Thelohania solenopsae (Microsporidia: Thelohaniidae) Infection in Reproductives of Red Imported Fire Ants (Hymenoptera: Formicidae) and Its Implication for Intercolony Transmission

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ABSTRACT The natural mechanism of intercolony transmission of *Thelohania solenopsae*, a pathogen of red imported fire ants, *Solenopsis invicta*, is unknown. However, *T. solenopsae* can be transmitted by introducing infected brood into an uninfected colony. We hypothesized that the transfer of brood among colonies during intercolony competition may be a mechanism for the horizontal transmission of *T. solenopsae*. Male and female reproductive caste alates, collected during the initiation of mating flights from infected colonies, had *T. solenopsae* infection rates of 93 and 75%, respectively. In addition, 47 field-collected, newly mated queens that were reared in the laboratory established *T. solenopsae*-infected colonies that contained egg, larvae, pupae, and adults. Because *T. solenopsae* is transovarially transmitted, this indicated that infected founding queens generated infected colonies. A life span of ≤52 wk was documented for 81% of the infected queens and 59% for uninfected queens. To determine whether imported fire ant colonies can become infected with *T. solenopsae* via colony raiding, seven pairs of *S. invicta* colonies consisting of large, uninfected and small, infected colonies were given access to each other in the laboratory. *T. solenopsae* infection was detected in four of seven of the large colonies. In the four large, infected colonies, brood levels declined an average of 64% after 22 wk in contrast to a 116% increase in the controls. Thus, there was evidence that incipient, *T. solenopsae*-infected colonies could provide a source of inocula for the horizontal transmission of *T. solenopsae* through the transfer of brood during colony raiding.

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

Thelohania solenopsae KNELL, ALLEN, and Hazard is a microsporidian pathogen of imported fire ants (Allen and Buren 1974, Allen and Silveira-Guido 1974). Infected queens have lower oviposition rates and die prematurely (Knell et al. 1977, Williams et al. 1999). Evidence of transovarial transmission of this pathogen has been reported by Briano et al. (1996) and Valles et al. (2002), but the natural mechanism of intercolony transmission is unknown. Infections of *T. solenopsae* can be initiated artificially in uninfected red imported fire ant *Solenopsis invicta* Buren colonies by introducing live, infected brood (Williams et al. 1999, Oi et al. 2001, Oi and Williams 2002). Because infected brood can be used for inoculations, natural mechanisms of brood transfer between colonies can potentially result in intercolony transmission.

Natural mechanisms of brood transfer among fire ants include brood raiding, in which incipient ant colonies steal brood from other incipient colonies (Tschinkel 1992a), and colony raiding, in which larger colonies overrun smaller colonies (Tschinkel 1993) and perhaps abduct brood, as reported for *Myrmecocystus mimicus* Wheeler (Hölldobler 1981). Our objective was to determine whether *T. solenopsae* was present in some of the necessary components of the brood- and colony-raiding processes. Specifically, we wanted to determine whether: 1) fire ant reproductives infected with *T. solenopsae* participate in mating flights; 2) infected newly mated queens could found infected colonies; and 3) uninfected colonies would abduct brood from a *T. solenopsae*-infected colony and become infected.

Materials and Methods

Infection in Alates. Flight traps modified from Morrill and Whitcomb (1972) were placed over nine *T. solenopsae*-infected colonies located in Gainesville, FL. Colonies appeared to be polygynous based on the preponderance of minor adult workers and relatively few major workers (Greenberg et al. 1985). Absolute determinations of monogyny or polygyny based on the number of inseminated queens (Porter et al. 1991) or by genotype (Valles and Porter 2003) were not conducted. To enhance collections, a metal rod was inserted into each nest such that the distal end extended

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beyond the collecting pan located near the apex of the trap. During the initiation of nuptial flights, alate reproductives climbed to the top of the rod and then began to fly above the collecting pan and thus were trapped more efficiently. Adult alate *S. invicta* were collected from the traps during the summer of 1998 and 1999. A sample of 18–20 alates from each colony was examined for *T. solenopsae* meiospores by macerating individual alates and preparing wet mount slides for examination under phase-contrast microscopy (Oi and Williams 2002).

