# Infection of Red Imported Fire Ant (Hymenoptera: Formicidae) Colonies with the Entomopathogen *Thelohania solenopsae* (Microsporidia: Thelohaniidae)

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**ABSTRACT** *Thelohania solenopsae* Knell, Allen, & Hazard is an entomopathogenic microsporidium that infects imported fire ants. We documented artificially initiated transmission of *T. solenopsae* among colonies of the red imported fire ant, *Solenopsis invicta* Buren. Microsporidian transmission was initiated by providing colonies with brood (mixture of eggs, larvae, and pupae) from infected *S. invicta* colonies. Inoculated laboratory colonies of *S. invicta* had significantly less brood and adults than control colonies; after 23–29 wk, there was at least 88% less brood in the inoculated colonies. Lower egg laying rates, queen weights, and queen survivorship was also documented from infected colonies. Thus, *T. solenopsae* slowly debilitates an *S. invicta* queen so that reproductive capacity diminishes and early death results. Artificial inoculations resulting in infection and spread of *T. solenopsae* among red imported fire ant colonies under field conditions was also demonstrated for the 1st time.

**KEY WORDS** *Solenopsis invicta*, fire ant, biological control, pathogen, microbial control, disease

IMPORTED FIRE ANTS are significant pests throughout the southern United States. These ants are composed of the black imported fire ant, *Solenopsis richteri* Forel; the red imported fire ant, *Solenopsis invicta* Buren; and their hybrid. Of the 3 ants, *S. invicta* is predominant (Vander Meer and Lofgren 1988). In addition, there are multiple-queen, or polygyne, colonies that are not territorial, and colonies that are monogyne, or have only 1 queen, that are territorial. Polygyny has been reported for *S. invicta* and the hybrid in the United States (Glancey et al. 1973, 1989), and for *S. richteri* and *S. invicta* in South America (Trager 1991). Areas infested with polygyne *S. invicta* have populations and colony nest site, or mound, densities that are 2–3 times that of monogyne infested areas (Porter 1992, Macom and Porter 1996). Because of their high reproductive capacity, aggressive foraging behavior, and lack of effective natural enemies in the United States, imported fire ants have become a dominant arthropod species and have reduced biological diversity in the areas they infest (Porter and Savignano 1990). In addition, 30–60% of people living in infested urban areas are stung by fire ants annually, and ≈1% of these people are hypersensitive to the venom (de Shazo et al. 1990). Fire ants also are responsible for losses of agricultural commodities, such as hay and soybeans (Adams et al. 1983, Barr and Drees 1996). A federal quarantine exists against the movement of untreated nursery stock from areas infested by fire ants to uninfested areas, resulting in added costs for nurserymen and regulatory agencies. Since their accidental introduction into Mobile, AL, in the early 1930s, fire ants have continued to spread rapidly and now infest >308 million acres in the southern United States and Puerto Rico (Code of Federal Regulations 1998).

Currently, the most common control method for fire ants is the use of chemical insecticides. While some insecticides are effective, they provide only temporary control and are often not economical for large areas, such as cattle ranches (Barr and Drees 1996). Recently there has been interest in biological control agents for imported fire ants in the United States (Porter et al. 1995, Cook et al. 1997, Williams et al. 1998). One of these agents is *Thelohania solenopsae* Knell, Allen, & Hazard, an entomopathogenic microsporidium that was first reported by Allen and Buren (1974) from alcohol preserved specimens of *S. invicta* collected in Brazil. *T. solenopsae* infections have since been confirmed in *S. richteri* in Argentina (Moser 1995). Spores of *T. solenopsae* are found in late stage pupae, adult workers, and queen ovaries, and vegetative stages are found in larvae, pupae, and queen ovaries (Knell et al. 1977). 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 of *T. solenopsae* on the longevity of adult workers and sexuals of *S. richteri*. However, in a field study in Argentina, Briano et al. (1995) reported an 83% decrease, over a 4-yr period, in the density of black imported fire ant colonies in a *T. solenopsae* infected area. We suspected that *T. solenopsae* reduced brood production because of the apparent lack of effect on adult workers and the slow reduction in colony densities.

