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Impact of a Microsporidian Pathogen, *Thelohania solenopsae*, on Red Imported Fire Ants

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Thelohania solenopsae Knell, Allen, & Hazard is an entomopathogenic microsporidium that was first reported by Allen and Buren (1974) from alcohol preserved specimens of the red imported fire ant, *Solenopsis invicta* Buren, collected in Brazil. *T. solenopsae* infections have since been confirmed in the black imported fire ant, *Solenopsis richteri* Forel, in Argentina (Moser 1995). Spores of *T. solenopsae* are found in fat bodies of adults, and vegetative stages are found in the fat bodies of larvae and pupae, and in queen ovaries. Vegetative stages are also found in eggs, and thus *T. solenopsae* is transovarially transmitted (Briano *et al.* 1996). Briano and Williams (1997) reported negligible effects on the longevity of adult workers and sexuals of *S. richteri*. Effects of *T. solenopsae* on the development times of immature stages of fire ants are unknown. However, in a field study in Argentina, Briano *et al.* (1995) reported an 83% decrease in the density of black imported fire ant colonies within 4 years. Because of the apparent lack of an effect on adult workers, and the slow reduction in colony densities, it was suspected that *T. solenopsae* reduced brood production.

In 1996, Williams et al. (1998) discovered *T. solenopsae* infected *S. invicta* in several locations in Florida. Limited surveys throughout the southern U.S. revealed that infections were present in Texas and Mississippi. With the discovery of *T. solenopsae* in the U.S., studies have been conducted on the impact of this pathogen on colonies of *S. invicta*. We report the effect of *T. solenopsae* on brood production in laboratory colonies, and the inoculation and infection of field colonies of *S. invicta*.

Material and Methods

Laboratory Inoculation 1. Monogyne laboratory colonies, reared from newly mated queens, were used to examine the effects of *T. solenopsae* on brood production. These colonies contained an average of 71,000 workers (range: 50k-95k), 68 ml brood (range: 30-90 ml), and 1 queen. To inoculate a colony, 1 gram of brood (an arbitrary mixture of eggs, larvae, and pupae) was separated from field collected *S. invicta* colonies that were infected with *T. solenopsae*. The brood was placed next to a colony so that it would be carried into the colony by worker ants. A total of ten colonies were inoculated, and 1 gram of uninfected brood per colony was also introduced into ten control colonies.

The brood volume and the number of adult worker ants per colony were catergorized visually, using procedures that were adopted from Banks and Lofgren (1991). In addition, to assess the effect of *T. solenopsae* infection on queen weights, pre- and post-inoculation weights were recorded for each queen. Colony population estimates and queen weights were obtained before brood introductions, 8 weeks later, and at 2 or 3 week intervals thereafter for 23 weeks. Brood volumes, worker populations, and queen weights from inoculated and control colonies were summed over sample dates and compared by *t*-tests.

Laboratory Inoculation 2. To examine the effect of *T. solenopsae* on oviposition rates, a set of five laboratory fire ant colonies were inoculated with brood from *T. solenopsae* infected colonies and another set of five colonies had uninfected brood introduced. These colonies had an average of over 80,000 workers, 59 ml of brood, and 1 queen. Eight weeks after brood introductions, queens were removed from the colonies, and the number of eggs laid in 1 hour was recorded. Queens were then returned to the colonies, and the procedure repeated at 12, 16, and 21 weeks after brood introductions. Zero eggs were assigned to queens that died after 8 weeks. Queen weights were also recorded when brood was introduced and when oviposition rates were determined. Oviposition rates and queen weights from inoculated and control colonies were averaged per colony over sample dates ≥8 weeks after brood introductions and then compared by *t*-tests.

Field Inoculation. Brood from infected or non-infected *S. invicta* colonies were introduced into polygyne *S. invicta* colonies located in a pasture in Alachua County, Florida. Introductions were made by making an opening in a mound with a shovel and pouring 5 gm of brood into the opening. A total of five mounds were inoculated with infected brood and five control mounds had uninfected brood introduced. Adjacent inoculated mound were located within 29 feet of each other, and adjacent control mounds were within 45 feet of each other. Inoculated and control mounds were grouped in separate areas that were a minimum of 135 feet apart. Brood introductions were made on May 8, 1997.

