since 1992.

MATERIALS AND METHODS

Longevity of Colonies

In January 1993, 38 colonies of S. richteri were collected along the roadsides of Rt. 12, km 104, Isla Talavera, Buenos Aires Province, Argentina. This sampling site was selected based on previous surveys that revealed high prevalence of T. solenopsae (Briano et al. 1995).
Weight of Workers

Sixty-seven infected and 65 healthy workers (not starved) of different sizes were selected at random from the colonies used in Test II. They were weighed (live weight) on an electronic balance (Precisa 120 A, PAG Oerlikon AG, Zurich, Switzerland), killed in 70% ethyl alcohol and their head widths measured under an ocular micrometer. Each worker was crushed on a microscope slide and examined under a phase-contrast microscope to confirm the presence or absence of *T. solenopsae*. The live weights of infected and healthy workers were compared and correlated with worker size.

Statistical Analysis

Mortality rate was analyzed with the logrank method, an application of the Mantel-Haenszel method (Mantel & Haenszel 1969). Longevity of colonies and survival of individual ants was analyzed with 2-sample t test. The simple linear regression model was used to correlate survival of workers with their size, and the curvilinear (cubic) model was used to correlate the live weight of workers with their size. Minitab Statistical Software (1991) was used for t tests and regressions. Means are reported ± 1 SD.

RESULTS AND DISCUSSION

Longevity of Colonies

Longevity of infected colonies was significantly shorter than in healthy colonies (Fig. 1). The cumulative mortality during the first 21 d was 64% for infected colonies and 24% for healthy colonies. After 3 mo, mortality was 92% for infected colonies and 49% for healthy colonies (Logrank method; $\chi^2 = 6.0; df = 1; P < 0.025$). In most colonies the queens died after the workers.

The different mortality rate between infected and healthy colonies suggests that *T. solenopsae* is lethal to stressed laboratory colonies of the black imported fire ant. Although mortality of healthy colonies is usually high under laboratory conditions, as this test showed, clearly this pathogen exerted additional stress and increased mortality. This is consistent with results of field work that showed a detrimental effect of this microsporidian on native fire ant populations and individual colonies of *S. richteri* (Briano et al. 1995a; 1995b). These results also agree with Knell et al. (1977) who reported that colonies of *S. invicta* infected with this microsporidian cannot be maintained under laboratory conditions as long as healthy colonies.

In this experiment we actually compared residual longevity because queens and workers were not newly enclosed when the test started. Comparisons are still valid because this also happened for healthy colonies. The actual life span of infected colonies compared to healthy colonies in the laboratory remains unknown and should be investigated.

Egg-laying started at day 11 in 2 healthy colonies and at day 14 in one infected colony. At day 21, 72% of the surviving healthy colonies and 80% of the surviving infected colonies showed worker brood production. The egg-laying rate of infected and healthy queens was not compared and deserves further investigation.

Survival of Workers. Test I

Mortality rate of workers from infected colonies was higher than that of workers from healthy colonies (Fig. 2). For small workers, after 3 d of starvation, mortality of individuals from infected colonies was 75% and mortality of healthy ones was 43%. At day 4, when all workers from infected colonies had died, 8% of healthy workers were still alive (Logrank method; $\chi^2 = 4.5; df = 1; P < 0.05$). On average, the survival of small workers from infected colonies was 8.8% shorter than that of healthy ones. The mean survival time was $3.1 \pm 0.2$ d for workers from infected colonies and $3.4 \pm 0.7$ d for healthy ones ($t = 2.691; df = 78; P < 0.005$).

For large workers, after 4 d of starvation, mortality of individuals from infected colonies was 95% and mortality of healthy ones was 33% (Fig. 2). At day 6, when all workers from infected colonies had died, 8% of the healthy workers were still alive (Logrank method; $\chi^2 = 16.45; df = 1; P < 0.001$). On average, large workers from infected colonies lived 29.2% less than healthy workers. The mean survival time was $3.4 \pm 0.7$ d for workers from infected colonies and $4.8 \pm 1.3$ d for healthy ones ($t = 5.933; df = 78; P < 0.0001$).

The difference in survival time both in small and large workers was underestimated because some workers from infected colonies could have been actually healthy. The difference was larger in large than in small workers (Fig. 2). It seems that *T. solenopsae* affected large workers more than small workers. This is consistent with the assumption that *T. solenopsae*, being a chronic disease, would affect more severely those individuals with longer life span such as large workers. The tasks performed by large workers in the colony (mound construction, foraging, territory defense, and transport of sexual broods) would be affected more severely than the tasks performed primarily by small workers. The actual impact that the high prevalence of infected
workers would have in field colonies remains unknown but is consistent with the findings reported by Briano et al. (1995a, 1995b).

**Survival of Workers and Sexuals, Test II**

The mortality rate of healthy workers was similar to that of infected workers (Fig. 3; logrank method; \( \chi^2 = 0.256; df = 1; P > 0.5 \)). Although the mean survival time of infected workers was shorter than that of healthy workers, no statistically significant differences were found. Infected minor workers survived 5.9 ± 5.3 d and healthy minor workers survived 6.5 ± 5.0 d (\( t = -0.457; df = 76; P = 0.648 \)). Infected medium workers survived 9.0 ± 9.3 d and healthy medium workers 10.0 ± 8.8 d (\( t = -0.276; df = 44; P = 0.782 \)). Infected major workers survived 11.5 ± 9.1 d and healthy major workers 12.9 ± 13.9 d (\( t = -0.313; df = 22; P = 0.766 \)).

