

Survival of Imported Fire Ant (Hymenoptera: Formicidae) Species Subjected to Freezing and Near-Freezing Temperatures

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ABSTRACT Survival at low temperatures is an important parameter determining distribution of imported fire ants in the United States. Supercooling points and survival at low temperatures, and the effects of species, individual size, and *Thelohania solenopsae* Knell, Allen & Hazard (Microsporida: Thelohaniidae) infection on these parameters, were examined. We tested *Solenopsis richteri* Forel, *S. richteri* X *invicta* hybrid, and *Solenopsis invicta* Buren. Great variation was observed in the supercooling points, which are not an appropriate measure of cold hardiness for imported fire ants. When exposed to near-freezing temperatures above their supercooling points, fire ants died at different rates depending on the species and *T. solenopsae*-infection status. Extended exposure to 4°C resulted in both the hybrid and *S. invicta* infected with *T. solenopsae* having significantly lower mortality rates than either the *S. richteri* or the uninfected *S. invicta*. At 0.5°C, the hybrids had significantly lower mortality than the uninfected *S. invicta*, but mortalities for *S. richteri* and *T. solenopsae*-infected *S. invicta* were not significantly different from each other or the hybrid. Ant mortality was 100% for all ant types after 7 d at -4°C. The uninfected *S. invicta* was consistently less cold-tolerant than the other ant types. The hybrid fire ants and the *T. solenopsae*-infected *S. invicta* had the lowest mortalities. These results support the hypothesis that extended cold injury causes winter kill of fire ants, and may partially explain the distribution of fire ant species in the United States.

KEY WORDS *Solenopsis invicta*, *Solenopsis richteri*, *Thelohania solenopsae*, red imported fire ant, black imported fire ant, hybrid fire ant

ORIGINALLY FROM SOUTH America, imported fire ants are well known pests throughout the southern United States and are found in 13 states and Puerto Rico, including 29 counties along Tennessee's southern border. The black imported fire ant, *Solenopsis richteri* Forel, is found in the northern portion of this range (Callcott and Collins 1996), an area approximately from 34.5 to 35.5° N (Shoemaker et al. 1996; R. Vander Meer, unpublished data) covering parts of northern Mississippi and Alabama, and in southern Tennessee from Shelby County to Giles County. The red imported fire ant, *S. invicta* Buren, occurs throughout the southern portion of the United States. The red/black hybrid, which is not found in South America, occurs in a band between the parent species east of the Mississippi (Callcott and Collins 1996, Shoemaker et al. 1996, Drees et al. 2000).

Originating from tropical and subtropical regions, imported fire ants were not expected to have mechanisms to survive extreme cold weather. Overwinter-

ing fire ant adults are nondormant and nonfreeze-tolerant (Francke and Cokendolpher 1986). Although imported fire ants may be active and forage on warm days during winter, they cease activities outside the mound at 10–15°C (Porter and Tschinkel 1987, Cokendolpher and Phillips 1990). Imported fire ants were not expected to infest areas north of the -12°C minimum January isotherm (Diffie et al. 1997), which runs ≈100 km south of Tennessee's southern border. However, the expansion of imported fire ants beyond this isotherm has led to speculation on the final limits of imported fire ant range in the United States.

Imported fire ants have a well developed sensitivity to temperature and humidity gradients (Porter and Tschinkel 1987, Cokendolpher and Phillips 1990), particularly when tending brood (Cokendolpher and Francke 1985, Porter and Tschinkel 1993). Imported fire ants move within the column of the nest soil depending on daily temperature changes (Pinson 1980), going deeper in the mounds to avoid cold (Morrill et al. 1978). Other factors, such as colony size (Green 1959, Callcott et al. 2000), clustering behavior (Kaspari and Vargo 1995), and nest construction/location (Hubbard and Cunningham 1977, Morrill et al.

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1978), may also play an important role in imported fire ant winter survival.

