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Authors: Briano, Juan A., Calcaterra, Luis A., Williams, DavidF., and Oi, David H.

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ATTEMPTS TO ARTIFICIALLY PROPAGATE THE FIRE ANT PARASITE *SOLENOPSIS DAGUERREI* (HYMENOPTERA: FORMICIDAE) IN ARGENTINA

JUAN A. BRIANO[1], LUIS A. CALCATERRA[1], DAVID. F. WILLIAMS[2] AND DAVID H. OI[2]
[1]USDA-ARS South American Biological Control Laboratory, Bolivar 1559 (1686) Hurlingham
Buenos Aires Province, Argentina

[2]USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology
P.O. Box 14565, Gainesville, FL 32604, U.S.A.

Since the 1960s, the workerless (permanent) parasitic ant *Solenopsis daguerrei* (Santschi) has been considered part of a complex of potential candidates for introduction into the United States for the biological control of the red and black imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel respectively (Lofgren et al. 1975, Jouvenaz 1990, Wojcik 1990). Pioneering work on *S. daguerrei* was conducted in Uruguay and Argentina by Silveira Guido et al. (1973; unpublished reports). More recent studies have been conducted at the USDA-ARS-South American Biological Control Laboratory (SABCL) since 1988 and more intensively since 1995. Studies included records on its occurrence and abundance in southern South America (Briano et al. 1997) and field and laboratory observations on its specificity, biology and behavior (Calcaterra et al. 1999, 2000, 2001). Also, many fire ant colonies from Argentina parasitized with *S. daguerrei* were shipped into quarantine at the USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Gainesville, FL, where complementary studies were conducted (unpublished information).

Although detrimental effects on fire ants were reported in Argentina (Calcaterra et al. 1999), further progress on the project was not achieved because of (1) the difficulty in rearing *S. daguerrei* under laboratory conditions and (2) the failure to introduce and establish the parasite into new host colonies.

A number of attempts to introduce and propagate *S. daguerrei* in laboratory and field host colonies were conducted in Argentina from 1996 to 1999 following different approaches. Some of this work was reported by Calcaterra et al. (2001). Although some tests showed limited success, *S. daguerrei* did not establish permanently in any of the target host colonies. Despite this failure, we believe that reporting the different approaches used in our studies will contribute to future research efforts.

Parasitized and nonparasitized colonies used in the tests were mostly multiple-queen colonies of *S. richteri* collected in four areas of Buenos Aires Province: (1) San Eladio (60 km W of Buenos Aires), (2) Saladillo (180 km SW of Buenos Aires), (3) Mercedes (100 km W of Buenos Aires), and (4) Suipacha (125 km W of Buenos Aires). The colonies were excavated in the field, brought to the laboratory in 10-liter buckets dusted with talc, separated from the soil by flotation (Banks et al. 1981) and placed in trays.

The basic approaches used in the laboratory tests were: #1) Transference of queens: Target host colonies were fragmented into microcolonies (n = 34) composed of one host queen, 50 workers and approximately 1 gr. of brood. These microcolonies were maintained in artificial nests (Bishop et al. 1980) in plastic rearing trays (40 × 25 × 7 cm) coated with Fluon® and fed adult house flies and/or canned Vienna sausage. A water source was always present. One active *S. daguerrei* queen separated from a parasitized colony was transferred to each microcolony. For about one month, the colonies were kept at different temperatures as follows: 10°C (five colonies), 10-15°C (eight colonies), 15°C (11 colonies), and 30°C (10 colonies). In five of these colonies kept at 30°C, the *S. daguerrei* queens were sprayed before transference with an extract of macerated workers from the target (receptor) host colony. The tests were monitored daily for the presence of the parasite in the receptor microcolonies. #2) Transference of sexuals: Similarly, target host colonies were fragmented into microcolonies (n = 22) as above and kept in trays at 15 °C. A total of 92 sexuals (alates) of *S. daguerrei* (3-9 per microcolony) was transferred and monitored daily. #3) Transference of pupae: Test 1. Target host colonies were fragmented (n = 6) as above and kept at 15°C. Six pupae of *S. daguerrei* were transferred to each microcolony. Test 2. Host colonies were fragmented into microcolonies (n = 4) composed of six host queens and 1 gr. of workers and maintained between 19-30°C. A total of 200 pupae of *S. daguerrei* was transferred to the microcolonies (six pupae per colony every 3-4 days for three weeks). #4) Entire colonies in contact: Test 1. A small plastic tray (40 × 25 × 7 cm, coated both inside and outside with Fluon®) with a parasitized colony was placed in a larger tray (50 × 40 × 12 cm) with a parasite-free colony. The colonies were monitored daily to observe the short flights of *S. daguerrei* sexuals into the larger tray and their survival. Test 2. Two trays (50 × 40 × 12 cm), the first with a parasitized colony and the second with a parasite-free colony were interconnected through plastic tubes (1 cm in diameter) to a third

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tray with a food source. The behavior of the colonies was observed daily.

