# Field Tests with Synthetic Components of the Queen Recognition Pheromone of the Red Imported Fire Ant, *Solenopsis invicta*

by

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## ABSTRACT

Three synthesized components of the queen recognition pheromone of the red imported fire ant, *Solenopsis invicta* Buren, were tested in combinations (both binary and tertiary) in the field in Florida, Georgia and Mississippi. The tertiary combinations and one of the binary combinations were as active as extracts of live *S. invicta* queens in eliciting responses from *S. invicta* workers from widely separated geographical populations. The materials were active over a range of 5 to 50 ng per surrogate queen. Three other *Solenopsis* spp. were not responsive to either the extracts from the live queens or to the synthetic compounds.

## INTRODUCTION

During the past few years, we have conducted a series of studies on the identification, isolation and synthesis of the queen recognition pheromone of the red imported fire ant, *Solenopsis invicta*. This pheromone, which is stored in the poison sac (Vander Meer et al. 1980) has been shown to control a series of stereotypic behaviors including attraction, recognition and tending of the queen (Jouvenaz et al. 1974, Glancey 1980, Glancey et al. 1983, Lofgren et al. 1983). The pheromone has also been found to be produced by virgin dealates which have undergone wing muscle histolysis (Glancey 1980). Recently, we published the synthesis of components A and B (Figure 1) of the queen recognition pheromone (Rocca et al. 1983 a,b). This paper presents the results of our field tests with 3 of the components of the pheromone, A, B and C. The sample of C (Figure 1) was kindly provided by Prof. M. Battiste and Dr. L. Strekowski of the Department of Chemistry, University of Florida, Gainesville.

## MATERIALS AND METHODS

The bioassay technique involved treatment of surrogate queens pre-

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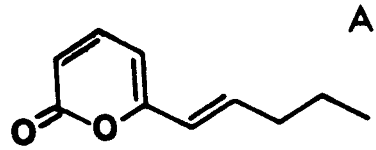
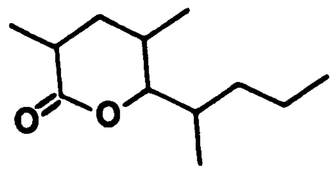
A



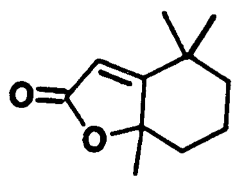
(E)-6-(1-pentenyl)-2H-pyran-2-one

B



tetrahydro-3,5-dimethyl-6-(1-methylbutyl)2H-pyran-2-one

C



dihydroactinidiolide

Fig. 1. Chemical structure of 3 of the components of the red imported fire ant queen recognition pheromone as determined by Rocca et al. 1983a,b.

pared from rubber-seal septa (see Lofgren et al. 1983 for a complete description). The surrogate queens were offered to colonies in the field using the distributed colony techniques described by Glancey et al. (1983). Essentially, a red imported fire ant (RIFA) mound was opened and a shovelful of soil containing ants and brood was distributed lightly and evenly over the floor of a large wooden box with one open end that butted against the mound. The septa were placed upon the soil and the ants' reactions were observed.

Past evaluations of five worker ant behavioral responses to the queen pheromone have involved subjective descriptions such as light to heavy, or all or none. In an effort to quantify the ants' responses so that the data could be analyzed statistically, we developed the following rating system. The five responses, which we used with live or surrogate queens (Glancey et al. 1983) are: (1) speed of attraction, (2) clustering, (3) moving brood to or around the queen, (4) development of a queen trail and (5) moving the queen into the nest. For each test we assigned a numerical rating of 1 to 5 for behaviors 1, 2 and 4 with a total lack of response being given a 1 and a maximum response being given a 5. For the behaviors 3 and 5, we

Table 1. Worker responses to surrogate queens treated with various concentrations of 3 components of the synthetic queen recognition pheromone.

| ng of each component per septum | Pheromone[1/] | Response x | SE | No. of tests |
|---|---|---|---|---|
| 0.5 | ABC[a] | 3.14 | .36 | 6 |
| | AB[a,b] | 2.82 | .7 | 3 |
| | AC[b,c] | 1.81 | .37 | 6 |
| | Solvent[c] | 1.22 | .25 | 6 |
| | BC[c] | 1.07 | .06 | 3 |
| 5 | AB[a] | 3.9 | .24 | 3 |
| | ABC[a] | 3.82 | .45 | 6 |
| | BC[b] | 1.77 | .54 | 3 |
| | Solvent[b] | 1.28 | .35 | 6 |
| | AC[b] | 1.27 | .27 | 6 |
| 50 | AB[a] | 3.66 | .32 | 3 |
| | ABC[b] | 2.67 | .34 | 6 |
| | AC[b,c] | 1.58 | .54 | 6 |
| | BC[c] | 1.23 | .14 | 3 |
| | Solvent[c/] | 1.02 | .02 | 6 |

1/ Test combinations with the same superscript are not significantly different (Duncan's multiple range test P=0.05). See Figure 1 for chemical names of pheromones which correspond to the capital letters above.

assigned a rating of 1 for no response and a 5 for a positive response. Then we multiplied the ratings by a decimal weighting factor which was based on a unit of 1. We assigned the highest weight (0.3) to clustering, the best indicator of queen recognition (Glancey et al. 1983). The next two most consistent responses of recognition, brood touching and trail formation, were assigned a value of 0.25 each. Speed of attraction and retrieval of the septum were weighted at 0.1 each. Although these factors are based on arbitrary units, we have found them extremely useful and replicable as indicated by the consistency of our test and experimental data.