The northward expansion of the imported fire ant range, the lack of correlation between supercooling points and the location of imported fire ant species, and the lack of information on imported fire ant ability to survive cold, nonfreezing temperatures, led us to study imported fire ant survival at low temperatures. Also, we were interested in the effects of the recently introduced biological control agent *Thelohania solenopsae* Knell, Allen & Hazard (Microsporida: Thelohaniidae) (Williams et al. 1999) on the imported fire ant ability to survive these low temperatures. The objectives of this research were to determine low temperature survival of *S. invicta*, *S. richteri*, and the *S. richteri* X *invicta* hybrid, and to examine the effects of seasonal acclimation and *T. solenopsae* infection on imported fire ant tolerance to low temperatures.

Materials and Methods

Solenopsis richteri, uninfected *S. invicta*, *T. solenopsae*-infected *S. invicta*, and the *S. richteri* X *invicta* hybrid are herein called ant "types" so the improper term "species" would be avoided when referring collectively to all ants tested.

Imported Fire Ant Colonies. *S. richteri* and hybrid colonies were collected from Tennessee's southern border counties. *S. richteri* was collected from Fayette and Hardeman counties, on 1 November 1999 (fall *S. richteri*), and 25 May 2000 (spring *S. richteri*). Hybrid colonies were collected from Bradley County on 29 November 1999 (fall hybrid) and 9 June 2000 (spring hybrid). Additional hybrid colonies from Sequatchie County, Tennessee, were collected on 11 January 2000 (winter hybrid).

Colonies of *S. invicta* were collected in Florida, from polygyne populations. Colonies infected with *T. solenopsae* were collected from Alachua County on 20 March 2000 (infected spring *S. invicta*). Uninfected *S. invicta* colonies were collected from Taylor County on 20 March 2000 (uninfected spring *S. invicta*). Five colonies of uninfected and five colonies of *T. solenopsae*-infected *S. invicta* were also collected on 13–16 December 1999 from the same locations (winter infected and uninfected *S. invicta*). At the time of collection, nest soil was shoveled into an 18.9-liter bucket. An intravenous drip-tube setup (Banks et al. 1981) was used to separate ants from nest soil. Floating ants were transferred to ant boxes (plastic containers 40.6 by 28.5 by 17.5 cm or 34.5 by 24 by 12.5 cm) (Rubbermaid, Wooster, OH) coated with Fluon (Asahi Glass Fluoropolymers, Chadds Ford, PA) to prevent escape.

Artificial nests (20 by 150-mm test tubes with moist cotton at the end or 150 by 15-mm petri dishes with dental plaster [Castone, Dentsply-Trubyte, Jacksonville, FL]) were provided in each ant box. Test tubes (20 by 150 mm) of deionized water plugged with cotton were used as a water source. Ants were fed to repletion on a 15% honey–15% dog food agar diet and live insects [*Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Popillia japonica* Newman (Coleoptera: Scar-

abaeidae), *Galleria mellonella* L. (Lepidoptera: Pyralidae), and *Periplaneta americana* L. (Blattaria: Blattellidae)]. Gas chromatographic analysis of venom and cuticular hydrocarbons (Vander Meer et al. 1985) was used to identify types at the USDA–ARS, CMAVE laboratory in Gainesville, FL.

Supercooling Tests. Five colonies of each ant type were selected for each test. Ten female alates (when available), small (head capsule <0.9 mm) and large (head capsule >0.9 mm) workers were selected at random from each colony and individually frozen to collect supercooling point data. Worker ants were selected from the smallest and largest ants in each colony. Based on preliminary data, supercooling point variation increased after a month or more exposure to laboratory conditions, thus ants were tested as soon after collection as possible (1 wk for Tennessee colonies, 2 wk for Florida colonies). Ants collected in fall 1999 also were tested after 3 mo in the laboratory to determine the effects of maintenance under laboratory conditions. Testing of large and small workers (10 ants for each size from five colonies) using both 0.01 and 0.1-mm thermocouples was conducted to evaluate the effect of thermocouple size on the supercooling point readings.