The regression of survival on worker size showed very low coefficients of determination for both healthy workers (\( r^2 = 0.07 \)) and infected ones (\( r^2 = 0.05 \)). The main reason for this was the high individual variability. Calabi & Porter (1989) also reported a high scatter in regression of longevity on worker size for S. invicta in the United States (\( r^2 = 0.02 \)). They speculated that the scatter was due to the absence of queens, brood and/or intercolony differences. In our experiment, an extra source of variability would be the undetermined age of the workers when the test started.

Considering the tests reported in this article, worker survival decreased about 60% when temperature increased from 22 to 27°C. The validity of this comparison may be questionable because the tests were conducted separately, the ants were collected in different locations and in different seasons. However, the information reported is consistent with studies conducted in the United States by Calabi & Porter (1989) showing that workers of S. invicta had an 80% reduction in longevity when the temperature increased from 17 to 30°C. It seems that at lower temperatures, the reduced activity and metabolic rate of the workers can reduce the debilitating effects of the infection. This should be investigated. We speculate that the detrimental effect of T. solenopsae could be more important in areas with warmer temperatures. According to Tanada & Kaya (1993), temperatures higher than 30°C can limit the infectivity of pathogens, but moderately high field temperatures accelerate the infectious process and result in quicker mortality.

The mortality rate of infected female sexuals was not significantly different from that of healthy ones (Fig. 4; logrank method; \( \chi^2 = 0.45; df = 1; P > 0.5 \)). However, the mortality during the first 10 d was much higher for infected individuals (\( \chi^2 = 6.36; df = 1; P < 0.025 \)). This means that infected sexual females (future queens) died quicker than healthy ones and might represent a negative effect of T. solenopsae on the colony.
Infected female sexuals — Healthy female sexuals

Fig. 4. Mortality of starved sexual females of S. richteri kept at 20.7°C.

Founding within infested areas. Again, this is consistent with field work showing a detrimental effect of this pathogen on S. richteri (Briano et al. 1995a, 1995b).

On average, infected sexuals survived 23.0 ± 21.0 d and healthy ones survived 32.2 ± 15.5 d, but this difference was not statistically significant ($t = -1.413; df = 29; P = 0.168$). This was probably due to the small sample size and high individual variability. Unfortunately, no more sexuals were available when the test started. This test should be replicated with larger sample size and at several temperatures.

As expected, mean survival time of sexuals was longer than that of major workers. This can be attributed in part to the fact that the ambient temperature was slightly lower in the test with sexuals, but the longer survival should be primarily attributed to their larger body size and their extra energy source provided by the histolytic breakdown of wing muscles. After 2-3 wk of starvation, all sexuals lost their wings.

Weight of Workers

The live weight of infected workers was very similar to that of healthy workers. Infected minor workers weighed 0.656 ± 0.225 mg (range 0.3-1) and healthy minor workers 0.653 ± 0.246 mg (range 0.3-1.3). Infected medium workers weighed 1.636 ± 0.362 mg (range 0.9-2.4) and healthy medium workers 1.628 ± 0.386 (range 1.2-7). Infected major workers weighed 3.665 ± 0.824 (range 2.2-5.4) and healthy workers 3.311 ± 0.747 (range 2.3-6).

As expected, the weight of workers was highly-positively correlated (cubic function) with their size (Fig. 5). The regression equation for infected workers was $y = 0.091 + 1.480 x^3 (r^2 = 0.93; F = 867.1; df = 1, 67; P < 0.0001)$ and for healthy workers was $y = 0.225 + 1.447 x^3 (r^2 = 0.93; F = 847.5; df = 1, 65; P < 0.0001)$. This agrees with Porter & Tschinkel (1985) who reported a similar relationship for workers of S. invicta in the United States. There was not any evidence that the presence of T. solenopsae affected the weight of the workers. As suggested by Knell et al. (1977) for S. invicta, we had speculated that the progressive destruction of the fat body produced by T. solenopsae, would have an impact on body weight in workers of S. richteri. However, a hypothetical loss of weight in infected workers could be balanced, at least in part, by the weight of the cysts totally filled with masses of Thelohania spores. This deserves further investigation.

We conclude that the microsporidium T. solenopsae affected the mortality rate and shortened the longevity of colonies of S. richteri reared under laboratory conditions. Survival of starved workers, mainly large workers, and initial mortality of sexual females was also affected. Temperature could be a regulating factor of this effect. These laboratory findings are consistent with results of field work reported by Briano et al. (1995a, 1995b), showing reduced mound volumes of infected colonies, less frequent presence of sexual brood in infected colonies and decreased mound density in a Thelohania-infested area of Argentina.

The introduction of a complex of natural enemies into the United States has been the ultimate goal of the imported fire ant control project. Among the several potential
candidates, T. solenopsea has been the first microorganism evaluated in South America as a potential biological control agent. Still, important aspects of its life cycle, such as the horizontal transmission and field propagation, remain unknown. After those studies are completed, T. solenopsea should be considered for the biological control of the imported fire ants in the United States.

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