Temporal Changes in a Red Imported Fire Ant (Hymenoptera: Formicidae) Colony Classification System Following an Insecticidal Treatment[1]

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ABSTRACT The decline of the red imported fire ant, *Solenopsis invicta* Buren (RIFA) following an application of fenoxycarb bait and the subsequent reinfestation from small, incipient colonies to large, mature colonies was studied in south Mississippi. Field colonies were rated using the numerical colony classification system of Lofgren and Williams (1982). The study site was considered RIFA free (98.9% decrease in pretreat population index) 3 months after a fenoxycarb bait treatment. Small incipient colonies categorized in class 7 (100-1,000 workers and worker brood present) appeared in the test site about 4 months after bait application. Mature colonies in classes 9 and 10 (10,000-50,000 workers present with worker brood and more than 50,000 workers with worker brood present, respectively) were detected after 12-15 months of development.

KEY WORDS Red imported fire ant, *Solenopsis invicta*, reinfestation, fenoxycarb.

Early in their development, small, incipient colonies of the red imported fire ant (RIFA), *Solenopsis invicta* Buren are comprised of a queen and less than 100 workers. Conversely, large mature colonies can contain over 230,000 workers plus reproductive and immature forms (Markin and Dillier 1971, Markin et al. 1973). Few studies have been undertaken to determine the time interval necessary for development from a single queen to a mature, reproductive RIFA colony. In those studies, maturity was determined using various criteria such as physical size of the mound, worker population, production of reproductive forms, or a combination of these. Markin et al. (1973) showed the time interval to produce mature colonies to be a minimum of 2.5 years and by 3 years, all colonies examined were fully mature. Lofgren et al. (1975) and Markin and Dillier (1971) estimated that colonies matured at 2 years. In these studies, maturity was determined not only by the size and population of the nest, but also by the presence of reproductive forms. Based on worker population alone, Tschinkel (1988) found that RIFA colonies reached maximum size at 4 to 6 years (ca. 220,000 workers). Many factors, including climatic conditions such as air and soil temperature, soil moisture and humidity, and environmental conditions such as food and site availability (Green 1967, Rhoades and Davis 1967, Lofgren et al. 1975), either inhibit or enhance the growth of a RIFA colony.

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Many researchers routinely use a numerical colony classification system, developed by Harlan et al. (1981) and modified by Lofgren and Williams (1982) to evaluate the effects of insecticides and insect growth regulators (IGR) upon RIFA populations (Table 1). This system is based on the estimated population of worker ants and the presence or absence of worker brood (larvae and pupae). Absence of worker brood suggests that a colony does not contain a normally functioning queen. A newly formed colony with worker brood present and 100 workers is numerically weighted as a "5" (colony class 6). Colonies of this rating are not easily visible in the field and thus rarely are detected. A large mature colony with worker brood and more than 50,000 workers is assigned a weighting factor of "25" (colony class 10). The population index for a particular site is calculated as follows:

$$\text{Population Index (PI)} = \sum_{K=1}^{25} K(N_K)$$

where N_k = the number of RIFA colonies in a given plot with a weighting factor of K where ($25 \geq K \geq 1$).

Studies to determine the time interval required for new colonies to attain the various classes defined by Lofgren and Williams (1982) have not been published. Markin et al. (1973) followed the growth of RIFA colonies in the field from newly mated queens to mature colonies and found those aged 2 months had an average of 66 workers, which on the Lofgren and Williams (1982) scale corresponded to a colony class 6. Colonies aged 3 and 5 months respectively, averaged over 200 workers, which would place them in class 7 and over 1,000 workers, placing them in class 8. Colony populations averaged more than 10,000 workers after approximately 1 year (i.e. class 9) and over 50,000 after 2.5 years, the equivalent of a class 10 colony.

Table 1. Colony classification system used to evaluate the effects of insecticides and insect growth regulators on RIFA populations.

	Worker brood absent		Worker brood present	
Number of worker ants	Colony class	Weighting factor	Colony class	Weighting factor
< 100	1	1	6	5
100 - 1,000	2	2	7	10
1,000 - 10,000	3	3	8	15
10,000 - 50,000	4	4	9	20
> 50,000	5	5	10	25

Developed by Harlan et al. (1981) and modified by Lofgren and Williams (1982).