To measure the supercooling point, ants were attached by means of petroleum jelly to either 0.01 mm (small workers) or 0.1 mm (large workers and alates) copper-constantan thermocouples (Omega Engineering, Stamford, CT) (Francke et al. 1986, Diffie and Sheppard 1989, Landry and Phillips 1996). Each thermocouple was attached to a 3-mm-diameter wooden dowel protruding through the center of a stopper that held the ant/thermocouple array in the center of a 48 by 15-mm glass vial. Vials with the ant/thermocouple arrays were placed in a test tube rack in a chest freezer set at -24°C . The temperature drop rate in the vials was $\approx 5.6^{\circ}\text{C}/\text{min}$ during the first 4 min, slowing to $\approx 3.5^{\circ}\text{C}/\text{min}$ by 10 min. Ant temperature changes were recorded through a Campbell Scientific CR10 data logger (Logan, UT) connected to a computer running Campbell Scientific PC208W data logger support software. The supercooling point of each insect was recorded as the lowest reading reached before the release of latent energy shown as an abrupt increase in the temperature. Frozen ants were saved for head capsule measurement and verification of infection by *T. solenopsae*.

To determine if ant size correlated with supercooling point, the head capsules were measured with a wedge micrometer (Porter 1983). Ants from *T. solenopsae*-infected *S. invicta* colonies also were checked individually for *T. solenopsae* infection by microscopic examination ($400\times$) of a wet mount slide of the ant abdominal contents. Because live ants do not show external signs of *T. solenopsae* infection, infection status could not be confirmed before supercooling tests. The supercooling points were compared between *T. solenopsae*-infected ants and apparently uninfected ants originating from infected colonies. This comparison allowed us to determine if the presence of *T.*

solenopsae infection in the colony affected the cold tolerance of the uninfected ants.

Extended Low Temperature Exposure Tests. The spring-collected colonies used in supercooling tests also were used in the extended cold exposure tests. Groups of ≈ 20 –30 (range, 14–85) worker ants of various sizes were collected at random and placed into test tubes (20 by 150 mm), with a Fluon ring to prevent ant escape and a base of moist Castone to maintain humidity. Tubes were closed with caps that allowed gas exchange and then placed in test tube racks. For each of five colonies from each ant type described above, one test tube was prepared for each of seven sampling days, for each of three temperature regimes (4 ± 0.5 , 0.5 ± 0.5 , and $-4 \pm 0.3^\circ\text{C}$), resulting in a total of 105 test tubes per test ant type. Mortalities at the different sampling dates were measured from independent ant samples (tubes), which were discarded after use. Thus, the observations were based on ants exposed constantly to the temperature regimes.

Ants were sampled after 1, 2, 3, 5, 7, 10, and 13 d at the test temperatures. To avoid preparation of excessive number of tubes, only enough tubes for four sample days were initially prepared. Tubes for days 7–13 were placed in refrigerators only after removal of initial samples, and only for those groups that had surviving ants after 5 d. The racks of ant tubes were placed inside Styrofoam coolers that had 4-cm walls and sand to a depth of ≈ 2 cm to provide temperature buffer against the refrigerator cycle.

All temperature regimes were above the fire ant supercooling points as determined in the previous experiments. At all these temperatures, cold coma was induced within 24 h; therefore, the ants were not provided with food and water. Without food or water, the ants kept at room temperature in preliminary tests had excessive mortality and this control treatment was not used subsequently. StowAway (Onset, Pocasset, MA) data logger units were used to record temperatures in each cooler in the refrigerators. Coolers were left open after being placed in the refrigerator, to facilitate the temperature drop in the test tubes, but were closed after 4.5 h to maintain uniformity at the desired temperature.

On sample days, ant tubes were removed from the coolers for counting of living and dead individuals. Ants were considered to be alive if, after being turned on their backs, they could return to an upright position and walk. Ants at the 0.5 and -4°C regimes were transferred into 60-ml plastic cups at room temperature after removal from refrigerators to prevent drowning in condensation formed in the tubes and to revive them more quickly. These ants were allowed 2 and 3 h to recover from cold coma. The ants kept at the 4°C regime recovered from cold coma within minutes at room temperature, and were not subject to the condensation problem. This experiment was repeated with ants from the same colonies except for two colonies of uninfected *S. invicta* and one colony each of the other three ant types, which were substituted by new colonies due to the low number of remaining ants.