Williams et al. (1998) discovered *T. solenopsae*-infected *S. invicta* in several locations in Florida in

1996. Limited surveys throughout the southern United States revealed that infections also were present in Texas and Mississippi. With the discovery of *T. solenopsae* in the United States, studies were initiated to assess the potential of this entomopathogen as a biological control agent. Our objective was to infect and examine the impact of *T. solenopsae* on laboratory *S. invicta* colonies. In addition, we document the inoculation and infection of *S. invicta* colonies under field conditions.

## Material and Methods

**Small Laboratory Colony Inoculation.** Small, monogyne laboratory colonies of *S. invicta*, reared from newly mated queens, were inoculated with brood (an arbitrary mixture of eggs, larvae, and pupae) from *T. solenopsae* infected colonies of *S. invicta* to determine if new infections could be initiated in uninfected colonies. Newly mated queens were collected in Gainesville, FL, and infected colonies were collected from Paynes Prairie in Alachua County, FL. Inoculated colonies contained an average of 8,450 workers (range, 8,000–9,000), 25.6-ml brood (range, 20–26 ml), and 1 queen. Because brood consisted of a mixture of immature stages of varying sizes, the number of immature ants was not estimated. Colonies reared from newly mated queens and the field-collected colonies used for inoculation were checked for the presence of *T. solenopsae* spores by examining, under phase contrast microscopy (400×), a wet mount slide prepared from groups of ≈50 worker ants that were macerated with water in a tissue grinder. To inoculate a colony, 0.5 g (≅1.2 ml) of brood was separated from field-collected *S. invicta* colonies that were infected with *T. solenopsae.* The brood was placed next to a nest cell (Banks et al. 1981) that contained a colony so that it would be carried into the nest by worker ants. The percent prevalence of *T. solenopsae* in the brood used to inoculate colonies was not determined. However, spores were readily observed in worker samples collected from colonies from which brood was obtained for inoculation. Before inoculation, spores were not detected in the newly mated queen colonies that were used for the study. A total of 5 colonies were inoculated, and 0.5 g of uninfected brood per colony was introduced into 5 control colonies. Colonies were maintained using standard laboratory rearing methods (Banks et al. 1981) for this and all subsequent laboratory studies.

The brood volume and the number of adult worker ants per colony were categorized visually, using procedures that were adopted from Banks and Lofgren (1991). Colony population estimates were obtained before brood introductions, 6 and 13 wk later, and at 2- or 4-wk intervals thereafter for 29 wk. Queens were weighed beginning with week 14 and at 3- or 5-wk intervals through week 30. To confirm infections in inoculated colonies, 10 larvae (4th instar) or prepupae per colony were smeared individually onto a slide, stained with Giemsa (Undeen 1997), and examined for vegetative stages of *T. solenopsae.* Because infected eggs develop into adults within 44 d (daily minimum–maximum of 25.6–30°C, D.H.O., unpublished data), larvae or prepupae used to confirm infections were collected at least 9 wk after brood introductions to ensure that brood used to inoculate colonies were not included in the larval/prepupal smears. Uninfected eggs develop into adults in 30.5 d at 30°C (Porter 1988). Brood volumes and worker populations obtained on each sample date were summed separately for each colony to provide a measure of the amount of brood and live workers observed per colony for the entire study period. Brood and worker sums from the inoculated and control colonies were compared by *t*-tests. Live queen weights from each of the inoculated and control colonies were averaged over the sample dates and compared by a *t*-test.

**Large Laboratory Colony Inoculation 1.** Monogyne, *S. invicta* 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, 50,000–95,000), 68 ml brood (range, 30–90 ml), and 1 queen. A total of 10 colonies were inoculated with brood from *T. solenopsae* infected colonies (1 g [≅2.4 ml] brood per colony). A gram of brood from uninfected colonies also was introduced into each of 10 control colonies. Newly mated queen colonies were confirmed to be uninfected before inoculations following the methods of the small colony inoculation study. The presence of *T. solenopsae* spores was detected in colonies collected from the locations described in the small colony inoculations and used as a source of infected brood.

The brood volume and the number of adult worker ants per colony were determined using the procedures described in the small laboratory inoculation. To assess the effect of *T. solenopsae* infection on queen weights, pre- and postinoculation weights were recorded for each queen. Colony population estimates and queen weights were obtained before brood introductions, 8 wk later, and at 1- to 4-wk intervals thereafter for 22 wk. Brood volumes and worker populations per colony were summed over sample dates as described in the small colony inoculation study and compared by *t*-tests. Queen weights before brood introductions from inoculated and control colonies were compared by *t*-test. After brood was introduced, live queen weights were averaged over sample dates for each colony, and these averaged weights from inoculated and control colonies were compared by *t*-test.

