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EFFECT OF THE MICROSPORIDIUM *THELOHANIA SOLENOPSAE* (MICROSPORIDA: THELOHANIIDAE) ON THE LONGEVITY AND SURVIVAL OF *SOLENOPSIS RICHTERI* (HYMENOPTERA: FORMICIDAE) IN THE LABORATORY

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ABSTRACT

The longevity of colonies of the black imported fire ant, *Solenopsis richteri* Forel, and the survival of starved workers and sexual females was compared between healthy colonies and colonies infected with the microsporidium *Thelohania solenopsae* 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. *T. solenopsae* should be considered for the biological control of the imported fire ants in the United States.

Key Words: *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 inhanición 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 sexuados fueron mantenidos sin alimento hasta su muerte. Se comparó el peso corporal de obreras enfermas y sanas. Después de 3 meses de cria 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 obreras de colonias enfermas fue mayor que la de obreras sanas. Obreras de 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) (Microsporida: Thelohaniidae). 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 microsporidia was later reported in surveys of fire ant natural enemies conducted in South America (Jouvenaz 1983, 1986; Jouvenaz et al. 1980, 1981; Wojcik et al. 1987; Briano et al. 1995). A comparative study conducted by Moser (1995) 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 1992, 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).

This microsporidium was detected in 16 colonies, being the other 22 colonies healthy. The colonies were separated from the soil by flotation according to the techniques described by Banks et al. (1981). Colonies with no queen were removed from the study, consequently, only 14 infected colonies were considered. Fifteen of the 22 healthy colonies collected at the same site were used as controls.

Because all colonies were polygyne, each one was fragmented into separate, equal subcolonies comprised of one queen selected at random, 50 small and 50 large workers. The fragmented colonies were kept in plastic rearing trays (40 × 30 × 15 cm) dusted with talc to prevent escape. The test was conducted in a walk-in rearing chamber (28.6 ± 1.3°C and 60-90% RH). The colonies were fed twice a week with approximately 100 adult house flies and ½ egg yolk; a water source and honey-agar cubes were always present in the rearing trays.

The egg laying of the queens was checked daily only to confirm their fertility. Mortality of the colonies was recorded and compared between infected and healthy colonies. A colony was considered dead when its queen was found dead. Growth of colonies and mortality rate of individual workers was not quantified.

Survival of Workers. Test I

In December 1995, 4 colonies of *S. richteri* (2 *Thelohania*-infected and 2 healthy) were excavated from Rt. 205, km 180, Saladillo, Buenos Aires Province. They were separated from the soil by flotation (Banks et al. 1981). Although the exact percentage of infected workers present in the infected colonies was not determined, based on previous work (Briano et al. 1996), we estimated that it was, on average, 88%. A total of 160 workers was selected from the colonies. Twenty small (head width: 0.67 ± 0.06 mm) and 20 large workers (head width: 1.08 ± 0.17 mm) were separated at random from each of the 4 colonies. The selected workers were put in groups of 10 in individual cells (4 × 4 × 2 cm) of plastic rearing trays (40 × 20 × 2 cm). A plastic lid covered each cell preventing escape. The workers were held without food until death. A small piece of moistened cotton was present in the cells as a source of moisture. The test was conducted in a walk-in rearing chamber (27.3 ± 1.6°C; 70-90% RH). Mortality was recorded daily and survival of small and large workers was compared.