Statistical Analysis. Comparison across ant types was done both with all ants in the supercooling tests, and with only those with head capsules within ranges shared by all ant types. The shared head width ranges were 0.70–0.73 mm (small ants), 1.27–1.40 mm (large ants), and 1.40–1.43 mm (female alates). Only data for these head-size selected groups are presented to maximize uniformity of tested groups. The supercooling point data were subjected to analysis of variance (ANOVA) followed by mean separation by Student-Newman-Keuls or contrasts procedure at a $P = 0.05$ level of significance using the SuperANOVA software (Abacus Concepts 1989). Because supercooling points were measured on individual ants (subsamples) from each colony (experimental unit), the colony within ant type factor was used as the error term for the main effect (ant type). The supercooling points of alates, and small and large workers were analyzed separately. Spring 2000-collected ants were compared with analogous fall- or winter-collected types to examine seasonal differences. Ant type and *T. solenopsae* infection effects were examined for ants collected in the same season. The supercooling points obtained 1 wk after collection were compared with those obtained 3 mo after collection to determine the effect of laboratory rearing on supercooling point. Pearson correlation coefficients between head capsule size and supercooling points were also calculated to determine any association between ant size and freeze-tolerance of ants within the same type/size category.

Percent mortality data from extended low temperature exposure tests were arcsine-transformed and ANOVA and mean separation procedures were performed as previously described. Different sampling dates were used as a repeated measurement factor in the analysis. Temperature, colony and experiments were used as main effects. Probit analysis using POLO-PC (LeOra Software 1987) was used to determine LD50 of cold exposure as measured in the number of days at the temperature regime.

Results

Supercooling Points. Thermocouple Size. Large ants consistently broke the small (0.01 mm) thermocouples; therefore, the use of larger, sturdier (0.1 mm) thermocouples was necessary for the large ants. No significant difference was found between supercooling points of small and large workers when tested on the small thermocouples (small workers = $-5.9 \pm 0.07^\circ\text{C}$; large workers = $-5.8 \pm 0.07^\circ\text{C}$), but supercooling points were significantly different ($F = 4.044$; $df = 1, 191$; $P = 0.031$) with large thermocouples (small ants = $-7.4 \pm 0.13^\circ\text{C}$; large ants = $-7.7 \pm 0.10^\circ\text{C}$). Also, the supercooling point measurements with large thermocouples were $\approx 1.5^\circ\text{C}$ lower than the data obtained with the more sensitive small thermocouple.

Ant Type Comparisons. No significant differences in supercooling point were observed when the subgroups of ants with similar head size were compared (Table 1). Mean head size varied considerably among the ant types tested. Despite the head size-selected

Table 1. Supercooling points (SCP) (mean \pm SEM $^{\circ}$ C) and head capsule sizes (HS) (mean \pm SEM mm) for head-size-selected groups of spring-collected *Solenopsis* ants

Ant type	n	SCP ($^{\circ}$ C) ^a	HS (mm)
Small workers ^b (head-size = 0.70–0.73 mm ^c)			
<i>S. richteri</i>	27	-6.9 \pm 0.23a	0.73 \pm 0.000b
Hybrid	19	-6.7 \pm 0.13a	0.72 \pm 0.003ab
Uninfected <i>S. invicta</i>	9	-8.1 \pm 0.43a	0.71 \pm 0.005a
<i>T. solenopsae</i> -infected <i>S. invicta</i>	7	-7.5 \pm 0.69a	0.71 \pm 0.006a
Large workers ^b (head-size = 1.27–1.40 mm ^c)			
<i>S. richteri</i>	31	-6.6 \pm 0.09a	1.31 \pm 0.007a
Hybrid	34	-7.7 \pm 0.35a	1.34 \pm 0.007a
Uninfected <i>S. invicta</i>	24	-6.9 \pm 0.21a	1.32 \pm 0.009a
<i>T. solenopsae</i> -infected <i>S. invicta</i>	6	-6.0 \pm 0.17a	1.30 \pm 0.021a
Female alates ^b (head-size = 1.40–1.43 mm ^c)			
<i>S. richteri</i>	37	-10.0 \pm 0.59a	1.42 \pm 0.003b
Hybrid	24	-8.0 \pm 0.25a	1.42 \pm 0.004b
Uninfected <i>S. invicta</i>	32	-8.8 \pm 0.42a	1.41 \pm 0.003ab
<i>T. solenopsae</i> -infected <i>S. invicta</i>	18	-7.6 \pm 0.93a	1.39 \pm 0.005a

^a Within a column, and within the ant size group, means followed by the same letter are not significantly different at the 0.05 level of confidence according to Student-Newman-Keuls means separation procedure.