**Large Laboratory Colony Inoculation 2.** To examine the effect of *T. solenopsae* on oviposition rates, 5 *S. invicta* colonies were inoculated with 1 g of brood per colony from *T. solenopsae* infected colonies and another 5 colonies were inoculated with uninfected brood. Colonies receiving brood were reared in the laboratory from newly mated queens, and contained an average of 93,500 workers (range, 80,000–105,000), 59 ml of brood (range, 20–75), and 1 queen. Newly mated queens and *T. solenopsae* infected colonies were

obtained from the locations described in the small colony inoculations.

Queens were removed from the colonies 8 wk after brood introductions, placed in separate containers, and the number of eggs laid in 1 h was recorded. Queens were then returned to the colonies, and the procedure was repeated at 12, 16, and 21 wk after brood introductions. Zero eggs were assigned to queens that died after 8 wk. Weights of live queens were recorded when brood was introduced and again each time oviposition rates were determined. Percentage reductions from initial (week 0) oviposition rates and queen weights were determined for each queen on each sample date. Percentage reductions were then averaged over sample dates and compared by *t*-test between inoculated and control queens. Pearson's correlation analysis also was conducted between percentage reductions in queen weights and oviposition rates obtained for individual queens from both inoculated and control colonies on each sample date.

**Field Inoculation.** To determine if field colonies of *S. invicta* could be artificially infected with *T. solenopsae*, brood from infected or uninfected *S. invicta* colonies were introduced into 10, polygyne *S. invicta* mounds located in a field in Alachua County, FL. Before brood was introduced, adult worker ant samples from these mounds were examined for *T. solenopsae* infection. No spores were detected in any of the samples. Brood was introduced into colonies by making an opening in a mound with a shovel and pouring 5 g (≅11 ml) of brood into the opening. Five mounds were inoculated with infected brood and 5 control mounds were inoculated with uninfected brood. Inoculated and control mounds were grouped in separate areas that were a minimum of 41.1 m apart. Adjacent inoculated mounds were located within 8.8 m of each other, and control mounds that were adjacent to each other were within 13.7 m. Brood introductions were made on 8 April 1997. On the 9th wk after brood introductions, and at 3- to 6-wk intervals thereafter for 40 wk, adult worker and brood samples were obtained from each mound to determine if colonies were infected. Adult workers were examined for spores and larval samples examined for vegetative stages using the procedures described for the laboratory inoculations.

On the 40th, 44th, 48th, and 66th wk 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. On the last 2 sample dates, mounds within a 0.116-ha circular plot (19.2-m radii) that encompassed the inoculated mounds were sampled to confirm that *T. solenopsae* had spread to colonies that were previously uninfected. The USDA population index ratings (Lofgren and Williams 1982) were determined for inoculated and control colonies on each worker/brood sampling date to assess effects of *T. solenopsae* on colony populations.

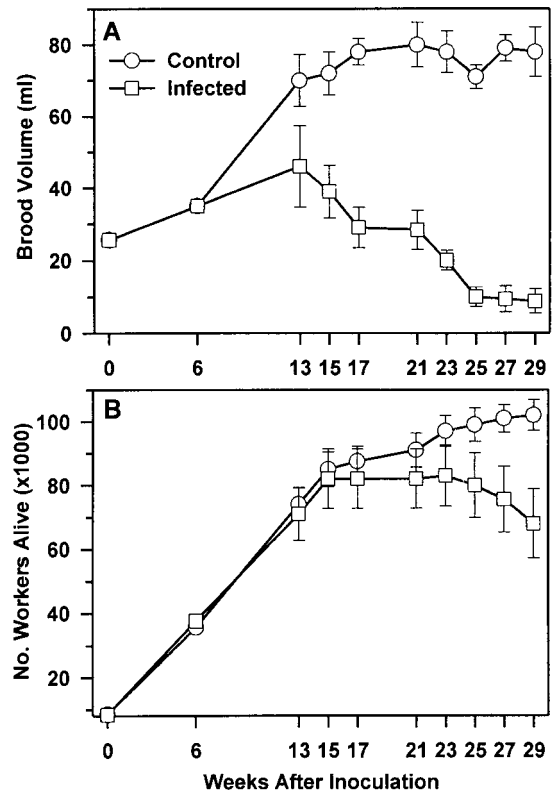


**Fig. 1.** Average (±SEM, $n = 5$) (A) brood volume per colony and (B) live adult workers per colony from small laboratory colonies of *S. invicta* inoculated with brood from uninfected (control) and *T. solenopsae* infected colonies.