Survival of Workers and Sexuals. Test II

In April 1996, 3 colonies of *S. richteri* (2 *Thelohania*-infected and a healthy one) were excavated in Moreno, Buenos Aires Province, and separated from the soil by flotation (Banks et al. 1981). Sixty-four workers of different sizes separated at random from each infected colony along with 13 female sexuals were put individually in cells of plastic rearing trays with moistened cotton as above. The trays were kept at room temperature (21.8 ± 1.4°C for workers and 20.7 ± 1.6°C for sexuals). The workers and sexuals were held without food until death. Only a small piece of moistened cotton was present in the cells as above. Workers of different sizes (n = 32) and sexuals (n = 18) separated from the healthy colony were used as controls. Once dead, the head width of each worker was measured under an ocular micrometer. To confirm infected and healthy individuals, the workers and sexuals were crushed individually in a drop of water placed on a microscope slide and checked under a phase-contrast microscope. Mortality was recorded daily and survival was compared between confirmed infected and healthy individuals. Arbitrarily, we considered minor workers those with head widths from 0.6 to 0.8 mm, medium workers those with head widths from 0.9 to 1.1 mm, and major workers those with head widths from 1.2 to 1.5 mm.

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 1959). 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 microspordium 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 microsporidium 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 eclosed 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

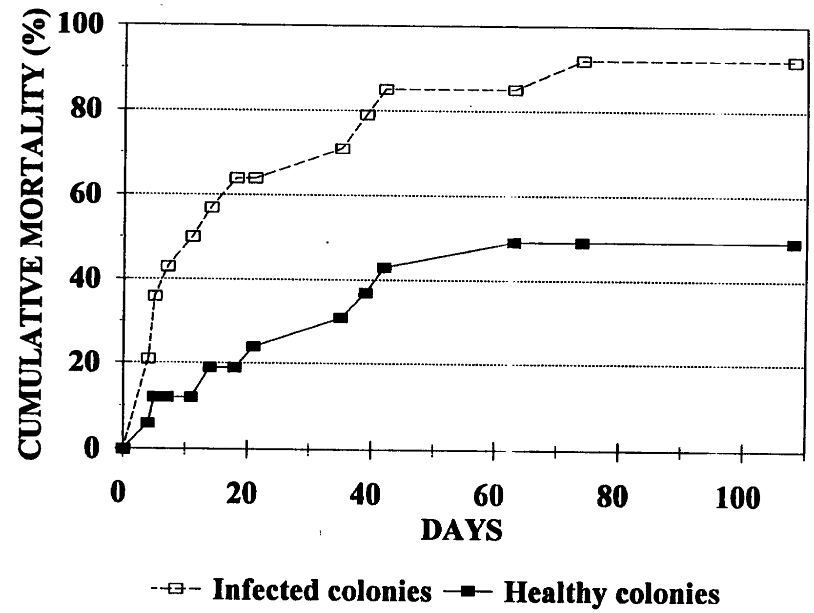

Fig. 1. Mortality of fragmented (1 queen and 100 workers) infected and healthy colonies of *S. richteri* reared at 28.6°C.

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 ± 0.2 d for workers from infected colonies and 3.4 ± 0.7 d for healthy ones ($t = 2.691$; df = 78; $P = 0.0087$).

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 ± 0.7 d for workers from infected colonies and 4.8 ± 1.3 d for healthy ones ($t = 5.633$; 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