^b Small workers were tested with a 0.01-mm thermocouple. Large workers and female alates were tested with 0.1-mm thermocouple.

^c Five colonies were tested per ant type. A maximum of 10 ants were tested from each colony but alates may not have been available in large numbers or in all colonies tested. Data is presented for head-size selected subgroups consisting of individuals with head capsule within a size range shared across all three species.

groups within specific ranges, significant differences in the mean head size also were observed for small workers ($F = 3.98$; $df = 3, 15$; $P = 0.03$), and female alates ($F = 8.4$; $df = 3, 11$; $P = 0.003$). No significant correlation (P varying from 0.052 for large *S. richteri* to 0.9688 for small *S. richteri*) was observed between head size and supercooling point for any of the ant types or castes.

Colonies of *S. invicta* were generally composed of smaller individuals. Head capsule widths for small workers ranged from >0.40 to <0.75 mm, and head capsules for large workers ranged from >0.90 to <1.45 mm. *T. solenopsae*-infected and uninfected *S. invicta* did not have significantly different head capsule sizes in any size group. Small *S. richteri* and hybrid ants had head width values of 0.60–0.87 mm, whereas large worker heads ranged from 1.07 to 1.50 mm.

Maintenance in the laboratory and collection season also affected supercooling point of fire ants. Large hybrid workers tested 1 wk after collection had significantly ($F = 4.3$; $df = 1, 9$; $P = 0.044$) lower supercooling point than ants tested 3 mo after collection from the field, but no significant difference existed for *S. richteri* ($F = 3.7$; $df = 1, 9$; $P = 0.063$) (Table 2). The ant type effect was not significant for time comparison of small workers ($F = 1.6$; $df = 9, 33$; $P = 0.17$). Seasonal differences in supercooling point were not significant for small *S. richteri* ($F = 0.063$; $df = 1, 33$; $P = 0.80$), hybrid ($F = 0.23$; $df = 1, 33$; $P = 0.63$), *T. solenopsae*-infected ($F = 2.1$; $df = 1, 33$; $P = 0.16$), and uninfected *S. invicta* ($F = 1.8$; $df = 1, 33$; $P = 0.19$). Fall-collected large *S. richteri* workers had significantly lower supercooling point than the spring-collected ants ($F = 24.8$; $df = 1, 33$; $P = 0.0001$), but other seasonal comparisons for large workers were not significant (hybrid, $F = 3.7$; $df = 1, 33$; $P = 0.063$; *T. solenopsae*-infected *S. invicta*, $F = 2.7$; $df = 1, 33$; $P =$

0.11; and uninfected *S. invicta*, $F = 0.30$; $df = 1, 33$; $P = 0.59$).

Extended Low Temperature Exposure. The results for the two extended low temperature exposure tests were not significantly different ($F = 0.001$; $df = 1, 95$; $P = 0.97$); thus, the results were combined for discussion (Fig. 1). Ant mortality rates increased as temperatures decreased ($F = 485.2$; $df = 2, 95$; $P = 0.0001$). Also, significant differences ($F = 18.7$; $df = 3, 95$; $P = 0.0001$) were detected among the four ant types tested. The hybrid ants were the most resistant to near-freezing temperature exposure. *S. richteri* and *T. solenopsae*-infected *S. invicta* had intermediate resistance to the low temperature regimes, and the uninfected *S. invicta* suffered the highest mortalities when exposed to cold. No significant interaction was observed among the main effects in the analysis.