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Lofgren and Williams (1985) used a variation of their 1982 population scale, along with population estimates from Markin et al. (1973), to study RIFA population dynamics after hydramethylnon bait treatments. Their study was concerned with nest and worker population density per unit area and did not distinguish between normal (brood present) and abnormal (brood absent) nests. Lofgren and Williams (1985) found no colonies in their Albany, GA plots 6 weeks after treatment with hydramethylnon. Assuming all colonies detected after that time developed from mating flights and contained brood, colonies corresponding to the Lofgren and Williams (1982) classes of 6, 7, and 8 were detected after 3 months or more of development. Colonies in classes 9 and 10 were found after at least 9 months of growth.

The present study, begun in the summer of 1989, aims to more closely define the time required for small, incipient colonies to develop into the various colony classes described by Lofgren and Williams (1982). We used only their criteria (estimated worker population and presence or absence of worker brood) to evaluate colonies. The study did not continue through the establishment of a stable RIFA field population, but only to the appearance of at least one representative of all colony classes.

Materials and Methods

A 4.05-ha mixed grass pasture (predominantly Bahia grass, *Paspalum notatum)* in Harrison County, MS was selected as the study site. Ten 0.1-ha circular subplots, each centered within a 0.4-ha square plot, were established within the site for use in evaluation of pretreatment and posttreatment RIFA populations. All nests in the subplots were opened using a shovel and evaluated by determining presence or absence of brood and estimating worker population. The classification of each nest according to Lofgren and Williams (1982) was recorded. Prior to treatment, the monogynous RIFA population in the study site averaged 138 colonies/ha and were predominantly colony classes 8-10 (Table 2, Fig. 1). The pre-existing population in the study site was reduced to a population of near zero by applying fenoxycarb bait (Logic®, Ciba-Geigy Corp., Greensboro, NC) at a rate of 1.7 kg/ha on June 13, 1989. The bait was applied broadcast using a custom built granular applicator mounted on a farm tractor (H.L.C., unpublished report).

Insecticide induced colony movement has been noted in a number of studies (Hillman 1977, Scarborough et al. 1982 and Williams and Lofgren 1983), and Lemke and Kissam (1987) observed relocation up to 5 m by treated colonies. Movement is also hypothesized to be related to food supply (Hays et al. 1982) and territoriality (Showler et al. 1990). While few researchers recorded distances moved, Showler et al. (1990) observed colonies that moved up to 6 m and Green (1967) stated that colonies may move up to 30 m under stressful conditions. Therefore, a 61 m "buffer zone" around the study site was treated with fenoxycarb in an effort to reduce movement of untreated RIFA colonies into the study site. This zone, along with the treated area outside each 0.1 ha subplot, provided a 75 m "buffer zone" which is 2.5 times the greatest distance recorded for RIFA colony relocation.

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Table 2. Temporal changes in colony classes present in test site after a fenoxycarb bait application.

Months post-treatment	Mean number of colonies ± SEM per 0.1-ha in indicated colony class					
	1	2	3	4	5	6
Pretreat	0.0 ± 0.0 a	0.0 ± 0.0 a	0.2 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
1	0.1 ± 0.1 a	1.7 ± 0.3 bc	2.5 ± 0.5 b	1.2 ± 0.3 b	0.1 ± 0.1 a	0.0 ± 0.0 a
2	0.2 ± 0.1 a	0.2 ± 0.5 c	3.2 ± 0.9 b	0.2 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a
3	0.1 ± 0.1 a	0.9 ± 0.3 abd	0.3 ± 0.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
4	0.0 ± 0.0 a	0.7 ± 0.3 ad	0.3 ± 0.2 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a
8	0.1 ± 0.1 a	1.0 ± 0.3 bd	0.2 ± 0.1 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a
13	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
16	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a

Months post-treatment	Mean number of colonies ± SEM per 0.1-ha subplot in indicated colony class				Mean number of colonies ± SEM/ 0.1-ha subplot
	7	8	9	10	
Pretreat	1.1 ± 0.4 ac	4.6 ± 0.7 a	5.4 ± 0.6 a	2.5 ± 0.5 a	13.8 ± 0.9 a
1	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	5.6 ± 0.7 bd
2	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	6.2 ± 1.0 be
3	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	1.3 ± 0.4 c
4	1.2 ± 0.4 ac	0.8 ± 0.3 b	0.4 ± 0.2 b	0.1 ± 0.1 bc	3.6 ± 0.8 bcd
8	0.9 ± 0.4 ac	0.4 ± 0.2 b	0.2 ± 0.1 b	0.0 ± 0.0 b	2.8 ± 0.4 cd
13	4.8 ± 0.8 b	3.7 ± 0.5 a	0.0 ± 0.0 b	0.0 ± 0.0 b	8.5 ± 1.0 e
16	2.2 ± 0.5 c	8.2 ± 0.9 c	7.2 ± 0.5 c	0.7 ± 0.3 c	18.3 ± 1.1 f

Means in a column followed by the same letter are not significantly different (Duncan's new multiple range test, $P = 0.05$).