## Results

**Small Laboratory Colony Inoculation.** Vegetative stages of *T. solenopsae* were detected in larvae or prepupae from all of the inoculated colonies. An average of 42.6% (range, 10–100%) of the larvae or prepupae examined per colony were infected. Inoculated colonies had significantly lower brood volume ($t = 8.64$, df = 8, $P < 0.01$), and reductions were evident by the 13th wk (Fig. 1A). There was 88.7% less brood in the inoculated colonies when the study was terminated on week 29. Inoculated colonies had an average of 8.8 ml (±3.3 SEM) brood per colony at 29 wk compared with 78.0 ml (±7 SEM) in the controls. Worker populations in inoculated colonies were not significantly lower than controls ($t = 1.30$, df = 8, $P = 0.23$), but worker populations were decreasing in the inoculated colonies. The control populations slowly increased during the last 4 wk of the study (Fig. 1B). The average weight per live queen for weeks 14 through 30 (Table 1) was 37% lower in the inoculated colonies ($t = 8.53$, df = 8, $P < 0.01$). By the end of the study (30 wk), 4 of 5 queens from inoculated colonies were not found and presumed dead, whereas all queens in the control colonies were alive.

**Table 1. Average ± SEM weight per live queen for specified weeks after brood introductions into small laboratory colonies of *S. invicta***

| Week | Avg wt (mg) per live queen | |
|---|---|---|
| | Infected | Control |
| 14 | 14.1 ± 0.7 | 20.1 ± 1.0 |
| 17 | 13.1 ± 0.7 | 21.9 ± 0.5 |
| 22 | 11.9 ± 0.4 | 22.1 ± 0.8 |
| 25 | 14.4 ± 1.1[a] | 21.6 ± 1.1 |
| 30 | 13.0[b] | 20.1 ± 1.3 |

$n = 5$.
[a] $n = 3$.
[b] $n = 1$.

**Table 2. Average ± SEM percent reduction in live queen weights for specified weeks after brood introductions into large laboratory colonies of *S. invicta***

| Week | Large lab. inoculation 1 | | Large lab. inoculation 2 | |
|---|---|---|---|---|
| | Infected | Control | Infected | Control |
| 8 | 18.2 ± 6.0 | −3.0 ± 3.5 | 8.3 ± 14.3[a] | −11.2 ± 4.4 |
| 10 | 31.2 ± 5.2 | −9.3 ± 2.1 | — | — |
| 12 | 31.6 ± 5.0 | −6.9 ± 2.6 | 31.6 ± 7.8[b] | −1.5 ± 8.4 |
| 15 | 32.4 ± 6.1[c] | 1.2 ± 3.5 | — | — |
| 16 | — | — | 23.2 ± 5.8[b] | 11.2 ± 10.9 |
| 18 | 40.3 ± 4.2[d] | −4.5 ±5.1[d] | — | — |
| 20 | 41.8 ± 4.3[e] | 4.5 ± 7.1[d] | — | — |
| 21 | — | — | 24.9 ± 0.0[f] | 1.8 ± 14.5 |
| 23 | 60.4 ± 3.1[d] | 18.3 ± 8.2[c] | — | — |

Average weight (mg) at 0 wk for inoculation 1 ($n = 10$), infected: 21.8 ± 0.5; control: 20.1 ± 0.5. Average weight at 0 wk for inoculation 2 ($n = 5$), infected: 19.8 ± 1.0; control: 18.2 ± 0.8.
[a] $n = 4$.
[b] $n = 3$.
[c] $n = 8$.
[d] $n = 9$.
[e] $n = 7$.
[f] $n = 2$.