Utilizing these techniques, we conducted experiments to compare (1) various combinations (over a range of concentrations) of the 3 synthetic pheromones (designated A, B, or C as in figure 1), (2) different ratios of pheromones A and B (over a range of concentrations), (3) the responses of populations of RIFA in Florida, Georgia and Mississippi (using various combinations at a given concentration) and (4) response of workers of *S. richteri*, *S. geminata*, and *S. xyloni* to the *S. invicta* surrogate queens (using

Table 2. Responses of worker ants to varying concentrations and ratios of pheromones A and B (see Figure 1).

| ng of total blend per septum[a/] | Pheromone ratio | | Response | |
|---|---|---|---|---|
| | A | B | $\bar{X}$ | SE |
| 2[b] | 20 | 80 | 2.23 | .28 |
| | 40 | 60 | 2.75 | .09 |
| | 60 | 40 | 2.70 | .12 |
| | 80 | 20 | 2.39 | .31 |
| 10[a] | 20 | 80 | 3.68 | .21 |
| | 40 | 60 | 3.68 | .23 |
| | 60 | 40 | 3.71 | .28 |
| | 80 | 20 | 3.81 | .23 |
| 50[a] | 20 | 80 | 3.40 | .80 |
| | 40 | 60 | 3.91 | .34 |
| | 60 | 40 | 3.95 | .35 |
| | 80 | 20 | 3.88 | .32 |
| 250[b] | 20 | 80 | 2.69 | .76 |
| | 40 | 60 | 2.82 | .84 |
| | 60 | 40 | 2.92 | .83 |
| | 80 | 20 | 2.79 | .76 |

a/ Concentrations with the same letter are not significantly different (Duncan's multiple range test, P<.001). Average of 4 replications.

various combinations at a given concentration). Analyses of variances (ANOVA) were run using the general linear model procedure of SAS (1982), and for Duncan's multiple range test.

## RESULTS

An ANOVA (Table 1) showed that workers responded most strongly to the combinations of pheromones A and B and A, B and C. Overall, the AB and ABC combinations elicited significantly greater responses than the other combinations (P<.001). However, the response to concentrations were not the same for all combinations. At a concentration of 5 ng of each component, the responses to the combinations AB and ABC are clear cut, they are statistically equivalent, and are the most effective. But at a low concentration of 0.5 ng per component or a high of 50 ng per component, the responses to the combinations are not so clear cut and there is an overlap of mean responses, e.g., ABC AB, but AB AC and AC solvent.

Worker ants did not discriminate among various ratios (20-80%) of pheromones A and B (Table 2). In fact, no differences among the ratios