Two approaches were used in the field trials: #1) Transplanting of entire colonies: Test 1. Fifteen colonies parasitized with *S. daguerrei* were excavated in the field, put in 10-liter buckets dusted with talc and transported to a natural pasture free of the parasite in the locality of Santa Coloma, Buenos Aires Province (130 km NW of Buenos Aires). Three circular plots (20 m in diameter) were established and five colonies per plot were poured in small holes dug in the ground, then covered with a dry dung pad. The plots were monitored 3-4 times per year for the presence of the parasite in the original colonies and for the dispersal to new host colonies. Test 2. Three parasitized colonies collected in the field were brought to SABCL and dyed with Calco-oil dyes (Pylam Products Company, Inc., New York) according to Bartlett and Lofgren (1961). The colonies were then deposited in small holes made in the ground of the SABCL yard and monitored for the survival of the transplanted colonies and the dispersal of *S. daguerrei*. The presence of the dyes was detected by crushing a sample of workers on a white sheet of paper (Bartlett and Lofgren 1961). #2) Transference of newly-mated queens: Buckets with parasitized colonies (n = 10) dug in the field were placed in a walk-in cage ($2 \times 2 \times 2$ m) inside a plastic greenhouse at the SABCL backyard (Calcaterra et al. 2001). Adult female sexuals of *S. daguerrei* were captured with an aspirator after they naturally flew out of the colonies. After dealation, 30 newly-mated queens of *S. daguerrei* were put in small plastic containers with a source of moisture and transported to a parasite-free pasture in Mercedes, Buenos Aires Province, with a heavy infestation with *S. richteri* (approximately 80 mounds per hectare). The *S. daguerrei* newly-mated queens were released on top of three fire ant mounds (10 queens per mound) and the area monitored 3-4 times per year for the establishment of the parasite.

Under laboratory conditions, the artificial propagation of *S. daguerrei* into parasite-free target colonies failed. At 10°C, no ant activity was observed, as expected; however, when the microcolonies were moved to warmer temperatures, the parasite queens were killed immediately. When queens were transferred at >10°C, total mortality of *S. daguerrei* was observed in 1 to 7 days; when sexuals were transferred total mortality was observed in 10 days and with pupae in 7 to 29 days (Table 1). When pupae of *S. daguerrei* were trans-

TABLE 1. APPROACHES TO ARTIFICIALLY PROPAGATE *S. DAGUERREI*.

Approach	Treatments	Results
Laboratory tests		
Transference of queens	10°C	No ant activity
	10-15°C	75% mortality at day 4 100% mortality at day 5
	15°C	91% mortality at day 4
	30°C	100% mortality at day 7
	30°C + spraying	100% mortality at day 1
Transference of sexuals	15°C	94% mortality at day 7 100% mortality at day 10
Transference of pupae	15°C	67% mortality at day 14 100% mortality at day 21
	19-30°C	Females: 50% mortality at day 4 100% mortality at 29 days Males: 100% mortality at day 7
Colonies in contact	Tray with parasitized colony in tray with nonparasitized colony	100% mortality as sexuals moved to nonparasitized colony
	Colonies interconnected	Nonparasitized colony invaded and killed parasitized one in <1 month
Field tests		
Transplanting of colonies	Natural pasture	6.6% of the transplanted colonies detected for 15 months. Parasite not established
	SABCL yard (dyed colonies)	Colonies not established
Transference of newly-mated queens	Natural pasture. Queens released on top of fire ant mounds	Parasite not established

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ferred, the individuals were killed after emergence of the adult parasites. Also, when parasitized and nonparasitized colonies were put in close contact, sexuals were killed when they moved to the nonparasitized colony. The parasite-free colony invaded the tray and nest of the parasitized colony and killed both the host and parasite in less than one month.

In the field plots, the presence of *S. daguerrei* was confirmed in 26.6% (4/15) of the original colonies after three months of transplanting and in 6.6% (1/15) after 15 months (Table 1). However, the dispersal of *S. daguerrei* to other colonies in the area was not observed. At the SABCL yard, only two of the dyed colonies released were observed for 1 to 7 days. The colonies did not establish and no dispersal of *S. daguerrei* was observed. The release of newly-mated queens of *S. daguerrei* on top of fire ant mounds was also unsuccessful. Whether the parasite queens were killed immediately or survived a certain time after introduction was not determined. After 2½ years of the release, the presence of *S. daguerrei* was not observed.

Our speculation that the F_1 generation of *S. daguerrei* could be adopted by the host colony if eggs were laid by transferred queens before being killed (Calcaterra et al. 2001), was not confirmed in these tests. Further research is needed to discover how this parasite disperses in nature. Based on the information reported here, future trials should be concentrated in the transference of pupae to host colonies and transplanting of entire parasitized colonies into field locations. This basic information will be essential to obtain the artificial propagation of *S. daguerrei*. Partial support for these studies was provided by Dr. Lynne Thompson at the University of Arkansas-Monticello, to whom we are deeply grateful.

Summary

Several laboratory and field tests were conducted to introduce and propagate *S. daguerrei* into parasite-free fire ant colonies. The basic approaches used were the transference of parasite queens, sexuals and pupae, the location of parasitized and non parasitized colonies in close contact and the transplanting of newly-mated queens or entire colonies to field locations. The artificial propagation of *S. daguerrei* failed. In the laboratory, total mortality of *S. daguerrei* was observed in less than one month. In the field, the presence of *S. daguerrei* in the original transplanted colonies was observed for 15 months but dispersal of *S. daguerrei* was not observed.

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