**Large Laboratory Colony Inoculation 1.** Inoculated colonies had significantly lower brood volume ($t = 6.67$, df = 18, $P < 0.01$), worker populations ($t = 5.74$, df = 18, $P < 0.01$), and queen weights ($t = 6.25$, df = 18, $P < 0.01$) than control colonies. Reductions in brood and workers were evident at 10 and 11 wk after inoculation, respectively (Fig. 2 A and B). At the end of the study (week 22), brood per inoculated colony averaged 6 ml (±2.3 SEM) compared with 50 ml (±13.4 SEM) of brood from the control colonies. Thus, there was 92% less brood in the inoculated colonies. Survivorship of queens was 90% for both inoculated and control colonies, however inoculated colony queens were smaller in size than control queens. Average percent reductions in live queen weights (Table 2) for the inoculated colonies (35.5 ± 4.2% SEM) were significantly greater than the controls (−0.6 ± 3.0% SEM) when weights were averaged for 8–23 wk after inoculation ($t = -6.9$, df = 18, $P < 0.01$). Vegetative stages of *T. solenopsae* were detected in 6 of the 10 inoculated colonies, with an average of 30.4% (range, 10–80%) of the 4th-instar larvae or prepupae infected per colony. By the end of the study, brood in these 6 colonies was reduced an average of 85% (±7.4% SEM) and workers 5% (±14.8% SEM) from preinoculation volumes and populations, respectively. In the 4 colonies where vegetative stages were not detected, average brood reduction was 95.5% (±4.5% SEM) and worker reduction was 69.5% (±12.2% SEM). In contrast, control colonies had an average 11.1% (±29.8% SEM) increase in brood volume and a 96% (±11.0% SEM) increase in the number of workers. Although vegetative stages were not detected in 4 inoculated colonies, the reductions in brood and workers were much greater than in the controls and suggested that these 4 colonies were infected. Detection of vegetative stages of *T. solenopsae* was difficult because the rapid brood reduction limited the number of samples that could be obtained. Examination of adult worker ants for *T. solenopsae* spores was inconclusive because infected workers can develop from the introduced brood. Queens were not examined for infections.

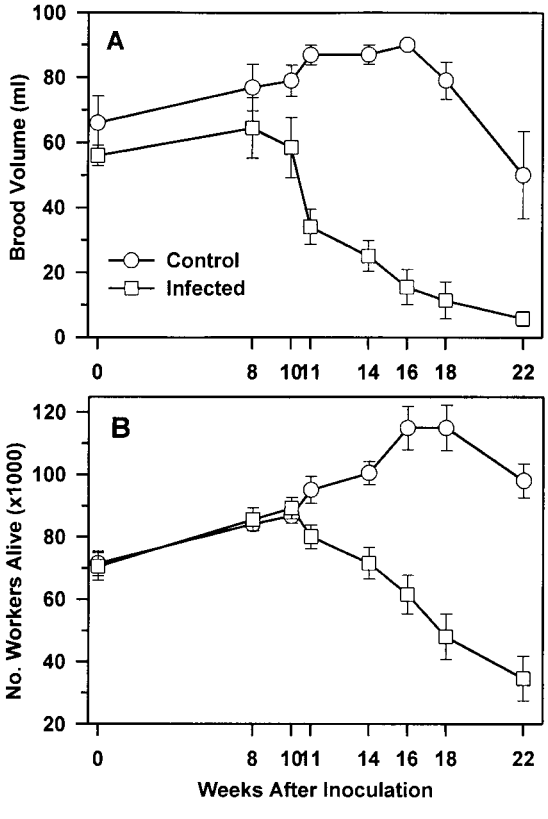


**Fig. 2.** Average (±SEM, $n = 10$) (A) brood volume per colony and (B) live adult workers per colony from large laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies (large laboratory colony inoculation 1).

**Large Laboratory Colony Inoculation 2.** Oviposition rates of queens from inoculated (85.3 ± 15.7 SEM eggs per hour) and control (65.8 ± 8.6 SEM) colonies at week 0 were not significantly different ($t = -1.151$, df = 7, $P = 0.29$). During the period from 8 to 21 wk