During the first 5 d at the 4 $^{\circ}$ C regime, no significant ($P > 0.05$) differences were observed as all four ant

Table 2. Mean supercooling points (mean \pm SEM $^{\circ}$ C) for head-size-selected groups of fall-collected *Solenopsis* workers, tested after maintenance in the laboratory over different periods of time

Ant type ^a	Test time	n	Small ^b	n	Large ^b
<i>S. richteri</i>	1 wk	19	-6.7 \pm 0.08a ^c	39	-8.7 \pm 0.12a
	3 mo	16	-6.6 \pm 0.15a	31	-8.0 \pm 0.14a
Hybrid	1 wk	27	-6.2 \pm 0.06a	38	-8.3 \pm 0.11a
	3 mo	29	-6.3 \pm 0.08a	39	-7.5 \pm 0.11b

^a Five colonies were tested per ant type, and a maximum of 10 ants were tested from each colony. Data is presented for head-size selected subgroups consisting of individuals with head capsule within a size range shared across all ant types.

^b Small workers were tested with a 0.01-mm thermocouple and large workers with 0.1-mm thermocouple.

^c Within a column, and within the ant type, means followed by the same letter are not significantly different at the 0.05 level of confidence (F test).

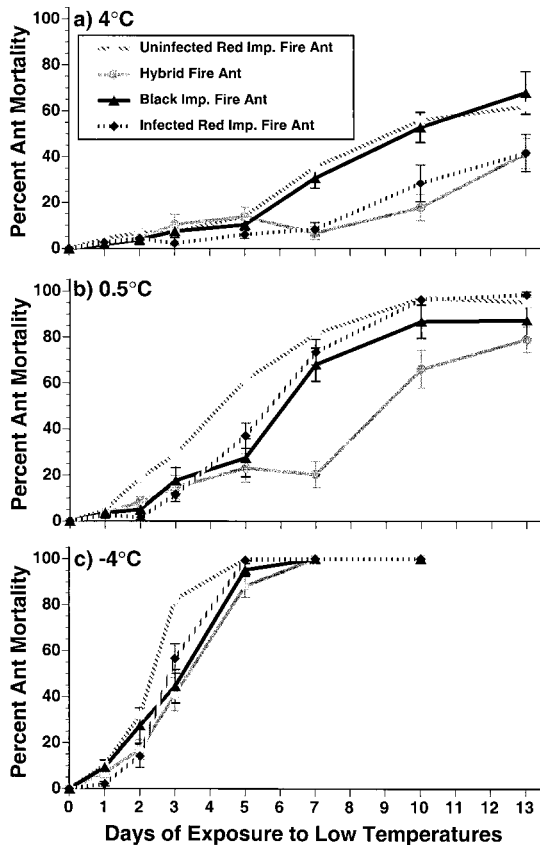


Fig. 1. Mean percent mortality of spring-collected workers of *S. richteri*, *S. richteri* X *invicta* hybrid, uninfected *S. invicta*, and *T. solenopsae*-infected *S. invicta* exposed to $4 \pm 0.5^\circ\text{C}$ (a), $0.5 \pm 0.5^\circ\text{C}$ (b), and $-4 \pm 0.3^\circ\text{C}$ (c) for up to 13 d. Data from two tests were combined. Bars represent standard error of the means.

types had low mortality (<15%) (Fig. 1a). By the seventh day, hybrid ants and infected *S. invicta* had lower mortalities (6.6 and 6.0%, respectively) than *S. richteri* or uninfected *S. invicta* (30.6 and 35.5%, respectively). These differences persisted for the duration of the experiment. LD50s could not be estimated for this temperature regime due to low mortalities in all treatments.

Significant ($P < 0.05$) differences in ant mortality were observed on the second day of exposure at the 0.5°C (Fig. 1b). After 2 d of near 0°C temperatures, uninfected *S. invicta* had 18.9% mortality compared with only 8.3% for the hybrid, 5.1% for *S. richteri* and 1.7% for *T. solenopsae*-infected *S. invicta*. By 5 d, the uninfected *S. invicta* mortality was approximately double that of the other three ant types. After 7 d, the hybrid ants had $\approx 20\%$ mortality, whereas all other ant types had mortality between 60 and 80%. Hybrid ant mortality approached that of other ant types only after 13 d of exposure to the 0.5°C temperature regime. The estimated LD50 was similar for the uninfected *S. invicta* (4.0 d), *S. richteri* (5.7 d), and *T. solenopsae*-

infected *S. invicta* (5.2 d), but the LD50 for hybrid ants was 8.6 d.