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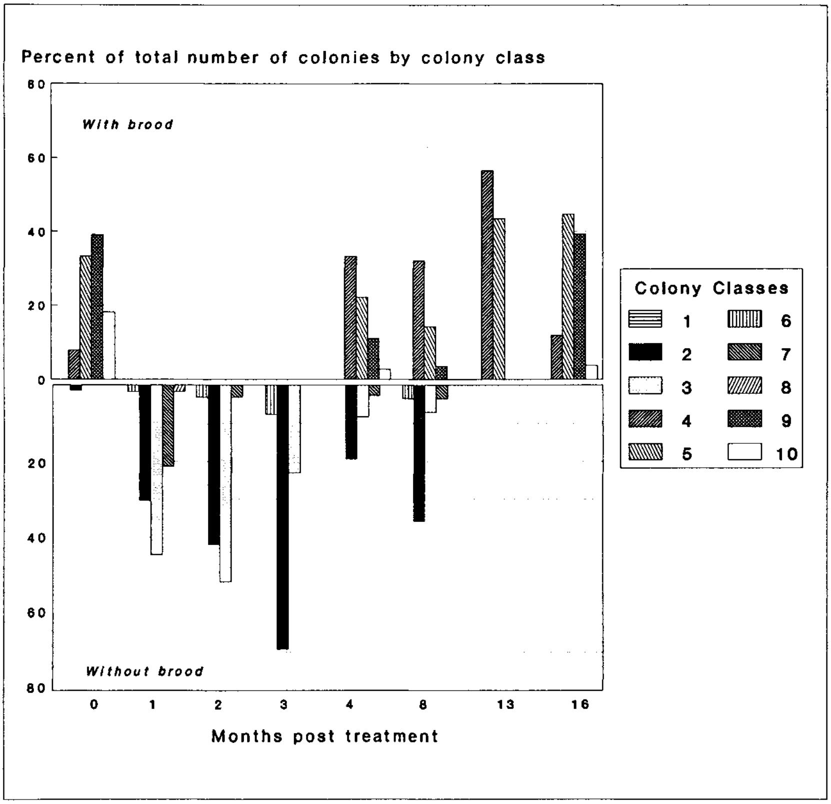

Fig. 1. Decline and reinfestation by RIFA colony classes following a broadcast application of fenoxycarb bait.

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Using the population index scale of Lofgren and Williams (1982), we rated population changes monthly in each subplot following application. When the pre-existing population reached its lowest level, the study began and henceforth the plots were rated quarterly. As reinfestation occurred, and incipient colonies grew and developed into the various colony classes, the types and numbers of colonies present were recorded. From these data, we determined the time required for colonies to develop into the various colony classes. Population index means at each posttreatment interval, and mean number of colonies in each class at each posttreatment interval, were compared using analysis of variance and Duncan's new multiple range test at $P = 0.05$ (Duncan 1955).

Results

A near zero population was attained approximately 3 months after the fenoxycarb treatment. At that time, the pretreatment population index was reduced by 97.2% or greater in all treated plots, with a mean reduction of 98.9% (Table 3). An

Table 3. Change in population index (PI) of RIFA colonies after a broadcast application of fenoxycarb bait.

Posttreatment interval (months)	PI $\bar{X} \pm$ SEM*	% Change in PI† $\bar{X} \pm$ SEM
0	249.1 ± 14.7 a	–
1	16.3 ± 2.0 bc	-93.3 ± 1.1
2	15.8 ± 2.8 bc	-94.0 ± 0.8
3	2.8 ± 0.9 c	-98.9 ± 0.3
4	36.7 ± 8.4 b	-83.7 ± 3.7
8	20.1 ± 4.1 bc	-91.5 ± 1.8
13	103.5 ± 12.1 d	-56.9 ± 6.6
16	306.5 ± 15.5 e	+25.9 ± 8.3

*Population index means followed by the same letter are not significantly different (Duncan's new multiple range test, P = 0.05).
†% change = (PI at indicated month - pretreatment PI)/pretreatment PI.

average of 1.3 colonies, all in classes 1-3, were present per 0.1-ha subplot compared to 13.8 colonies, predominantly in classes 8-10, present before treatment (Table 2). A gradual reinfestation of the study site occurred over the next 13 months. Twelve months after initial reinfestation was detected, the mean population index was significantly greater than the index of the original population (Table 3).