Before brood introductions, colonies were checked for the presence of *T. solenopsae* by looking, under phase contrast microscopy, for spores in a supernate obtained from groups of about 50 worker ants that were macerated with water in a tissue grinder. On the 8th week after brood introductions, and at 3 to 6 week intervals thereafter for 40 weeks, adult worker and larval samples were obtained from each mound to determine if colonies were infected. Adult workers were examined for spores as described above. Larval samples (10 larvae per colony) were individually smeared onto a slide, stained with Giemsa, and examined for vegetative stages of *T. solenopsae*. On the 40th, 44th, and 48th weeks after brood introductions, adult worker samples from mounds surrounding the inoculated and control mounds were also examined for *T. solenopsae* spores to check for evidence of spread. To assess effects of *T. solenopsae* on colony populations, the USDA population index ratings (Lofgren & Williams 1983) were determined for inoculated and control colonies on each worker/larvae sampling date.

Results and Discussion

Laboratory Inoculation 1. Inoculated colonies had significantly lower brood volume (t = 6.67, df = 18, $P \leq 0.0001$), worker populations (t = 5.74, df = 18, $P \leq 0.0001$), and queen weights (t = 6.25, df = 18, $P \leq 0.0001$) than control colonies. Reductions in brood and workers were evident at 10 and 12 weeks after inoculation, respectively (Fig. 1 and 2). At the end of the study (week 23), there was an average of 6 ml brood per colony from inoculated colonies in contrast with 50 ml in the controls. Queen weight reductions in inoculated colonies were evident at the initial post-inoculation sample at 8 weeks (Fig. 3). The slow decline in brood and worker populations, along with the concomitant reduction

in queen weights suggested that *T. solenopsae* caused a decrease in brood production in colonies.

Laboratory Inoculation 2. The average number of eggs laid per queen per hour from inoculated colonies was 66% lower than control colonies (t=2.03, df=7, P=0.0816) during the period from 8 to 21 weeks after brood introductions (Fig. 4). This period was compared because previous studies indicated that the effects of *T. solenopsae* infections generally became detectable 8 weeks after inoculation. Oviposition rates between inoculated and control colonies at week 0 were not significantly different (t= -1.151, df=7, P=0.2874).

Pre-inoculation queen weights did not differ significantly (t=-1.287, df=7, P=0.2389) between inoculated and control colonies. After brood introductions, average live queen weights were 18% lower in the inoculated colonies than in controls (t=1.457, df=7, P=0.1884). However, 3 out of the 5 queens from inoculated colonies were dead by week 16 and thus their weights were excluded from the average when they died. The lack of significantly lower queen weights from the inoculated colonies might be attributed to the death of queens in these colonies, and consequently, only heavier surviving queens were available for the queen weight average. All control queens were alive on the 21st week.

Field Inoculation. *T. solenopsae* infections were detected in four of the five inoculated colonies 18 weeks (Sept. 1997) after brood introductions. Both meiospores in adults and vegetative stages in larvae were recovered from these four colonies throughout the study. *T. solenopsae* was not found in any of the control colonies. After 11 months, all inoculated colonies were still active, however, there was a 30% reduction in the average population index for the inoculated colonies in contrast to a 4% increase in the controls.

At 40 and 44 weeks after inoculations (Jan. and Feb.1998), eight additional colonies surrounding the inoculated mounds were found to be infected with *T. solenopsae*. In week 48 (March 1998), *T. solenopsae* was found in 12 more colonies near the inoculated mounds. Infected colonies found in weeks 40 and 44 were still active and infected in week 48. However, because non-inoculated colonies that were later found to be infected were not confirmed to be uninfected by *T. solenopsae* prior to infection, evidence for the natural spread of *T. solenopsae* is not conclusive.

This is the first documentation of artificially initiated horizontal transmission of *T. solenopsae* infections among fire ant colonies. The laboratory inoculations showed significant reductions in brood and workers from infected colonies. Lower oviposition rates, queen weights, and queen survivorship was also documented from infected colonies. Reductions were first noticeable 8 to 12 weeks after inoculations in monogyne, laboratory colonies. Artificial inoculations resulting in infections of red imported fire ant colonies under field conditions was also demonstrated for the first time. After 48 weeks, a 30% reduction in colony populations was observed in the infected colonies. Since field inoculations were made in polygyne, *S. invicta* colonies, the impact of *T. solenopsae* may have been slowed or reduced because it is possible that all queens may not be infected. The ability to artificially infect *S. invicta* colonies with *T. solenopsae* should facilitate the assessment and development of this pathogen as a biological control agent of imported fire ants.

