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AN IMPROVED AND QUANTIFIED TECHNIQUE FOR MARKING  
INDIVIDUAL FIRE ANTS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Individual fire ants, *Solenopsis invicta* Buren, were marked with dots of Markal brand Ball Point Paint Markers. Marked ants were returned to the colony while the paint was still wet. The marks were slowly lost over a 100-day period, with one mark lasting 119 days. No mortality could be attributed to the paint. The proportion of ants retaining marks over time can be expressed as a simple exponential decay model: proportion with marks =  $0.7665 * \exp(-0.0175 * \text{time in days})$ . The technique will be useful in assessing basic behavioral characteristics, such as polygyne versus monogyne interactions, nestmate discrimination, foraging profiles, and in assessing impact of candidate biological control organisms on behavior and survivorship of fire ants.

Key Words: *Solenopsis invicta*, methods, model, paint, longevity, non-linear regression

## RESUMEN

Hormigas coloradas (fire ants) *Solenopsis invicta* Buren fueron marcadas con bolígrafos Markal® y devueltas a su colonia cuando la pintura aún estaba fresca. Las marcas desaparecieron lentamente en un período de 100 días (una marca duró 119 días). La pintura no causó la muerte a las hormigas. Se desarrolló un modelo exponencial simple para predecir la proporción de hormigas que retienen la marca de pintura al paso del tiempo. Esta técnica permitirá evaluar el comportamiento de las hormigas, por ejemplo, las interacciones polígamas vs. monógamas, la discriminación dentro del nido y los hábitos alimenticios de esta especie. También será posible evaluar el impacto de organismos con potencial de control biológico en el comportamiento y supervivencia de estas hormigas.

Marking insects is important in research in biology, ecology, and behavior (Southwood 1978). However, the nature of the insect cuticle presents problems with adhesion and durability of marks, especially for insects with very smooth cuticle (Walker & Wineriter 1981). Mass marking of red imported fire ants (*Solenopsis invicta* Buren) for population studies using spray paints has been documented (Bhatkar et al. 1991), but this method can cause ant mortality and cannot be used to mark a particular individual chosen for study. Other methods used for marking individual ants, can be inappropriate, cumbersome, or unsuitable, particularly for small fire ant workers (Kruk de Bruin et al. 1977, Miranda & Vinson 1979, Verron & Barreau 1974, Porter & Jorgensen 1980, Stuart 1986, Showler et al. 1989, 1990, Porter 1991). Longevity of marks is often inadequate when used in long term studies in fire ant colonies because of the chemical constituents of fire ant cuticle (Lok et al. 1975 and their intense grooming behavior (Wilson 1962) removes paint marks. Since fire ant workers can live 60 to 90 days (Vinson 1997), a marking system was needed which would last for a large portion of the ants life-span. The purpose of this study was to develop a method of marking individual fire ants which lasted for most of the ants life span.

## MATERIALS AND METHODS

Red imported fire ant colonies were collected in Gainesville, FL, separated from the soil, and maintained in the laboratory using standard procedures (Banks et al. 1981). In preliminary screening, over 150 dyes, stains, or paints were tested for 1) lack of mortality, 2) ease of use and 3) longevity of marks. Only Markal ball point paint markers (Markal Co., 1201 Pratt Blvd, Elk Grove Village, IL 60007) met these criteria and were studied further to determine the longevity of the marks on worker ants. This paint was initially chosen for testing because it is designed for marking on oily surfaces and freshly cut wood, particularly pine. Preliminary studies indicated that if ants were returned to their colonies when the paint had dried, these paint marks would only last a few days. For the marks to last longer, the ants had to be returned to their home colony while the paint was still wet. The ants were grasped with forceps and the top of the gaster touched into a drop of paint. Dots of blue, red, white, yellow, orange, or green paint were placed on seven individual workers for each color (one color per ant). Forty-two ants were marked in each of 6 queen-right colonies (each colony contained one queen) for a total of 252 marked ants. The marked ants were returned to the colony while the paint was still wet. No attempt was made to mark newly-enclosed workers. The colonies were checked daily for marked ants for three days, then twice weekly until less than one percent of the marked ants were recovered in two consecutive examinations.

We desired to develop an equation that could be used to predict the expected proportion of ants retaining their marks as a function of time since application. Because the proportion retaining marks appeared on visual inspection to be constant over time, we fitted a simple exponential decay model using the combined data set of all replicates using a standard statistical package (Jandel's SigmaStat, ver. 2.0; Jandel Corporation, P. O. Box 7005, San Rafael, CA 94912-7005).