Infection in Newly Mated Queens. Newly mated queens were collected from parking lots and sidewalks at various locations in Gainesville (Alachua County), FL. Because of predation and dispersion into cryptic nest sites, queens probably were collected within 24 h of a nuptial flight. Each queen was confined to a separate, capped, nest tube, which was a modification of newly mated queen cells described by Banks et al. (1981). A nest tube consisted of a glass test tube half filled with water, then plugged with a layer of cotton, followed by a layer of dental plaster (Castone; Dentsply Trubyte Division, York, PA). Queens laid and reared eggs to adulthood within the nest tube. When adult worker caste ants eclosed, tubes were uncapped and placed in plastic boxes ($7.5 \times 23 \times 5$ cm) with the inner sides coated with Fluon (ICI Fluoropolymers, Downington, PA) to prevent ants from escaping. Ants were provided a diet of frozen, farm-raised crickets; water; and a 10% sucrose solution.

T. solenopsae has been detected in all developmental stages and castes of fire ants (Briano et al. 1996, Valles et al. 2002), and in eggs and larvae obtained directly from infected *S. invicta* queens (D.H.O., unpublished data). Thus, the presence of spores observed under phase-contrast microscopy of wet-mount slides prepared from macerated adult workers (Williams et al. 1999) served as an indication of *T. solenopsae* infection in queens. Dead queens that could be recovered from colonies reared from newly mated queens were also checked for infection using the method described above. Survivorship of queens from infected colonies was monitored weekly. We made paired comparisons of the ages of the infected queens with the maximum age of uninfected queens (as of 31 January 2003 for queens still alive) with a paired *t*-test (SAS Institute 2001). Paired queens had identical collection locations and dates; hence, they were reared under the same conditions during the same time frame. We also took an arbitrary census of the ages of queens collected in 1999–2002 that founded uninfected colonies to provide an indication of their life spans under our rearing conditions.

Brood volume and the number of adult workers from four surviving infected colonies and four healthy, uninfected colonies, reared from newly mated queens collected on similar dates, were compared with a *t*-test (SAS Institute 2001). Brood volume and worker populations were estimated visually using procedures modified from Banks and Lofgren (1991). Brood and worker estimates were obtained on 2 December 2002, which was between 26 and 185 wk after collection of queens.

Brood Abduction from Infected Colonies. Seven small colonies of *T. solenopsae*-infected *S. invicta*, which contained an average of 1,100 (±412 SD) adult worker caste ants, 6.3 (±3.9) ml of brood, and 1 queen, were held in nest cells (Banks et al. 1981) placed in fluon-painted containers (32 cm L × 18 cm W × 8 cm H). Five of the small colonies were subcolonies of a polygyne, field-collected *T. solenopsae*-infected colony. This colony had an infection rate of 30% based on microscopy examinations for vegetative stages of *T. solenopsae* of 10 individual Giemsa-stained larval or prepupal smears (Undeen 1997, Williams et al. 1998). The remaining two small colonies were reared in the laboratory from newly mated queens in which *T. solenopsae* was detected in the adult workers (larval infection rates were not determined). Seven large, uninfected *S. invicta* colonies that contained an average of 5,571 (±1,134 SD) adult worker caste ants, 25.7 (±45 SD) ml of brood, and 1 queen were housed in nest cells in separate containers. These large colonies were individually reared from field-collected newly mated queens. Queens from each colony were marked with paint to indicate their colony source. In addition, seven pairs of uninfected small (1,057 ± 331 SD workers, 5.9 ± 3.2 ml brood) and large colonies (5,286 ± 1,800 SD workers, 26.4 ± 3.8 ml brood) that were reared in the laboratory from newly mated queens were used as controls. Small and large colonies were paired and were provided access to each other by a bridge suspended between the containers. Water and sucrose solution were provided to each colony; however, frozen crickets and chicken egg yolk were placed only in the container with the small colony to facilitate interaction between the colonies.