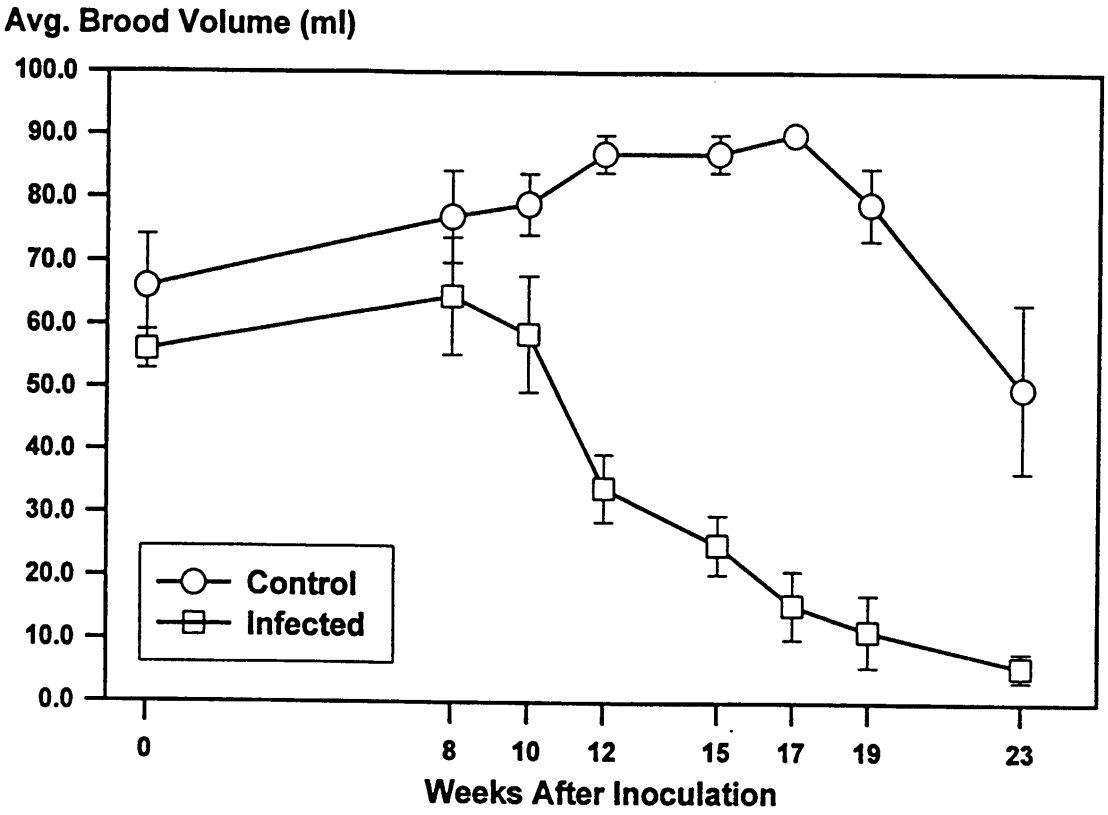

Fig. 1. Average (bar: ±1 SE, N=10) brood volumes from laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies.

