Comparison of the Control of Monogynous and Polygynous Forms of the Red Imported Fire Ant (Hymenoptera: Formicidae) with a Chlorpyrifos Mound Drench^{1, 2}

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J. Entomol. Sci. 25(2): 317-324 (April 1990)

ABSTRACT Single (monogynous) and multiple (polygynous) queen mounds of the red imported fire ant, *Solenopsis invicta* Buren, were treated according to label directions with individual mound drenches containing chlorpyrifos. Reduction of marked 30 mound sets and mound densities per unit area were monitored. Treated polygynous mounds were rendered inactive as easily as monogynous mounds. Percent reduction of polygynous mound densities was initially greater but mound reinfestation after approximately 10 weeks occurred at a higher rate for the polygynous ant. The higher mound density may be due to the survival of satelite mounds. Further, the density of polygynous mounds remained numerically higher than that of the monogynous mounds throughout the study period. In effect, more labor and insecticide was required to treat polygynous populations, because of higher initial mound densities.

KEY WORDS Red imported fire ant, monogynous, polygynous, control, Solenopsis invicta..

The red imported fire ant (RIFA), Solenopsis invicta Buren (Hymenoptera: Formicidae), introduced to the United States in the late 1930 or early 1940's, has generally been considered a monogynous species (Lofgren et al. 1975, Holldobler and Wilson 1977). The ant constructs mounds (colonies) that contain several hundred thousand ants and a single queen (Glancey et al. 1973). However, reports over the last decade have shown the existence of multiple queen (polygynous) mounds (Glancey et al. 1973, Fletcher 1983, Hung et al. 1974, Lofgren and Williams 1984) that differ from monogynous colonies in a number of parameters. Greenberg et al. (1985) reported that the workers of polygynous colonies were generally smaller than monogynous workers and that the density of mounds per ha was greater in polygynous than monogynous infested areas: however, both forms are presently considered to be the same species (Ross et al. 1987).

Although there are many options available for the control of the RIFA (Drees and Vinson 1988), the development of these control tactics and the label recommendations are all based on data presumably from the monogynous form (Francke

¹ Accepted for publication 15 February 1990.

² Approved as TA22928 by the Director of the Texas Agricultural Experiment Station. Supported in part by Expanded Research funds in Pest Management.

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1983, Morrill 1977). Many anecdotal reports and "conventional wisdom" suggest that the control of polygynous mounds with a mound drench are more difficult than control of monogynous mounds since more queens must be destroyed. Lammers (1987) found that the survival of polygynous mounds treated with an experimental juvenile hormone analog bait to be greater than that of monogynous mounds, suggesting that the polygynous form may resist this type of insecticide.

Field experiments were developed to determine if the use of individual insecticide mound drenches might be more or less effective on individual multiple queen mounds compared to single queen mounds. Although mound drenches are fast acting and have a short residual, we wanted to determine if the use of a mound drench can be expected to reduce the number of mounds in an area over the longer term (2½ months). Because the number of polygynous mounds per ha can be five-fold-higher than the density of the monogynous form (Lammers 1987, Greenberg et al. 1985), the levels of control of the two forms of the ant on an area basis in Texas using the insecticide chlorpyifos (Dursban[®] 4E) were also compared. Since colonies may migrate after various types of disturbance (Francke 1983, Hayes et al. 1982), the locations of all mounds in the treated study area were mapped.

Materials and Methods

Study sites: Two sites were selected:: 1) a pasture in Brazos County, TX, suspected of being infested with polygynous RIFA because of smaller worker size and higher mound density before confirming studies, and 2) a pasture in Montgomery County, TX, suspected of being monogynous because of relatively larger RIFA worker size and lower mound density.

Conformation of ant form inhabiting site: Since head width of worker ants are accurate indicators of single or multiple queen mounds, worker ants from 10 randomly selected mounds were collected from each site, and head widths were measured from a minimum of 25 worker ants from each mound before calculating average values for determining colony type (Greenberg et al. 1985). The student's t test was used to compare mean worker head width. To further substantiate the type of RIFA in each location, 10 colonies were collected from plot areas. Dealate female (gynes) collected from each colony were dissected to observe the presence of sperm in the spermathaeca. The presence of sperm is used as an indicator of reproductive cabability since any one queen from a polygynous mound may lay more or less eggs than another queen, rendering the use of ovarian development as an indicator of reproductive activity questionable. Thirty randomly selected mounds at each location were also measured for mound height and diameter.