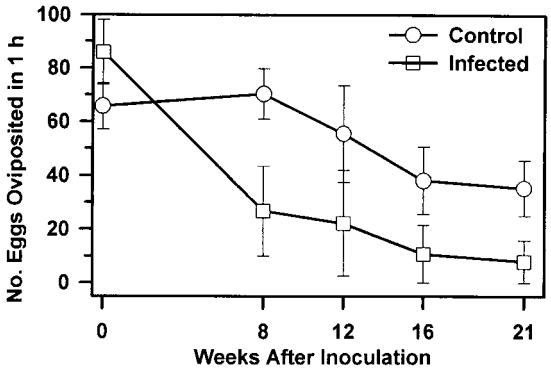


**Fig. 3.** Average (±SEM, $n = 4$ infected, $n = 5$ control) oviposition rates of queens from large laboratory colonies of *S. invicta* provided with brood from uninfected (control) and *T. solenopsae* infected colonies (large laboratory colony inoculation 2).

after brood introductions, there was an average 81.4% (±9.7 SEM) reduction in the number of eggs laid per queen per hour as compared with the week 0 oviposition rate for queens from inoculated colonies. This was significantly greater than the 22.3% (±17.5 SEM) reduction recorded from the control colonies ($t = -2.74$, df = 7, $P = 0.03$). This sampling period was compared because previous studies indicated that the effects of *T. solenopsae* infections generally became detectable 8 wk after inoculation. During this 8- to 21-wk sampling period, the average number of eggs laid per queen per hour from inoculated colonies was 66% lower than control colonies ($t = 2.03$, df = 7, $P = 0.08$) (Fig. 3).

Live queen weights before brood introductions did not differ significantly ($t = -1.29$, df = 7, $P = 0.24$) between inoculated and control colonies. After brood introductions, live queen weights were reduced 26.3% in the inoculated colonies and 0.1% in the controls ($t = -2.21$, df = 7, $P = 0.06$) (Table 2). Vegetative stages were observed from 3 of 4 colonies with a 10% infection rate per colony (brood production declined too fast to be collected for examination from a 5th colony). Three of the 5 queens from inoculated colonies were dead by week 16, whereas all control queens were alive on week 21. Two of the dead queens were from colonies in which *T. solenopsae* infections were confirmed by the detection of vegetative stages, whereas the remaining dead queen was from the colony in which brood samples could not be obtained (infections in queens could not be confirmed). Percent reductions in queen weights were positively correlated with percent reductions in oviposition rates ($r = 0.79$, $n = 32$, $P < 0.01$).

**Field Inoculation.** *T. solenopsae* infections were first detected in 4 of the 5 inoculated colonies 22 wk (September 1997) after brood introductions and 27.5% of the larvae examined were infected. In subsequent samples, both spores in adults and vegetative stages in brood were recovered from these 4 colonies. *T. solenopsae* was not found in any of the control colonies.

**Table 3. Average ± SEM percent reductions in *S. invicta* population indices of colonies inoculated with either infected or uninfected brood, and the number of colonies with *T. solenopsae* infection from a field study in Alachua County, FL**

| Weeks after inoculation | Inoculated plot | | Control plot | |
|---|---|---|---|---|
| | Avg % reduction in pop. index[a] | No. colonies Infected/ Sampled[b] | Avg % reduction in pop. index[a] | No. colonies infected/ sampled[b] |
| 9 | 18.4 ± 18.4 | 0/5 | 4.0 ± 4.0 | 0/5 |
| 14 | 8.0 ± 8.0 | 0/5 | 3.0 ± 8.3 | 0/5 |
| 18 | 8.0 ± 8.0 | 0/5 | 3.0 ± 8.3 | 0/5 |
| 22 | 36.0 ± 4.0 | 4/5 | 16.0 ± 4.0 | 0/5 |
| 26 | 36.0 ± 4.0 | 2/5 | 16.0 ± 4.0 | 0/5 |
| 32 | 4.0 ± 4.0 | 3/5 | −5.0 ± 5.0 | 0/5 |
| 35 | 16.0 ± 11.7 | 4/5 | −5.0 ± 5.0 | 0/5 |
| 40 | 19.2 ± 19.2 | 2/10 | −5.0 ± 5.0 | 0/23 |
| 44 | 19.2 ± 19.2 | 5/12 | −5.0 ± 5.0 | 0/24 |
| 48 | 30.4 ± 19.3 | 14/53 | −5.0 ± 5.0 | 0/19 |
| 66 | 98.4 ± 1.0 | 18/31[c] | 78.6 ± 9.9 | 0/4[c] |

The population index at week 0 was 50 and 49 for the inoculated and control plots, respectively. No colonies were infected at week 0.

[a] $n = 5$.

[b] Includes inoculated colonies.

[c] Ant samples difficult to obtain due to hot, dry conditions.