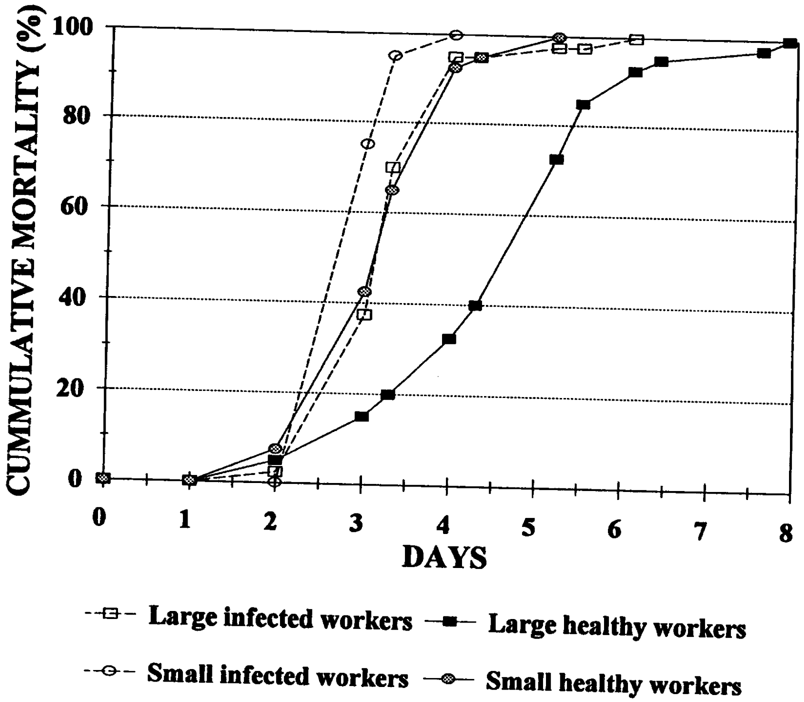

Fig. 2. Mortality of starved small and large workers from infected and healthy colonies of *S. richteri* kept at 27.3°C.

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 ($t = -0.278$; 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; $P = 0.756$).

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,

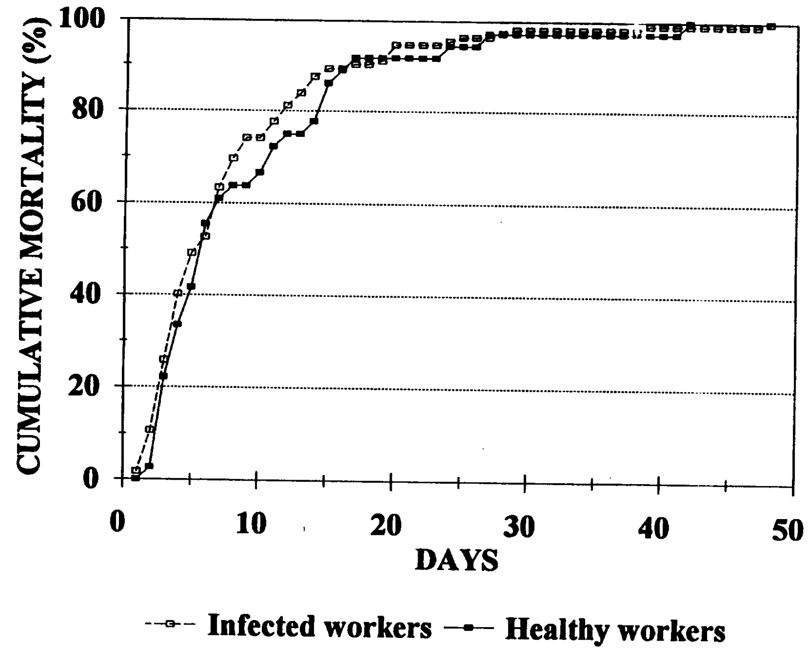

Fig. 3. Mortality of starved infected and healthy workers of *S. richteri* kept at 21.8°C.