Study design: To determine the efficacy of the individual mound treatments on the two forms, 30 mound sets were marked in each area. All active mounds in circular treatment plots were mapped in order to determine if treatment caused splitting or mound movement, thus influencing the density of mounds per unit area.

After conformation of the ant type present at the two locations, circular plots were established. In the Brazos County polygynous mound location, 0.05 ha circular plots were marked with a center stake and located so that each plot was at least 9 m apart to provide a buffer zone. All mounds within the plot were mapped and 30 mounds were randomly selected and marked with numbered canning jar

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lids (Kerr®) nailed into the ground using 12.7 cm long nails. The metal lids could be relocated with a metal detector at later dates. One treatment and one control plot each of 30 mounds was established each week for 4 weeks (four replicates) beginning July 16, and concluded on August 7, 1986.

In Montgomery County, populated predominately with monogynous mounds, larger 0.1 hectare (¼ acre) plots were established due to the lower mound density at this site. Further, in order to mark and number 30 mounds per replicate, three to six circular plots were required for each replicate. One control and one treatment plot (consisting of several circular plots per replicate) each of 30 mounds was established each week for 4 weeks beginning July 14 and concluded on August 7, 1986.

Treatment: After plot establishment, all mounds, including the 30 marked mounds within each mapped circular plot(s), were treated with either 7.4 ml chlorpyrifos (Dursban[®] 4E) in 3.8 liters water or the same amount of plain water using plastic sprinkler cans (Drees and Vinson, 1988). In addition, all mounds in a 3.0 m wide area around each circular mapped plot(s) were treated to produce a similarly treated buffer zone.

Evaluation and data analysis: Mounds were evaluated pre-treatment and 7 and 73 ± 7 days post-treatment. Since the use of insecticidal mound drenches such as chlorpyrifos are predicated on ant contact and rapid kill (Francke 1983, Hillman 1977, Morrill 1976) evaluations for control were conducted 7 days post-treatment. A second evaluation was made approximately 10 weeks post-treatment to evaluate reinfestation or recombining of satelite mounds.

Evaluations were conducted early in the day when summer temperatures were moderate to warm. Because of interest only in elimination of mound activity and not insecticidal mode of activity, progressive minimal mound disturbance method of evaluation similar to that used in earlier individual mound treatment evaluations was used (Francke 1983). This minimized possible mound movement due to evaluation methods. Individual mounds were first minimally disturbed by tapping followed by penetration of the mound with a plot flag to detect worker ant activity. If 25 or more worker ants rapidly emerged from the colony, that colony was considered to be active. If no activity was detected, the mound was further disturbed by digging a hole in the mound. The mound was determined to be inactive if no ant activity could be detected after escavating a quarter to a half of the colony. This procedure generated a value for 1) the total number active mounds out of the 30 marked mounds for each plot, and 2) the total number of active mounds in the treated area.

The mean number of active colonies of the 30 treated in each replicate was compared to controls using the student's t-test to determine the effectiveness of each mound drench in eliminating ants from the treated mound. To determine if the individual mound treatment reduced the population of mounds, the total number of active mounds in the treated plots were compared to the initially mapped and treated mounds and to controls using the student's t-test. Since the density of mounds of the two ant forms (monogyne and polygyne) differed greatly, the percent reduction of active mounds per unit area was first calculated using Henderson's formula (Henderson and Tilton 1955). The data were transformed (arcsin) and compared using student's t-test.

Average head capsule width (mm)				
Brazos County	Montgomery County			
(presumed polygynous)	(presumed monogynous)			
$0.7680 \dagger$	0.8827			
0.6250	0.8995			
0.7164	0.9782			
0.7056	0.7214‡			
0.6408	0.8059			
0.6920	0.8249			
$0.7992 \dagger$	0.8112			
0.6712	0.8011			
0.6912	0.8309			
0.7152	0.8105			
$0.7025 \pm 0.0527 \text{SD}$ §	$0.8366 \pm 0.0692 { m SD} \S$			

Table 1. Average head capsule widths of 25 red imported fire ant workerscollected from each of 10 mounds in the Brazos and MontgomeryCounties, TX, plots 1986.*

* Greenberg et al. (1985) reported maximum head width of monogynous colonies to be > 0.74 mm while polygynous colony worker head widths were < 0.71 mm. Since then Dr. Greenberg (personal comm.) has refined these limits with additional data to >0.789 mm for monogynous and <0.74 mm for polygynous forms.

† May be monogynous based on revised data.