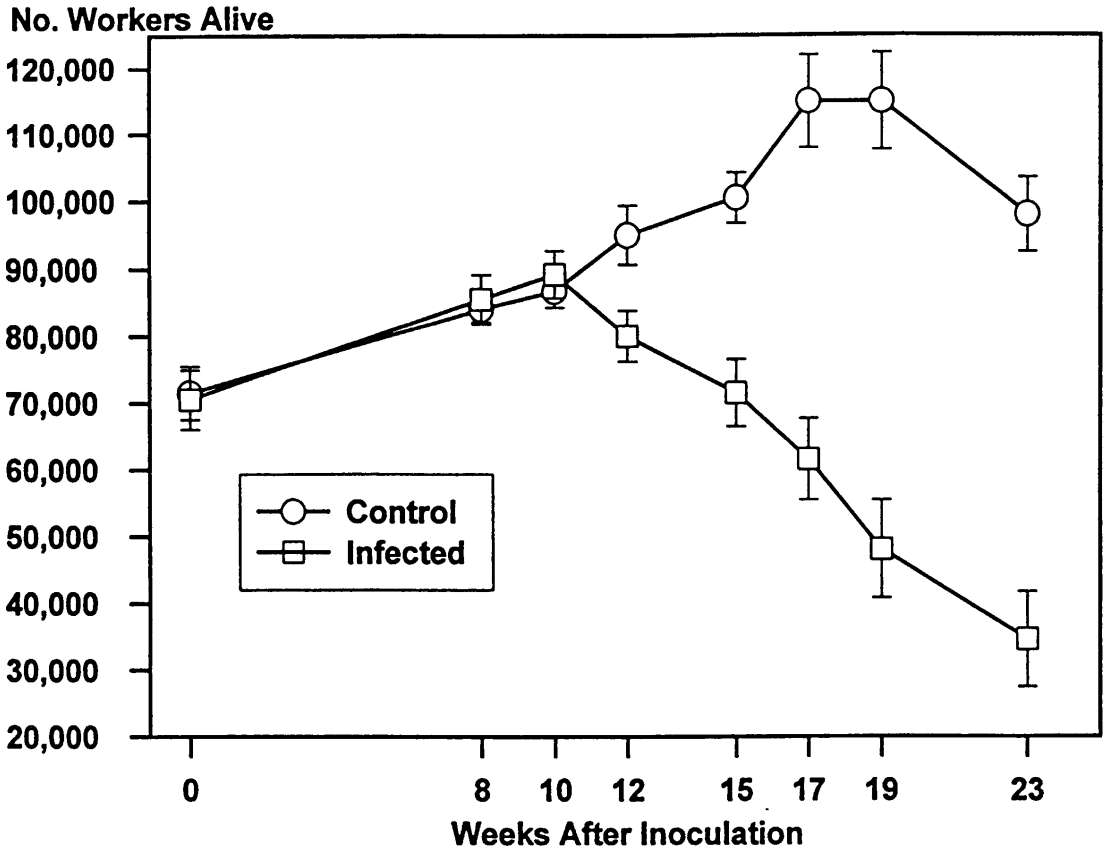

Fig. 2. Average (bar: ±1 SE, N=10) number of live adult workers from laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies.