Figure 1 clearly shows a trend in colony class changes following a fenoxycarb bait application. The IGR activity of fenoxycarb disrupts egg laying and brood development of RIFA queens (Banks et al. 1983 and 1988). Banks (1990) stated that colonies which ingest IGRs may survive over a year in the laboratory and 4 to 6 months in the field. In this study, 98% of the pretreatment colonies were in colony class 7 and above (Fig. 1). Worker brood was not found in any colony up to 3 months posttreatment (PT). Colonies without worker brood persisted up to 8 months PT, with a total of 14 present at that time (Table 2).

Reinfestation began to appear 4 months PT with over 50% of colonies categorized as classes 7 or 8 (Fig. 1). The exact time repopulation of a site begins is difficult to determine because small, incipient class 6 colonies are not easy to detect. At 4 months PT, four colonies in class 9 and one in class 10 were detected. These colonies are not believed to be the result of natural colony growth and development in the study site but rather the result of mature colonies outside the treated area relocating, over time, through the "buffer zone" into the outer edges of a plot, or perhaps more likely large broodless colonies (i.e. class 4 or 5) having adopted a newly mated queen. As previously mentioned, relocation of colonies is well documented. The significant decrease in RIFA populations afforded by the fenoxycarb treatment would encourage mature colony movement into the site through a lack of intercolony competition and an increase in available food sources. The other more plausible explanation for these class 9 and 10 colonies is adoption of newly mated queens into orphaned colonies. In the laboratory, orphaned monogynous colonies may reject or execute a number

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of queens before seemingly arbitrarily accepting one (Banks et al. 1981). Glancey and Lofgren (1988) also found that polygynous field colonies adopt newly mated queens. However, large major workers, or repletes, are capable of storing the oil solutions of IGRs in their crop for several months and may then pass the toxicant, through trophallaxis, to these adopted queens (Banks and Schwarz 1980). As stated previously, fenoxycarb disrupts the queen's reproductive system, and in the laboratory, the quantity of worker brood is diminished within one week after a fenoxycarb treatment, but does not entirely disappear for 4-8 weeks (Banks et al. 1988). The appearance of the class 9 and 10 colonies in our study at 4 months PT may have succeeded the adoption of newly mated queens, which initially produced worker brood. However, the inadvertent poisoning of the queens by workers retaining fenoxycarb, resulted in the subsequent disappearance of these colonies at 8 and 13 months.

Eight months PT (Feb. 1990), there was a slight, although not significant, decrease in the mean number of colonies present in the site (Table 2). In relation to the previous count, a greater percentage of these had no brood present (Fig. 1), which may have been a seasonal effect. Markin and Dillier (1971) found that the colony development and brood production were greatly reduced in RIFA colonies in south Mississippi during the winter months.

Thirteen months, PT, all colonies were rated as classes 7 and 8 and were present in significantly greater numbers than in all prior evaluations, except the 2 month PT count (Table 2). By 16 months PT, some of these class 7 and 8 colonies had developed into class 9 and 10 colonies, but the majority, 84.1%, were in classes 8 and 9 (Fig. 1).

Discussion

From the findings of Markin et al. (1973), we assume that small class 6 colonies were growing and developing 1-2 months prior to detection in the field. Incorporating this assumption into the data presented here, we find that class 7 and 8 colonies first appeared approximately 3 months after their inception, and classes 9 and 10 first appeared after 12-15 months of development. Markin et al. (1973) found that colonies required 2.5 years to obtain the equivalent of a class 10 colony (>50,000 workers), but Lofgren and Williams (1985) detected colonies equivalent to class 10 after about 9 months of development. These differences in the time required for a class 10 colony to develop were probably a result of those previously mentioned biotic and abiotic factors that can influence colony growth. Estimation of worker population also varies from one evaluator to the next, especially in categorizing high class 9 colonies (ca. 40,000 workers) and low class 10 colonies (ca. 50,000 workers).

This study concluded with the appearance of class 10 RIFA colonies as defined by Lofgren and Williams (1982). These class 10 colonies developed over a 13-month interval from a near zero population and we considered them mature because we observed reproductive forms present in many of the colonies. However, at 1 year these colonies had not reached the ultimate worker population and, according to Markin and Dillier (1971), Markin et al. (1973) and Tschinkel (1988), they are likely to require additional time to do so. Presumably, colonies in the lower class categories will continue to grow in size and develop into higher classes.

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Reinfestation by RIFA at the study site progressed as expected; from single, undetected mated queens to small, undetected, incipient colonies (100 workers with worker brood), and then through the small but visible colony 10 classes, to large mature colonies (>50,000 workers with worker brood).

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