‡ May be polygynous or a young colony.

§ Statistically different using student's test (t = -4.8762; P = 0.0001, df = 18).

Results and Discussion

Confirmation of ant form infesting each site: Diameters of 30 randomly selected mounds measured from the Montgomery and Brazos Counties plots were similar (46.36 \pm 10.13 SD cm diameter in Montgomery County vs 50.11 \pm 13.06 SD cm in Brazos County), indicating that neither population consisted of small incipient colonies.

Samples of worker ants randomly collected from the Brazos County RIFA plots showed that the population was predominantly polygynous (80 %) (Table 1). In addition, 80 % of the mounds collected from the Brazos County plots contained more than one mated female gyne. The Montgomery County colonies were predominantly (90 %) monogynous. Furthermore, 25% of the mounds were found to contain a single mated female. The remainder contained numerous unmated dealates females thought to have lost their wings after mounds were collected from the field. Female reproductives soon drop their wings in the absence of the queen (Fletcher and Blum 1981). Thus, the absence of mated females in these cases is interpreted as supportive evidence that these were monogynous mounds. Pretreatment plot mound densities between the polygynous (1635 ± 573.5 mounds per ha) Brazos County and monogynous $(72 \pm 19.0 \text{ mounds per ha})$ Montgomery County sites differed significantly (t = 5.4478; dF = 6; P = 0.0079). Mound densities in the polygynous colony site were 22.7-fold higher than those in the monogynous site. Equivalent percent reductions of mounds in each of these areas would obviously not result in numerically identical levels of control.

Table 2.	Number of active red imported fire ant mounds following a
	drench treatment with either water (control) or chlorpyrifos (treat-
	ment) in a polygynous (Brazos County) or monogynous (Mont-
	gomery County) colony area, TX, 1988.

Evaluation	Polygynous colonies		Monogynous colonies	
Period	Control	Treatment	Control	Treatment
Pre-treatment	30	30	30	30
7-day post-treatment	29.8*	0*	$23.3 \dagger$	0.3†
Percent reduction 7-day	100		99.1	
73 ± 7 day post-treatment	16	0	13	0.5
Percent reduction 73 ± 7 day	100		96.2	

* Significant using the student's t test ($P \le 0.05$) (t = 4.2534; dF = 6; P = 0.0027).

† Significant using the student's t test (P \leq 0.05) (t = 3.9537; dF = 6; P = 0.0038).

Effect of chlorpyrifos drench on RIFA mound activity: Table 2 presents the results of treating four replicates of 30 marked polygynous and monogynous RIFA mounds. One week following treatment, the ant activity in treated mounds was significantly reduced compared with untreated mounds of both RIFA forms. Further, no significant differences were found between the percent reduction of mounds between the two ant forms. No activity was detected in treated polygynous mounds and only two active treated monogynous mounds were detected after 73 ± 7 days. Thus, it appears that reinfestation of treated marked mounds, with the two exceptions noted above, did not occur throughout the study period. The number of active marked mounds in the control for both forms decreased somewhat over time although more marked control mounds in Montgomery County became inactive. This is an indication that perhaps more natural movement of colonies from one mound site to another occurs in the monogynous form.

Effect of chlorpyrifos drench of RIFA mound density: Treatments significantly reduced the number of active mounds of both types 7 days following treatment (Table 3). The percent reduction was significantly greater in the polygynous population (95.3%) than in the monogynous population (82.6%). Although we did not find any ant activity in the 30 marked and treated polygynous colonies (Table 2), a few surviving mounds within the polygynous treated area and new small mounds were found (Table 3). These new mounds were presumably the result of RIFA movement from treated mounds, mounds escaping treatment or migration into treatment plots. In the monogynous area, several of the 30 marked mounds (Table 2) and a few additional mounds within the treated area survived, and a few new mounds appeared (Table 3). The density of active mounds 7 days post-treatment remained higher for the treated polygynous (76 mounds per ha) than monogynous (13 mounds per ha) RIFA infested area. Thus, while chlorpyrifos drench treatments resulted in a greater percent reduction of active polygynous RIFA mounds 1 week after treatment, the absolute number of mounds per unit area was still 5.8 times greater than the number of monogynous RIFA mounds because of the greater initial (pre-treatment) density of polygynous mounds. The presence of 13 mounds per hectare in the monogynous populations is generally more acceptable than 76 mounds per ha in the treated polygynous area.