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

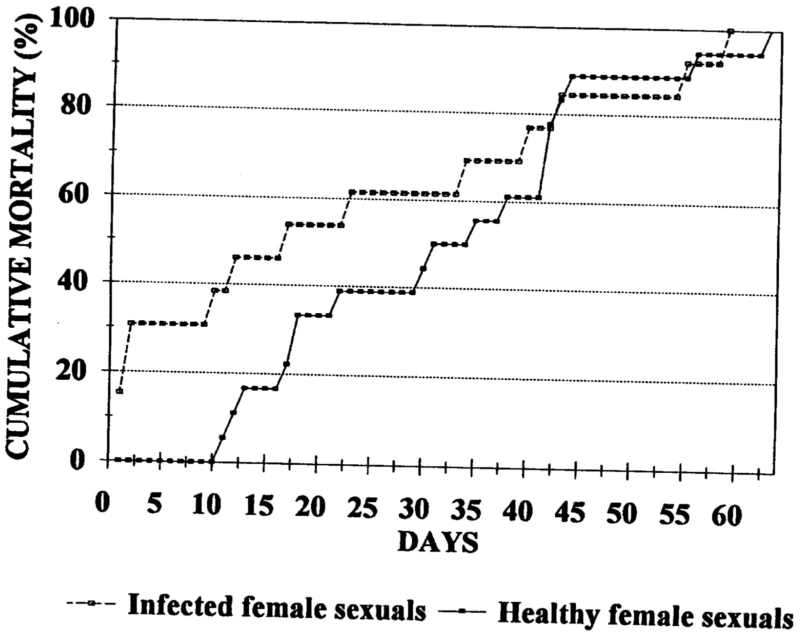

Fig. 4. Mortality of starved sexual females of *S. richteri* kept at 20.7°C.

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 histolysis 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 2.3-5).

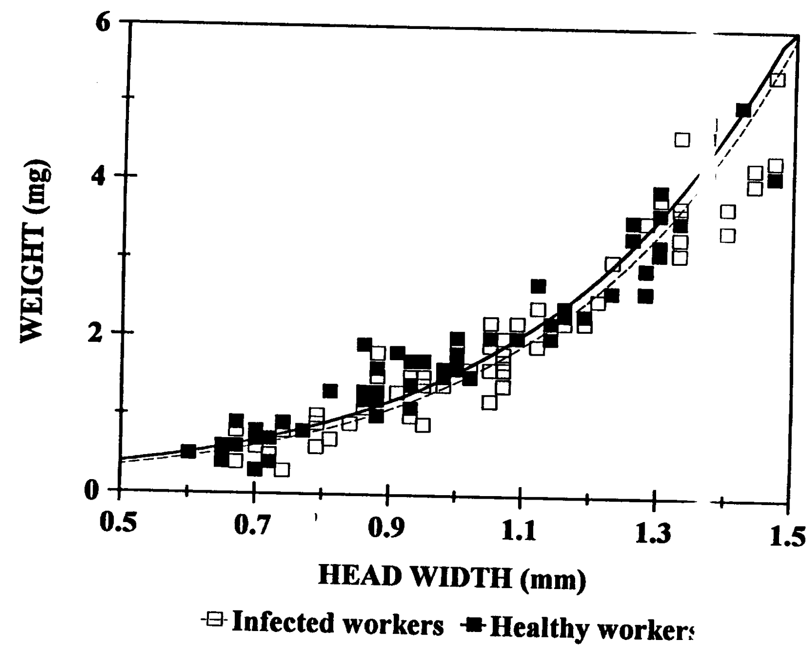

Fig. 5. Relationship between live weight and size (based on head width of healthy and infected workers of *S. richteri*.

As expected, the weight of workers was highly-positive correlated (cub function) with their size (Fig. 5). The regression equation for infected workers was = 0.091 + 1.480 x^3 (r^2 = 0.93; F = 857.1; df = 1, 67; P < 0.0001) and for healthy work s was y = 0.225 + 1.447 x^3 (r^2 = 0.93; F = 847.5; df = 1, 65; P < 0.0001). This agrees ith Porter & Tschinkel (1985) who reported a similar relationship for workers of *S. in cta* in the United States. There was not any evidence that the presence of *T. lenopsae* affected the weight of the workers. As suggested by Knell et al. (1977) fo *S. invicta*, we had speculated that the progressive destruction of the fat body produ l by *T. solenopsae*, would have an impact on body weight in workers of *S. richteri*. owever, a hypothetical loss of weight in infected workers could be balanced, at least part, by the weight of the cysts totally filled with masses of *Thelohania* spores. Th deserves further investigation.

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

The introduction of a complex of natural enemies into the United Stat has been the ultimate goal of the imported fire ant control project. Among the sever potential candidates, *T. solenopsae* 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. solenopsae* should be considered for the biological control of the imported fire ants in the United States.

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