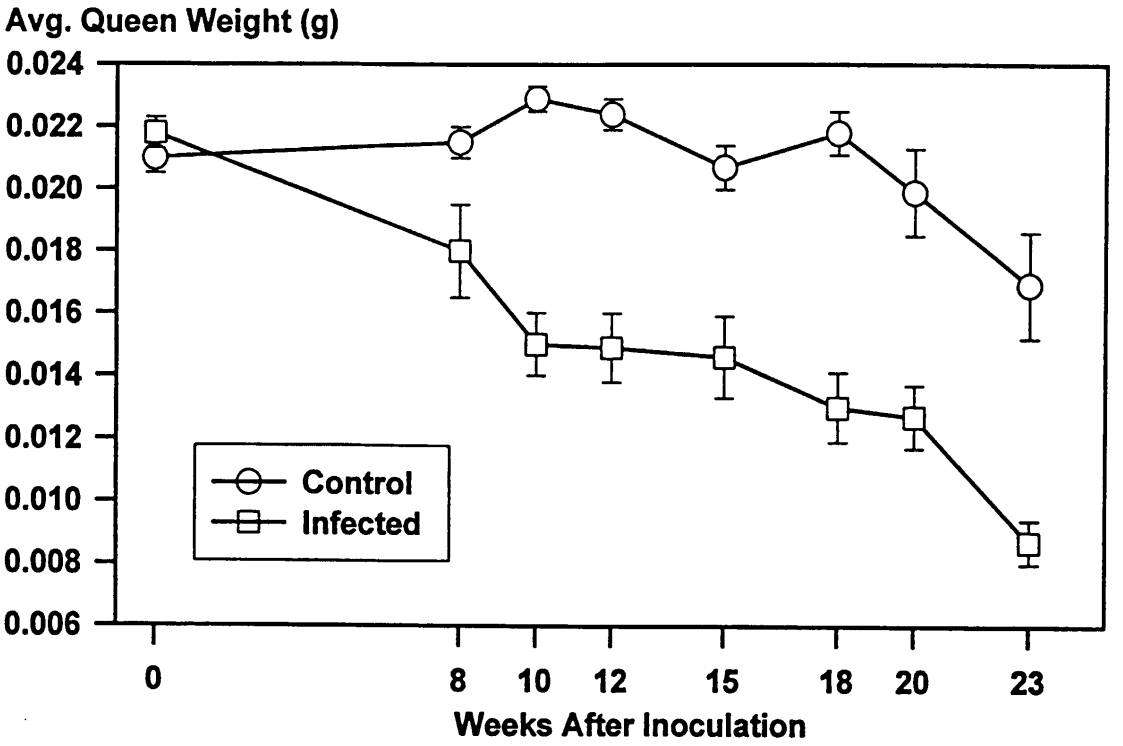

Fig. 3. Average (bar: ±1 SE, N=10) queen weights from laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies.

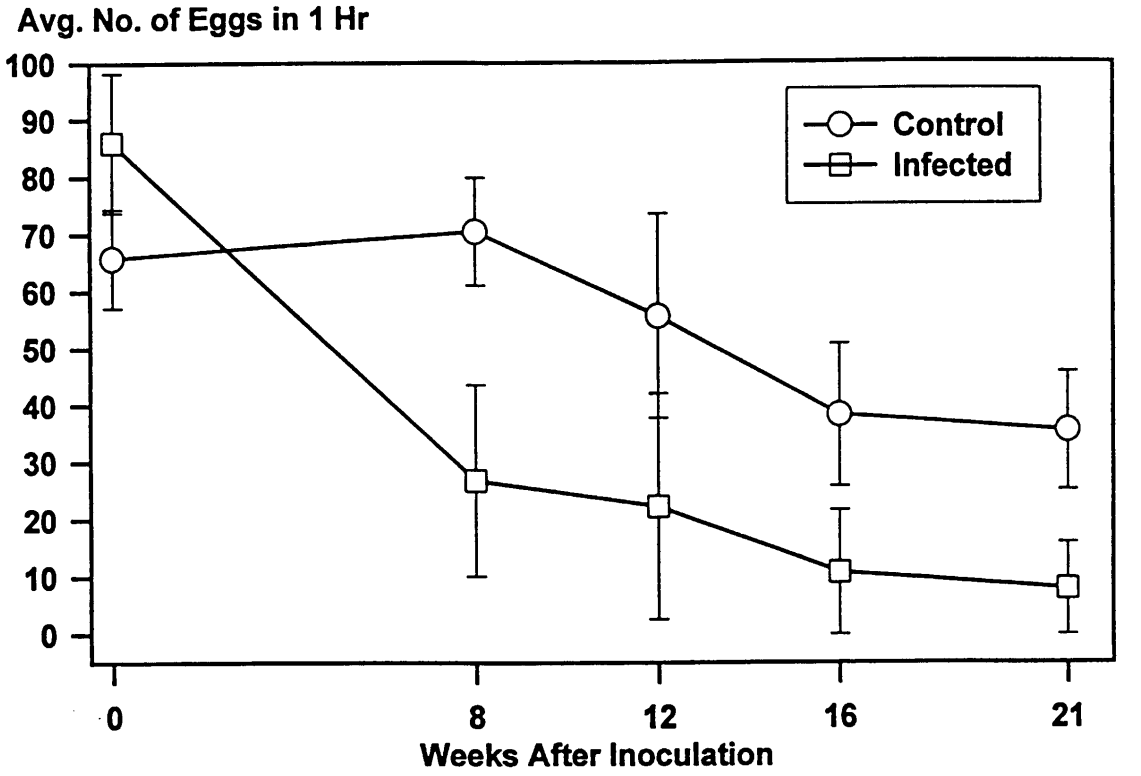

Fig. 4. Average (bar: ±1 SE, N=4 infected, N=5 control) oviposition rates from laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies.

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