Aproximately 10 weeks following application, the number of active mounds in treated plots at both sites increased significantly from their 7 day post-treatment levels (Table 3). In polygynous mound plots, active mounds, which based on our mapping data were in new locations, increased from 76 to 486 per ha, a 6.4-fold increase, while in monogynous mound plots, new active mounds increased from 13 to 73 per ha, a 5.6-fold increase. This is an indication that "reinfestation" in previously treated areas may occur more rapidly with polygynous populations, either by invasion from outside the treated area or through the development of small queen right colony fragments that escaped the treated mound and were undetected at the 7 day evaluation.

Conversely, considering the long term $(2\frac{1}{2} \text{ months})$ effect of the treatment of the two ant forms, mounds in the polygynous colony plots were reduced from 1636 pre-treatment to 486 per ha, 10 weeks post-treatment, while active mounds in monogynous colony plots returned to pre-treatment levels by 10 weeks (72 and 73 mounds per hectare, respectively). Return of active mound densities to pre-treatment levels in monogynous colony plots most likely resulted from immigration or other factors discussed by Francke (1983). Thus, it appears that some control was maintained for 10 weeks in the treated polygynous RIFA area relative to the monogynous area. A possible reason for the continued higher mound suppression in the polygynous colony area is that many of the potentially suitable mound locations within the plots had been treated and, as indicated with the treated marked mounds (Table 2) and through the evaluation of mapped mounds (data not shown), ants were not found to reinfest treated mounds during the period of this

Table 3. Number of active red imported fire ant mounds per ha and
percent reduction* following drench treatment with either water
(control) or chlorpyrifos (treatment) in a polygynous (Brazos
County) or monogynous (Montgomery County) infested area, TX,
1986.

Evaluation Period	Number active mounds per hat				
	Polygynous colonies		Monogynous colonies		
	Control	Treatment	Control	Treatment	
Number pre-treatment	1196	1636	144	72	
Number 7-day post-treatment	1456a‡	76ab	165c	13cd	
Percent reduction 7-day post-treatment	95.3		82.6		
Number 73 ± 7 day post-treatment	1060	486.3b	210	73d	
Percent reduction 73 day post-treatment	59	1.8e	29).9e	

* Calculated using Henderson's Formula (Henderson and Tilton 1955).

† The number of mounds of both forms of the imported fire ant are presented on a one hectar basis for comparison.

[‡] Mean values with the same letter are significantly different using the student's t test. Percent reduction in parenthesis was analyzed following arcsin transformation of the percent values. In all cases dF = 6 and P \leq 0.05). For a (t = 5.5046, P = 0.0008), b (t = -4.9299, P = 0.0013), c(t = 6.3311, P = 0.0004), d(t = -3.2095, P = 0.0092), e(t = 2.3554, P = 0.0283).

study. At the end of the study period, there was a 6.6-fold difference between the polygynous and monogynous RIFA plots, well below the 22-fold difference prior to the treatment. Although the individual mound drench treatment narrowed the difference in the population densities between the two forms of ant, the treatment failed to reduce the polygynous mound population below monogynous colony pre-treatment levels.

Labor, cost and the environmental effect of excessive product usage must be considered in selecting methods for control. Mixing, hauling and drenching individual mounds is labor intensive and treatment of mounds in polygynous RIFA areas requires additional labor and time. Polygynous RIFA mound densities required 640 individual mound drenches per 0.4 ha, at a chemical cost of \$96.87 (1.25 gal Dursban[®] 4E at 1986 cost of \$77.50/3.75L or \$20.67/L). Only 29 individual mound drenches at a chemical cost of \$4.39 per 0.4 ha were required to treat monogynous ant mounds. Current instructions on the product labels do not allow for adjustments in the needed amount of active ingredient or water volume relative to mound size or RIFA colony type. The label also does not suggest a maximum number of mounds to be treated per unit area for economic purposes or to avoid excessive environmental contamination of treated areas. Yet, in the urban environment where polygynous colonies are common (at least in infested areas of Texas), the individual mound drench technique is one of the more common methods of RIFA control.

Acknowledgments

We thank S. Johnson and J. Greathouse for assisting in conducting this research and W. MacKay and A. P. Bhatkar for reviewing earlier versions of this manuscript. We are grateful for assistance from G. McIlveen, L. R. Sprott, M. E. Heimer, and T. R. LeRoy. We also appreciate materials provided by J. Bertholf of Dow Chemical, USA, and financial support from the Texas Agricultural Experiment Station Expanded Research Funds.

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