Close to 100% mortality was reached by 5 d in all ant types exposed to -4°C and no survivors remained after 7 d (Fig. 1c). By day 3, the uninfected *S. invicta* displayed significantly ($P < 0.05$) higher mortality (82.1%) than all other types (hybrid = 41.1%, *S. richteri* = 44.6%, and *T. solenopsae*-infected *S. invicta* = 65.6%). The LD50 was estimated to be between 2 and 3 d for all ant types.

Discussion

Supercooling points were not an adequate measurement of cold tolerance because they are highly variable even after groups of ants were selected with similar head-capsule size. Also, the differences in results obtained with the 0.01- and 0.1-mm thermocouples prevent comparisons with previous supercooling point studies with fire ants (Francke et al. 1986, Taber et al. 1987, Diffie and Sheppard 1989, Landry and Phillips 1996), in which larger thermocouples (0.33–0.59 mm) were used. Often, colonies of *S. richteri* and hybrid fire ants from Tennessee are composed of larger individuals than *S. invicta* colonies as demonstrated by head capsule comparisons. Whether larger ant size is an adaptation to colder weather, or a consequence from slower development of larvae (Porter 1988), cannot be resolved by our results.

Avoidance is usually the first line of defense in cold weather survival of insects (Denlinger and Lee 1998). Imported fire ants escape damaging cold temperatures by going deeper into their nests (Morrill 1977), but ants may become trapped in the upper layers of the nest if a cold front moves in too rapidly (Green 1959, Morrill et al. 1978, Diffie et al. 1997). In the extended low temperature experiments, no structural protection was available to the ants and they had succumbed to cold coma within 24 h. Thus, cold avoidance behaviors were not factors in these experiments and the results represent a measurement of physiological, not behavioral, cold hardness.

Short-term exposure to 4°C had little effect on ant mortality as fire ants may have derived some benefits, such as lower energy use, from cooler temperatures (Calabi and Porter 1989, Porter and Tschinkel 1993). However, without protective mechanisms, cellular functions eventually are affected by continued low-temperature exposure (Denlinger and Lee 1998). With exposure to colder temperature, the deleterious effects occurred sooner.

A winter-mortality study of an isolated population of *S. invicta* in eastern Tennessee (Callcott et al. 2000) corroborates our results from the extended low temperature exposure tests. In this study, the greatest mortality (87.5%) occurred during winter 1993–1994, with seven consecutive days at $\leq 1.1^\circ\text{C}$. In our study, 81.4% mortality of uninfected *S. invicta* was observed after 7 d at the $+0.5^\circ\text{C}$ regime. In the field, sunny days allow nest soil to warm up, reducing the extent of the exposure to low temperatures and ant mortality, but cloudy days may prolong exposure of ants to low

temperatures within the nest. In the future, monitoring of nest microclimate in the field may allow better understanding of ant survival during cold weather, and would be useful in modeling the likelihood of fire ant colonization (Wiley et al. 2000). Further benefit may be obtained from studies focusing on *S. richteri* and the *S. richteri* X *invicta* hybrid and on possible cell-level advantages of hybrid ants that determine cold tolerance.

The geography of *S. richteri* infestations in both North and South America, and a lack of previous evidence of hybrid advantage (Diffie and Sheppard 1989, Diffie et al. 1997), led to the expectation that the black imported fire ant had an improved cold hardiness. However, the survival of *S. richteri* exposed to extended periods of cold temperatures was not significantly different from that observed for the more tropically oriented *S. invicta*. Despite the lack of significant differences in cold tolerance between *S. invicta* and *S. richteri*, the hybrid was more resistant to extended exposure to low temperature than both parent species.

Within their current range, fire ants would rarely be exposed to temperatures close to their supercooling points inside the nest. However, exposure to occasional extended periods of low temperature may occur frequently. Results from extended exposure to low temperatures support the theory that the distribution of *S. richteri*, *S. invicta*, and their hybrid is linked to cold hardiness. As the fire ant range expands north, the chances of fire ants encountering damaging temperatures are increased. Based on our extended low temperature results, we expect that the northern range expansion may favor the hybrid fire ant over the parent species.

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