Project no.: 99-DO-o0
Project title: Biological Control of Ants
Project Leader: David Oi, USDA-ARS, Center for Medical, Agricultural, & Veterinary Entomology, Gainesville, Florida
Cooperating Personnel: Walt Bentley (UCCE/KAC)

Objectives:

1) Determine if the imported fire ant pathogen, *Thelohania solenopsae,* can infect two major ant pests of almonds, the southern fire ant, *Solenopsis xyloni* and the pavement ant, *Tetramorium caespitum*.

2) If *T. solenopsae* infections occur, evaluate the impact on colony populations of these ants.

Justification of objectives:

In the 1970's a microsporidium (protozoan) was found infecting several fire ant species in the genus *Solenopsis* in South America. A field study in Argentina reported an 83% reduction in black imported fire ant, *S. richteri*, populations (Briano et al. 1995). In 1996, *T. solenopsae* was found in the red imported fire ant, in Florida (Williams et al. 1998). Laboratory results with this pathogen have been excellent. *T. solenopsae* infects the queen's ovaries so that her egg production drops and she eventually dies (Williams et al. 1999). After 3 years of an ongoing field study in Florida, the artificial inoculation and natural spread of this pathogen has resulted in a 62% maximum reduction in red imported fire ant populations (D.H.O. unpublished data).

T. solenopsae represents a potential, non-chemical, self-sustaining biological control agent for imported fire ants and possibly other ant species. This study is an initial effort to assess the potential of *T. solenopsae* as an alternative control for pest ants of almonds. If *T. solenopsae* does not infect pavement and southern fire ants, this study will help define its host range. Host range information is important for the potential use of *T. solenopsae* in California for the control of red imported fire ants, which have been found in California almond orchards.

Summary of progress:

To accomplish objectives, pavement ant and southern fire ant colonies consisting of queen(s), brood (immature ants), and adult ants were collected in June and August of 1999 with the assistance of Walt Bentley (UCCE/KAC). Ants were separated from the soil using dripping water, and then transported to the USDA-ARS Center for Medical, Agricultural, & Veterinary Entomology in Gainesville, Florida. While summer is often too hot and dry to collect ant colonies, it was thought that irrigated orchard floors would make ant colonies accessible for collection. Over 30 colonies of both species were shipped to Gainesville. However, only six southern fire ant colonies had viable, or reproducing, queens, and no viable pavement ant queens were collected. The following studies were conducted in 1999 and 2000.

Southern fire ant inoculation study.

Methods: Collected colonies were allowed to establish and grow in artificial nests for 8 weeks. Three southern fire ant colonies were inoculated with the pathogen by placing 0.75

grams of *T. solenopsae* infected red imported fire ant brood near each colony. Three other colonies were not inoculated and served as controls. Infection levels of inoculum were determined by examining individual geimsa stained smears of larvae and prepupae samples for vegetative stages of *T. solenopsae* using phase contrast microscopy. For two replicates, inoculum infection levels were 70%, and 100% for the remaining replicate. To detect infection, adult, pupal, and larval ant smears were examined for *T. solenopsae* spores and vegetative stages at 12, 20, and 24 weeks after inoculations. Colony impact was assessed at 2 or 4-week intervals by estimating adult worker populations and the amount of brood using standard fire ant visual rating scales. To confirm that the inoculum could initiate infections, three laboratory reared red imported fire ant colonies were inoculated using the same procedures described for the southern fire ants. Three other red imported fire ant colonies were not inoculated and used as controls. Colony impact observations were terminated after 24 weeks because substantial declines of infected red imported fire ant colonies have occurred in other studies within 6 months (Williams et al. 1999).