All inoculated mounds were still active after 48 wk; however, there was a 30% reduction in the average population index for the inoculated colonies in contrast to a 5% increase in the controls (Table 3).

At 40 and 44 wk after inoculations (January and February 1998), 2 and 1 additional colonies, respectively, surrounding inoculated mounds were found to be infected with *T. solenopsae.* In week 48 (March 1998), *T. solenopsae* was found in 12 colonies of 48 colonies sampled (excluding the 5 inoculated colonies) within the 0.116-ha plot that encircled the inoculated mounds. Infected colonies found in weeks 40 and 44 were still active and infected in week 48. Sixty-six weeks after inoculations (July 1998), 18 of 31 colonies that were not originally inoculated were infected within the inoculated plot. Two of the 5 inoculated colonies were still active but were rated as having <100 ants each. The 5 control colonies that received uninfected brood remained active and uninfected with an average population rating equivalent to ≈16,000 ants per colony. Locations of 4 of the 12 infected colonies from the March 1998 sampling corresponded to infected colony locations in July 1998. The remaining 8 infected colonies from the March 1998 samples may have moved or died. Hence, the 6 new infected colonies found in July indicated that *T. solenopsae* had spread. In addition, there was a 98% reduction in the population index from inoculated colonies. However because of the hot, dry weather, the population index in the control colonies had declined by 78% (Table 3).

## Discussion

This article documents artificially initiated horizontal transmission of *T. solenopsae* infections among fire ant colonies. The laboratory infections resulted in significant reductions in brood and workers. Lower

oviposition rates, queen weights, and queen survivorship also were documented from infected colonies. Tschinkel (1988) demonstrated reductions in oviposition rates of *S. invicta* queens in the absence of 4th-instar larvae and this may contribute to the decline of infected colonies. However, because *T. solenopsae*-infected larvae can develop into adults (D.H.O., unpublished data), colony reduction is probably initiated by *T. solenopsae* infection in queens rather than reductions in the numbers of 4th-instar larvae. Briano and Williams (1997) reported that all sizes of adult *S. richteri* workers that were infected with *T. solenopsae* and then starved, had died <1.5 d faster than uninfected workers. Thus, infections in the worker caste does not seem to be a cause of queen debilitation. Worker populations may have declined faster in the large colony inoculations than in the small colony inoculations (Figs. 1B and 2B) because of the age of the colonies when the studies were initiated. Average longevity of adult, *S. invicta* workers range from 18 to 36 wk at 24°C (Hölldobler and Wilson 1990). The large colonies were 46 wk old when the study was initiated and thus probably had a greater percentage of workers approaching senescense, in contrast to the small colonies which were ≈26 wk old. The effects of *T. solenopsae* in monogyne, laboratory colonies were noted 8–12 wk after inoculations. 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. These results suggest that *T. solenopsae* slowly debilitates an *S. invicta* queen resulting in diminished reproductive capacity and death.

Artificial inoculations resulting in the infection and spread of *T. solenopsae* in field colonies of *S. invicta* occurred in a polygyne population. The impact of *T. solenopsae* may have been slowed or reduced when compared with monogyne colonies infected in the laboratory. A possible reason for this difference is that some queens in polygyne colonies may not become infected (D.F.W., unpublished data).

The mechanism by which a colony becomes infected when infected brood is introduced is not known. Introductions of various formulations of spores in attractive foods, and the addition of dead, infected adult workers, or macerated infected brood to colonies have not resulted in infections (D.F.W., unpublished data). The results of our studies and the prevalence of *T. solenopsae* in polygyne fire ant populations (Williams et al. 1998) suggests that the exchange of live brood or brood raiding is one pathway for the natural spread of *T. solenopsae.*

Natural epizootics have been observed in ant colonies (Marikovsky 1962, Allen and Buren 1974, Evans 1989); however, artificial inoculations resulting in sustained infections in field colonies have not been reported. In general, entomopathogens and nematodes previously studied for fire ant control have required direct application and contact with individual ants for infections to occur (Drees et al. 1992, Oi et al. 1994). Usually, only ants that contacted the organism became infected and infections did not spread to other fire ant colonies. In contrast, *T. solenopsae* inoculations of *S. invicta* colonies have resulted in noninoculated progeny becoming infected, and infections have spread to noninoculated colonies. 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.

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