Colonies were examined every 1–3 d for the first 3 wk to monitor colony interactions and movement, and then at 1- to 2-wk intervals for 22 wk to document colony vigor by estimating the number of adults, volume of brood, and presence of queens, as described previously. *T. solenopsae* infections were ascertained from examination of 10 larval smears per colony at 8 and 10 wk after colonies had access to each other. At the end of the study, infection of surviving queens from infected colonies was determined by searching for *T. solenopsae* meiospores in smears prepared with queens (Oi and Williams 2002). Estimates of worker populations and brood volume were averaged over all sampling dates and compared between the infected and control colonies using a one-way analysis of variance (ANOVA) (SAS Institute 2001).

Results and Discussion

Infection in Alates. *T. solenopsae* infection was detected in 93.3% of the 45 males examined from 7 colonies (3–10 males per colony) and 75.2% of the 133 females examined from 9 colonies (10–20 females per colony) that were collected during the initiation of mating flights. The average infection rate of both males and females per colony was 79.9% (±22.6 SD).

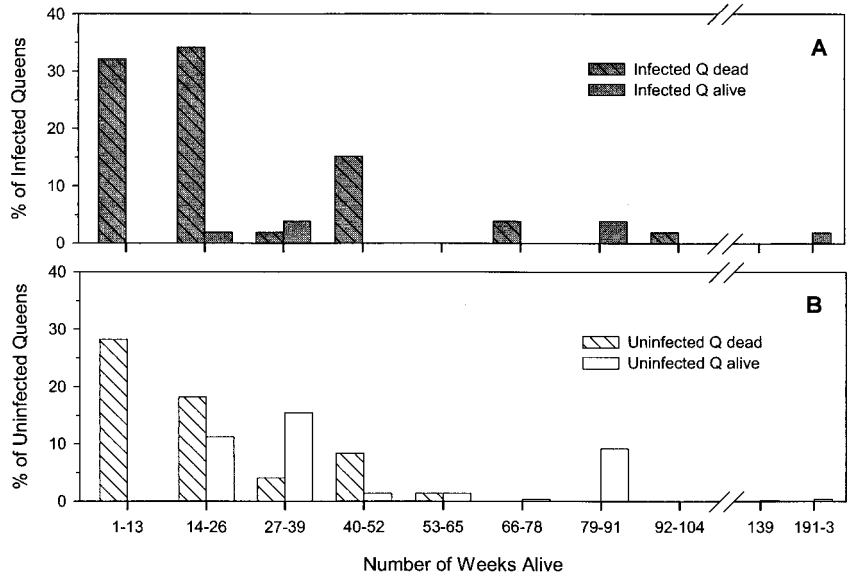

Fig. 1. Percentage of age distribution of (A) *T. solenopsae*-infected ($n = 47$) and (B) uninfected ($n = 510$) newly mated queens that established colonies in the laboratory.

The alates examined above were a subsample of a total collection of 63 males from 7 colonies and 394 females from 9 colonies. From these results, it was evident that *T. solenopsae*-infected alate reproductives could participate in nuptial flights. Cook et al. (2003) also reported infection in male and female *S. invicta* alates after nuptial flights with males and females having infection rates of 20.5 and <25%, respectively. Briano et al. (1996) documented infection rates of *T. solenopsae* in *S. richteri* alate females (75%) and males (80%) extracted from nests in Argentina. Our alate infection rates, however, were higher than the 46 and 31% reported for inseminated queens collected from polygyne, *S. invicta* colonies (Oi and Williams 2002, Williams et al. 1998) and the 34% reported from *S. richteri* (Briano et al. 1996).