Results: *T. solenopsae* infections were not detected in the southern fire ant colonies. The number of live, adult worker caste ants and brood volume per southern fire ant colony during the study are shown in Figures 1A and 1B, respectively. Average ant populations ranged between 16,000 and 32,000 for the inoculated colonies, and 15,000 and 30,000 for the controls. Brood volumes ranged from 16,000 to 23,000 ml for the inoculated colonies and 7,000 to 17,000 ml for the controls. Percentage reduction in adult ant populations from pre-inoculation levels in the inoculated and control southern fire ant colonies were not significantly different (t-value=1.76, df=46, P=0.0854). Percentage reduction in brood volume was significantly greater in the control than in the inoculated colonies (t-value= 2.24, df=37.9, P=0.0309). However, the average reduction in the control was minor at 30.7% and 11.1% in the inoculated colonies. All queens remained viable throughout the study.

T. solenopsae infection was detected in one of the three inoculated red imported fire ant colonies. No significant differences in percentage reductions occurred for adult and brood levels between the inoculated and control colonies. This lack of difference was most likely due to the large variation in brood levels in the controls beginning at week 20 (Fig. 2B) because queens died in two of the control colonies for unknown reasons.

Pavement ant inoculation study.

Because no viable pavement ant queens were collected, a study was conducted to determine if groups of worker caste adults would adopt *T. solenopsae* infected red imported fire ant brood. Studies with red imported fire ants have indicated that the adoption of live infected larvae by uninfected colonies resulted in infection (D.H. O. unpublished data). It was hypothesized that if pavement ant workers would adopt and rear infected red imported fire ant brood, it would be supportive of potentially infecting pavement ants by this method. Four groups of adult pavement ants (range: 70-110 ants) were each provided 0.25 grams of infected red imported fire ant brood (infection level of 60%) and 4 groups (range: 36-110 ants) were provided uninfected brood. All pavement ant worker groups adopted the red imported fire ant brood, however newly emerged fire ant adults were executed by the pavement ants. It is encouraging that infected brood was adopted by the pavement ant worker groups and not rejected immediately. Thus, further efforts to collect pavement ant colonies with viable

queens are warranted to determine if pavement ant colonies can be infected with *T. solenopsae*.

Conclusions: In general, inoculated southern fire ant colonies did not exhibit drastic reductions in adult populations or brood. Thus, *T. solenopsae* impact was not evident in the three, inoculated colonies. Because the number of southern fire ant colonies was limited, and infections were detected in only one of the three red imported fire ant colonies, more replications are needed to confirm these results. The adoption of red imported fire ant brood infected with *T. solenopase* by pavement ants is supportive of possibly infecting pavement ants with this pathogen. However, to determine actual infection and impact will require colonies with reproducing queens. More colonies of southern fire ants and pavement ants are being collected this spring, to provide additional replications.

References Cited:

Briano, J. A., R. S. Patterson, & H. A. Cordo. 1995. Long-term studies of the black imported fire ant (Hymenoptera: Formicidae) infected with a microsporidium. Environ. Entomol. 24: 1328-1332.

Williams, D. F., G. J. Knue, & J. J. Becnel. 1998. Discovery of *Thelohania solenopsae* from the red imported fire ant, *Solenopsis invicta*, in the United States. J. Invertebr. Pathol. 71: 175-176.

Williams, D. F., D. H. Oi, and G. J. Knue. 1999 Infection of red imported fire ant (Hymenoptera: Formicidae) colonies with the entomopathogen *Thelohania solenopsae* (Microsporidia: Thelohaniidae). J. Econ. Entomol. 92: 830-836.

