

# Red Imported Fire Ant Colony Diagnostics Using Worker Head Widths

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## Introduction

Two social forms, monogyne (single queen) and polygyne (multiple queens) of *Solenopsis invicta* Buren coexist throughout much of the southern U.S. Several methods (in field queen counts, colony flotation, worker sizes, and PCR) are currently employed by researchers to determine social form. Detecting social form based on differences in worker size (maximum head widths) is appealing because sampling is relatively noninvasive to the colony, and measurements can be made the same day as sampling with nothing more than an ocular micrometer. Greenberg *et al.* (1985) found distinct differences in mean head widths of workers between social forms.

In 2003, our objective was to determine if differences in worker size were indicative of social form for *S. invicta* colonies in Louisiana. We found significant differences between head width means of social forms. We also concluded that additional sites needed to be sampled for better resolution of differences and an increased sampling effort was needed within sites to fine-tune site specific differences. We chose to continue this study based on our previous recommendations.

Samples were screened for *Thelophania solenopsae*, a naturally occurring microsporidia and potential biological control agent of *S. invicta*, in case infection influenced mean size distributions (Valles *et al.* 2002, Valles and Porter 2003).

## Materials and Methods

**Fencerow Survey:** Twenty samples from each of four Louisiana Agricultural Experiment Stations and five privately owned farms (Figure 1) were collected in summer 2003 and winter 2005, respectively. Specimens were obtained by inserting a Teflon® coated vial into *S. invicta* mounds located along pasture fencerows (Figure 2). Samples were preserved in 95% ethanol.

**Fences vs. Centers Survey:** Forty samples, 20 from fencerows and 20 from pasture centers were collected from each of the five privately owned farms. Collecting was conducted as above.

Twenty ant heads from each sample (fencerow n=3600; fences vs. centers n=4000) were mounted onto double stick tape, and maximum head widths were recorded using Image-Pro Plus v4.5 (Figure 3). Statistical analyses were conducted with SAS Proc Mixed ANOVA using Kenward-Rogers adjustment on the degrees of freedom.

PCR methods for determining social form of each colony and detection of *T. solenopsae* infection were described by Valles *et al.* (2002) and Valles and Porter (2003). PCR of the Gp-r alleles were not multiplexed. Presence of *T. solenopsae* within *S. invicta* colonies were indicated on figures to show distribution within genotypes.

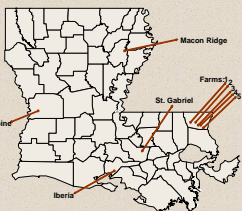


Figure 1. Louisiana Agricultural Experiment Stations and five farms from Washington Parish where *S. invicta* samples were collected.



Figure 2. Teflon® coated vial inserted into *S. invicta* mound for collecting specimens.



Figure 3. *S. invicta* worker heads, excluding antennae, mounted for head width measurements.

## Results

**Fencerow:** Mean head widths did not present an apparent separation between social forms, even though polygyne workers tended to have smaller heads (Figure 4). After adjusting for site and social form  $x$  site variances as random effects in our ANOVA, we detected significant mean differences between social forms in 2003 and 2005, respectively ( $F_{1,85,9}=38.99$   $p<0.0001$ ,  $F_{1,8,48}=20.70$   $p=0.003$ ). In 2003 the difference in mean head widths between social forms was  $0.16\pm 0.03$ , and in 2005 it decreased to  $0.1\pm 0.02$  ( $\pm$ SE) (Figure 5). Median head widths for monogyne and polygyne workers were 0.91 and 0.73, respectively, in 2003. In 2005 median head widths were 0.86 for monogyne workers and 0.76 for polygyne workers. Size differences between social forms appeared to be smaller with increased sampling.

The percentage of the polygyne form sampled among sites varied from 0 to 80%. *Thelophania solenopsae* was rarely detected in monogyne workers but frequently found in polygyne samples (Figure 4).

**Fences vs. Centers:** Worker head widths from monogyne colonies in centers of pastures were significantly larger than those from fencerow colonies ( $F_{1,8,82}=10.47$   $p=0.01$ ). No significant differences were detected from polygyne worker head widths between locations even though a similar trend was apparent ( $F_{1,387}=1.95$   $p=0.17$ ) (Figure 6).

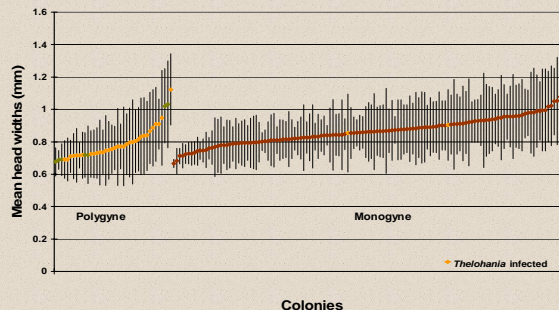


Figure 4. Fencerow Survey: means and standard deviations of *S. invicta* worker head widths with separation of polygyne and monogyne social forms. (n=180)

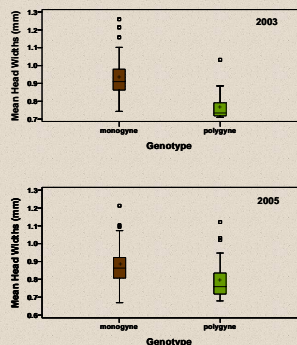


Figure 5. Box plots of *S. invicta* worker head width means from fencerows for 2003 and 2005. (n=80, n=180)

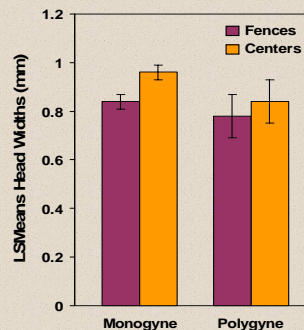


Figure 6. LSMeans  $\pm$  SE worker head width estimates by location of mounds. (n=200)

## Discussion

Contrary to Greenberg *et al.* (1985), worker head widths were not mutually exclusive among the two social forms, even though means were significantly different. Data from 2003 showed that a polygyne worker in the 90th percentile based on head width had a 25% chance of being misclassified as monogyne. In 2005, we found that a polygyne worker in the 90th percentile had a 75% likelihood of being incorrectly labeled as monogyne.

Several factors have led us to conclude that use of worker head widths as a diagnostic tool is not recommended. First, head widths vary from site to site, and between fencerows and centers within sites. Second, the overlap of sizes between social forms increased with additional sampling, while the difference between means of social forms decreased.

Methods used in this study did not allow us to show a direct correlation between worker head widths and *T. solenopsae* infection. In addition, social form appears to be confounded with *T. solenopsae* infection.

## Literature Cited

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- Valles, S.M., D.H. Oi, O.P. Perera, and D.F. Williams. 2002. J. Invertebr. Pathol. 81: 196-201.
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## Summary

Identification of monogyne and polygyne *S. invicta* colonies by differences in worker size (head widths) were compared against PCR discrimination of alleles for colony social form. Means of maximum head widths were significantly different between the two social forms both in 2003 and with additional data in 2005. However, reliable assignment of social form based on head widths was not possible because of considerable overlap in sizes between the two groups.



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