## RESULTS AND DISCUSSION

The marks were slowly lost over a 100-day period, with one mark lasting 119 days (Fig. 1). No mortality was attributed to the paint. Large chips of paint were rarely found (three times). Dead marked ants were found for 9% of the marked ants (23 out of 252 workers). No differences were found in the longevity of any one color. In three instances, unmarked ants acquired paint marks; presumably they came into contact with the marked ants before the paint dried. Returning marked ants to the colony while the paint was still wet, possibly allowed the paint to acquire the colony odor, thereby making it less objectionable to other worker ants. Fire ant workers that have been isolated from the colony are intensely scrutinized upon return to the colony tray. Normally any foreign material is vigorously removed by the examining workers

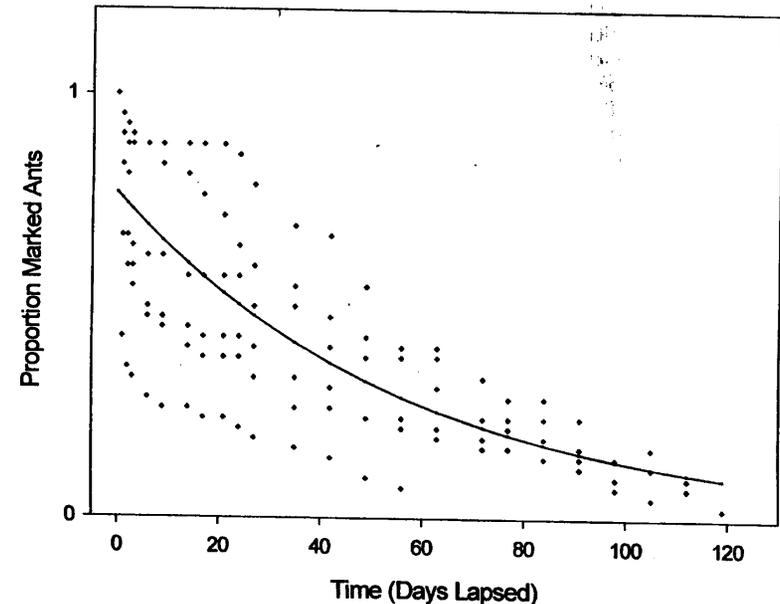


Fig. 1. Survivorship of marks on *Solenopsis invicta* workers made with Markal paints. Observed and predicted proportion of ants retaining marks over time. The dots indicate observed proportions and the line represents the exponential decay function developed with non-linear regression using all replicates. See Table 1 for statistics on fit and coefficients.

(Mirenda & Vinson 1979). This is the critical period for paint marks on the ants. If the marked workers can reenter the colony and the marks survive the initial period of scrutiny, the marks have an enhanced survival rate.

The proportion of ants retaining marks over time can be estimated with the following expression:

$$\text{Proportion with marks} = 0.7665 * \exp(-0.0175 * \text{time})$$

where time indicates the elapsed number of days since the marks were applied (Fig. 1). The proportion of the variability in Proportion with marks that could be explained by reference to time (i.e., the coefficient of determination,  $R^2$ ) was 0.62. Table 1 provides the full accounting of the analysis. Glancey & Lofgren (1988), using this technique, recovered marked fire ant queens 9-months after marking and releasing into a polygyne field colony. This technique will be useful in various aspects of fire ant and other ant research in a variety of studies including: polygyne versus monogyne colonies, nestmate discrimination, foraging, and impact of biological control organisms.

#### INTERPRETIVE SUMMARY

Fire ants occur in parts of 11 southern states, and cause hundreds of millions of dollars of damage to domestic animals, wildlife, and the infrastructure (roads, electrical systems, equipment). Additionally, several deaths of humans occur each year. Understanding the biology and behavior of fire ants is critical to developing new management strategies. Researchers at the ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL, developed a simple system to mark individual ants with a small dot of special paint so that their foraging profiles and interactions with other nestmates could be studied and characterized. If the paint was still fresh when the ants were reintroduced into the colony, nestmates were less likely to try and remove it than if it was dry at the time of reintroduction. Consequently, in the absence of active grooming, marks lasted up to 119 days and did not appear to cause mortality. This technique will be useful in learning how fire ants interact with their

TABLE 1. STATISTICS FOR THE NON-LINEAR REGRESSION OF "PROPORTION RETAINING MARKS" AS A FUNCTION OF TIME SINCE APPLICATION.

Statistics for coefficients:					
Coefficient	Std. Error	t	P		
0.7665	0.0301	25.4847	<0.0001		
0.0175	0.0017	10.3777	<0.0001		
Analysis of Variance:					
	DF	SS	MS	F	P
Regression	1	5.9422	5.9422	195.1055	<0.0001
Residual	122	3.7157	0.0305		
Total	123	9.6579	0.0785		

nestmates and ants of other nests. This is expected to provide insight into how colonies function, and more importantly, how we may be able to disrupt critical interactions so that colonies do not survive.

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