Fig. 1

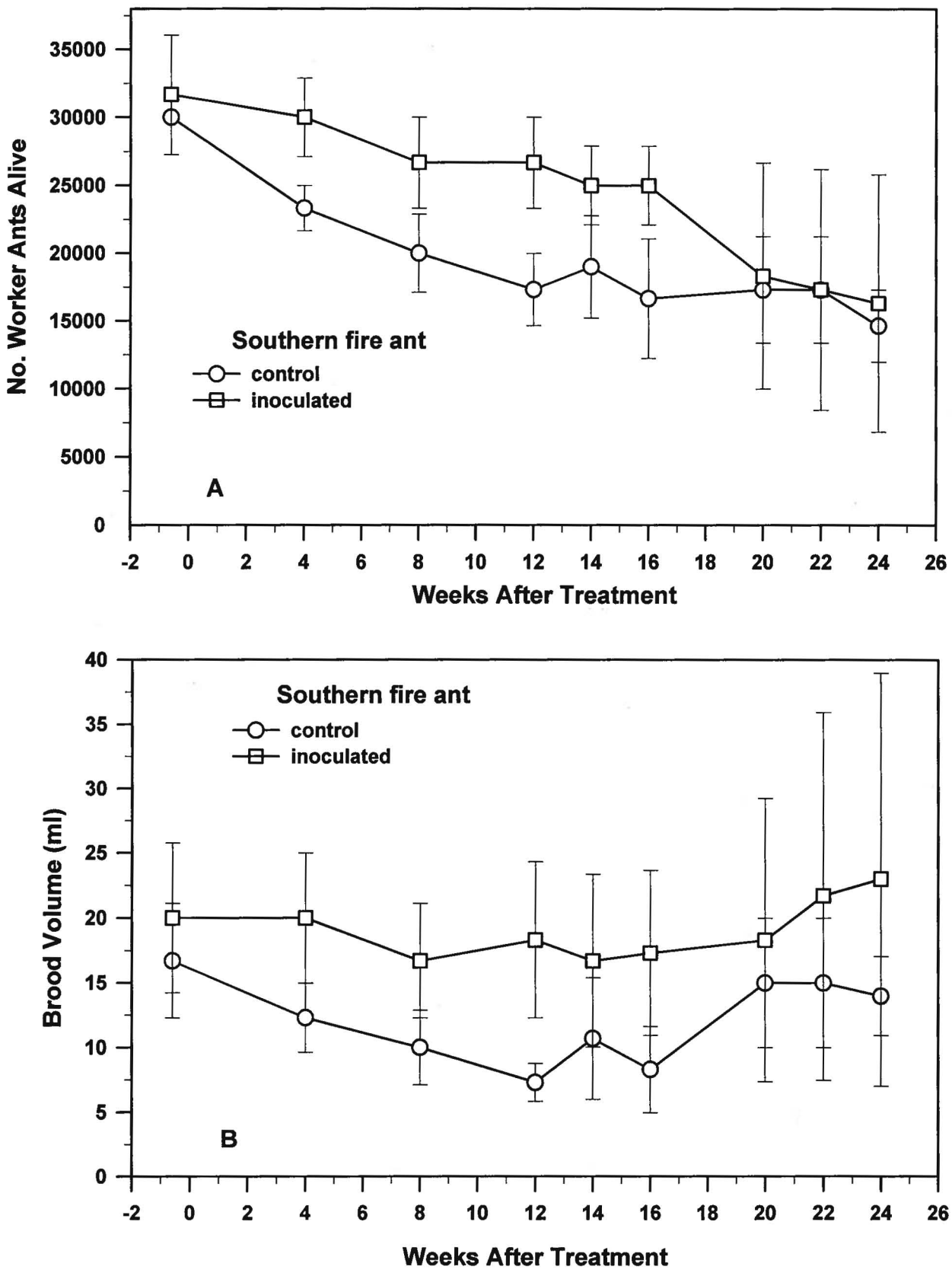

Fig. 1. (A) Average number of live, adult worker caste Southern fire ants per *T. solonopsae* inoculated and control colonies. (B) Average amount of brood (ml) per inoculated and control colonies. Vertical bars represent ± 1 standard error (n=3) .