Infection in Newly Mated Queens. We were able to confirm *T. solenopsae* infection in 47 incipient colonies of 659 colonies reared from newly mated queens collected over a 4-yr period. Average age of dead and alive (as of 31 January 2003) infected queens that established colonies containing all worker caste life stages (i.e., eggs, larvae, pupae, and adults) was 215 d (±233 SD, $n = 47$). The maximum average age of dead and alive uninfected queens that were collected on dates and locations identical with the infected queens was significantly ($t = 3.48$, df = 46, $P < 0.01$) older at 398 d (±276 SD, $n = 47$). A life span of <52 wk was recorded for 81% (38 of 47) of all infected queens with 6 (12.8%) infected queens still alive (as of 31 January 2003) ranging in age from 24 to 191 wk. In the census of laboratory colonies reared from uninfected newly mated queens, 59% (301 of 510) of these queens died within 52 wk and 39.6% (202 of 510) were still alive. Ages for 9.8% (50 of 510) of uninfected queens ranged from 80 to 193 wk (Fig. 1). Because many vigorous, uninfected colonies were used for other studies within 1 yr of newly mated queen collections and not included in the census, the queen longevity depicted in Fig. 1 is a conservative distribution of the life span of laboratory-reared colonies. Uninfected *S. invicta* colonies reared in the laboratory from newly mated queens can thrive for 3 or more yr (D.H.O., unpublished data).

Four of the forty-seven infected queens that were still alive after 181, 500, 550, and 1,273 d had significantly less ($t = -6.06$, df = 6, $P < 0.001$) brood volume in the infected colonies than in the uninfected colonies of a similar age with 8.3 ml (±5.8 SEM) and 46.3 ml (±2.4), respectively. The average number of worker ants was 16,875 (±1,206 SEM) in the infected colonies and 48,750 (±3,750) in the uninfected colonies ($t = -2.37$, df = 6, $P = 0.055$). Thus, it was evident that these *T. solenopsae*-infected newly mated queens that produced infected colonies grew less than colonies established by healthy queens.

Queens that founded infected colonies had shorter life spans than the potential 5–7 yr estimated for healthy queens (Tschinkel 1987). Earlier queen death also has been reported for colonies that were inoculated with *T. solenopsae* in the laboratory (Williams et al. 1999). Because of desiccation and microbial contamination of dead queens, we were unable to obtain an accurate infection rate of all newly mated queens that failed to initiate colonies. However, ≈40% of 1,790 newly mated queens collected from 1999 to 2002 established colonies with a minimum of ≈800 adults. Survivorship of queens and colony establishment in

Table 1. Summary of results of laboratory colony raiding between pairs ($n = 7$) of a small, *T. solenopsae*-infected colony and a large uninfected colony (Treatment) and pairs ($n = 7$) of uninfected controls (Control)

	Treatment	Control
No. dead queens from small colonies[a]	6/7	6/7
Average (±SD) no. of days to queen death	13.2 ± 3.1	7.7 ± 5.2
Occurrence of brood transfer	6/7	6/7
Average (±SD) no. days to complete brood transfer	8.8 ± 4.9	6.5 ± 2.3
No. of infected large colonies after raiding	4/6[b]	0/6[b]
Average max infection level in large colonies	80 ± 34%	0%

[a] All queens from large colonies survived.
[b] One pair of colonies did not transfer brood.

the laboratory can be affected by many factors, such as injury during collection, improper rearing, queen weight and fecundity (Keller and Ross 1993, Tschinkel 1993), insemination, and possibly infection by *T. solenopsae* or other microorganisms. Thus, the rate of successful colony establishment cannot be attributed solely to the presence or absence of *T. solenopsae.*

Brood Abduction from Infected Colonies. After an average of 8.8 d (±4.9 SD), all brood was found within a single colony in six of the seven pairs of the infected small and uninfected large colonies. Six of the seven queens from the small, infected colonies died within 16 d, while all of the queens from the large colonies survived for at least 22 wk, with the exception of one queen that was dissected before natural death at 18 wk. *T. solenopsae* infection was detected from larvae in four of the seven large or coalesced colonies. Average maximum larval infection rate for these colonies was 80% (±34 SD) (Table 1). Infection was found in one of two queens recovered from infected large colonies. Infection was not detected in either of the two large colonies paired with small infected colonies that were reared from newly mated queens. However, brood infection rates from these colonies were not obtained, and it is possible that *T. solenopsae* titers may have been insufficient for inoculation. The last uninfected large colony remained separate from the small infected colony.

In the controls, all brood was found within the large colony after 6.5 d (±2.3 SD) in six of seven pairs, and six of seven queens from the small colonies died. *T. solenopsae* was not detected in any of the control colonies (Table 1). Over the duration of the study, worker populations ($F = 12.4$; df = 1, 8; $P = 0.0079$) and brood volume ($F = 23.7$; df = 1, 8; $P = 0.0012$) were significantly lower in the infected large colonies than in the controls. After 22 wk, in the large colonies in which *T. solenopsae* was detected, brood levels declined 64% from levels at the start of the study. In contrast, there was a 116% brood level increase in the controls and a 78% increase in the two colonies that did not become infected despite being exposed to a small infected colony (Fig. 2). This further indicated that *T. solenopsae* infection had established and was impacting the fire ant colonies inoculated through colony raiding.

In these studies, we showed evidence of intercolony transmission of *T. solenopsae* via colony raiding. We documented that *S. invicta* male and female reproductive caste alates, infected with *T. solenopsae*, can initiate mating flights. In addition, field-collected, infected, newly mated queens founded colonies that were smaller and contained infected brood. It also was demonstrated that the abduction of brood from infected colonies can result in the transmission of *T. solenopsae* to uninfected *S. invicta* colonies. Competition among newly founded, or incipient, fire ant colonies often involves the stealing of brood and is referred to as brood raiding (Tschinkel 1992b, Adams and Tschinkel 1995a, Adams and Tschinkel 1995b). This competition is a major mechanism of early mortality among incipient colonies (Tschinkel 1992b). The transfer of brood between older colonies as a result of competition has not been specifically documented in fire ants under field conditions. However, it has been observed that larger colonies will overrun smaller colonies (Tschinkel 1993). Reductions in colony numbers after the period of brood raiding among incipient colonies (Lofgren and Williams 1985, Adams and Tschinkel 1995c) also provide circumstantial evidence of continued competition among nonincipient *S. invicta* colonies. Larger colonies of *Myrmecocystus mimicus* Wheeler (Hölldobler 1981) have been observed to kill adults and take brood from smaller colonies. The ongoing competition among older *S. invicta* colonies, which was depicted in our laboratory ab-

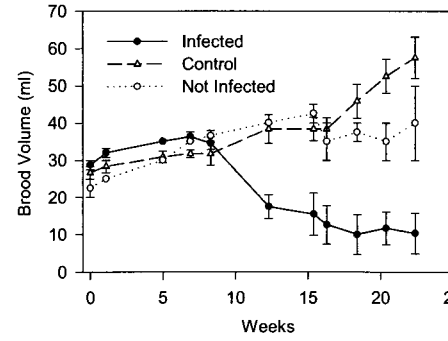

Fig. 2. Average (±SEM) brood volume of *S. invicta* colonies that became infected ($N = 4$), or remained uninfected (Not Infected, $N = 2$) after access to small *T. solenopsae*-infected colonies. Also shown is the average brood volume of colonies that raided uninfected small colonies (Control, $n = 6$).

duction study, represents an opportunity for the introduction of infected brood into uninfected colonies. For transmission to occur, infected newly mated queens must survive long enough to produce a sufficient quantity of infective brood. In addition, adoption by established colonies of *T. solenopsae*-infected, newly mated queens (Glancey and Lofgren 1988, Vander Meer and Alonso 2002) may also serve as a means of transmission through the oviposition of infected eggs within the adopting colony. Thus, infected, *S. invicta* female alate reproductives can provide a means for *T. solenopsae* transmission by mating and producing brood that can serve as inocula.

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