Fig. 2

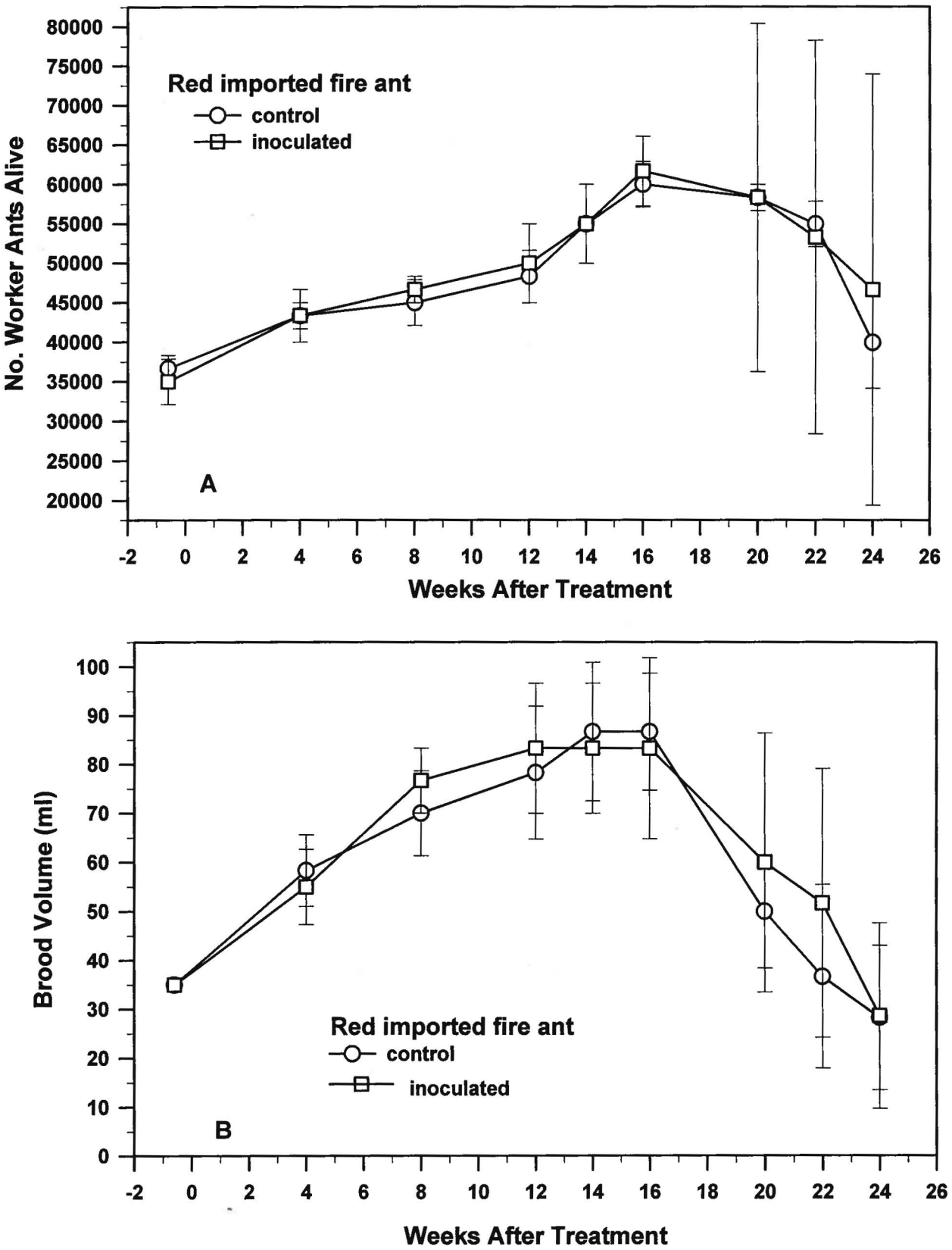

Fig. 2. (A) Average number of live, adult worker caste red imported fire ants per *T. solonopsae* inoculated and control colonies. (B) Average amount of brood (ml) per inoculated and control colonies. Vertical bars represent ±1 standard error (n=3).