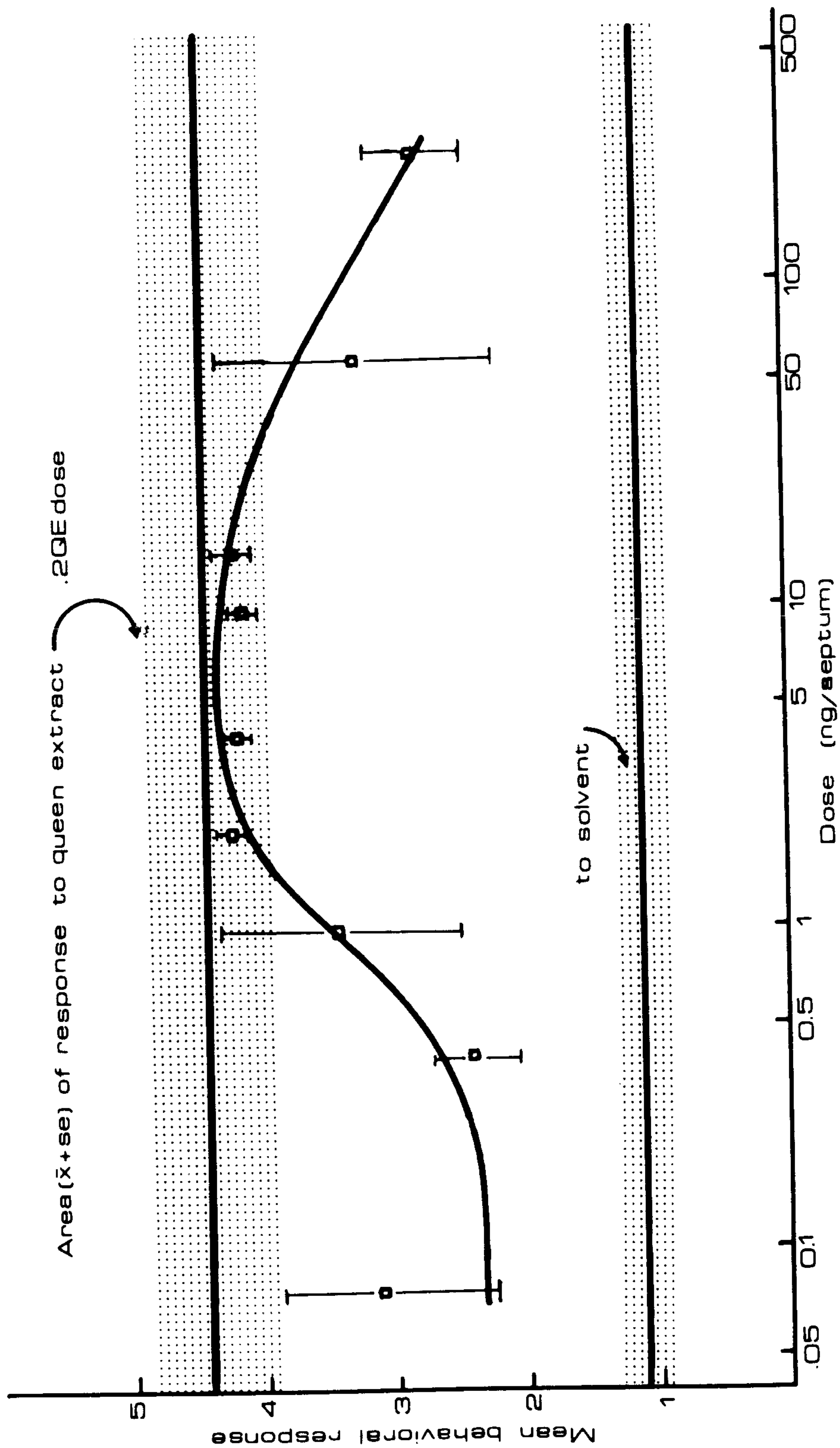



Fig. 2. Response of worker ants from disrupted field colonies to varying doses of a combination of 2 pheromone components (AB) on surrogate queens.

Table 3. Response of colonies of *S. invicta* to surrogate queens treated with natural and synthetic queen recognition pheromone.

| Sample | Dose[1/] | $\bar{x}$ Response | SE | Reps |
|---|---|---|---|---|
| | | Gainesville, FL | | |
| Queen extract | 0.2QE | 3.88[a] | .37 | 6 |
| ABC | 5:7:5ng | 3.82[a] | .45 | 6 |
| AB | 5:7ng | 3.90[a] | .24 | 6 |
| AC | 5:5ng | 1.27[b] | .27 | 6 |
| BC | 7:5ng | 1.77[b] | .54 | 3 |
| SBO | 0.5mg | 1.12[b] | .12 | 6 |
| Solvent | 25ul | 1.07[b] | .06 | 12 |
| | | Sanford, FL | | |
| Queen extract | 0.2QE | 4.29[a] | .07 | 27 |
| ABC | 25:35:25ng | 3.93[a] | .19 | 12 |
| AB | 25:35ng | 4.24[a] | .04 | 4 |
| AC | 25:25ng | 2.35[b] | .47 | 4 |
| BC | 35:25ng | 1.55[c] | .42 | 4 |
| Solvent | 25ul | 1.22[c] | .09 | 12 |

1/ Quantity of each component per surrogate. Means within a column followed by the same letter are not significantly different. Each location analyzed separately, Duncan's multiple range test.

were found at any concentration. The effect of increasing the concentration was to increase the response up to 50 ng per surrogate and to decrease the response beyond 50 ng per surrogate. The most significant responses ($P<.001$) were obtained at the 10-50 ng range.

The responses of worker ants to varying quantities of the pheromone combinations AB and ABC are presented in Figures 2 and 3. It is estimated that 1 QE (queen equivalent) of the crude queen extract contains about 1 ng of A, 4 ng of B, and 1 ng of C. The ants responded maximally to the AB and ABC combinations at doses of 2-40 ng per surrogate. In both cases the responses to the synthetic pheromones fell within the mean responses range for a 0.2 QE concentration of the queen extract, but did not exceed the mean response in either case. Lower or higher dosages resulted in responses with large standard errors of the mean.

The responses of *S. invicta* populations from widely separated geographical sites are given in Tables 3-7. Tests conducted in the Gainesville and Sanford, Florida areas were run singly, whereas the other tests were run in pairs with one of the other *Solenopsis* species. Responses to low rates (Gainesville) and higher rates (Sanford) show clearly that these *S. invicta* populations respond best to the queen extract and the synthetic combinations of AB and ABC (Table 3). A possible enhancing effect of C

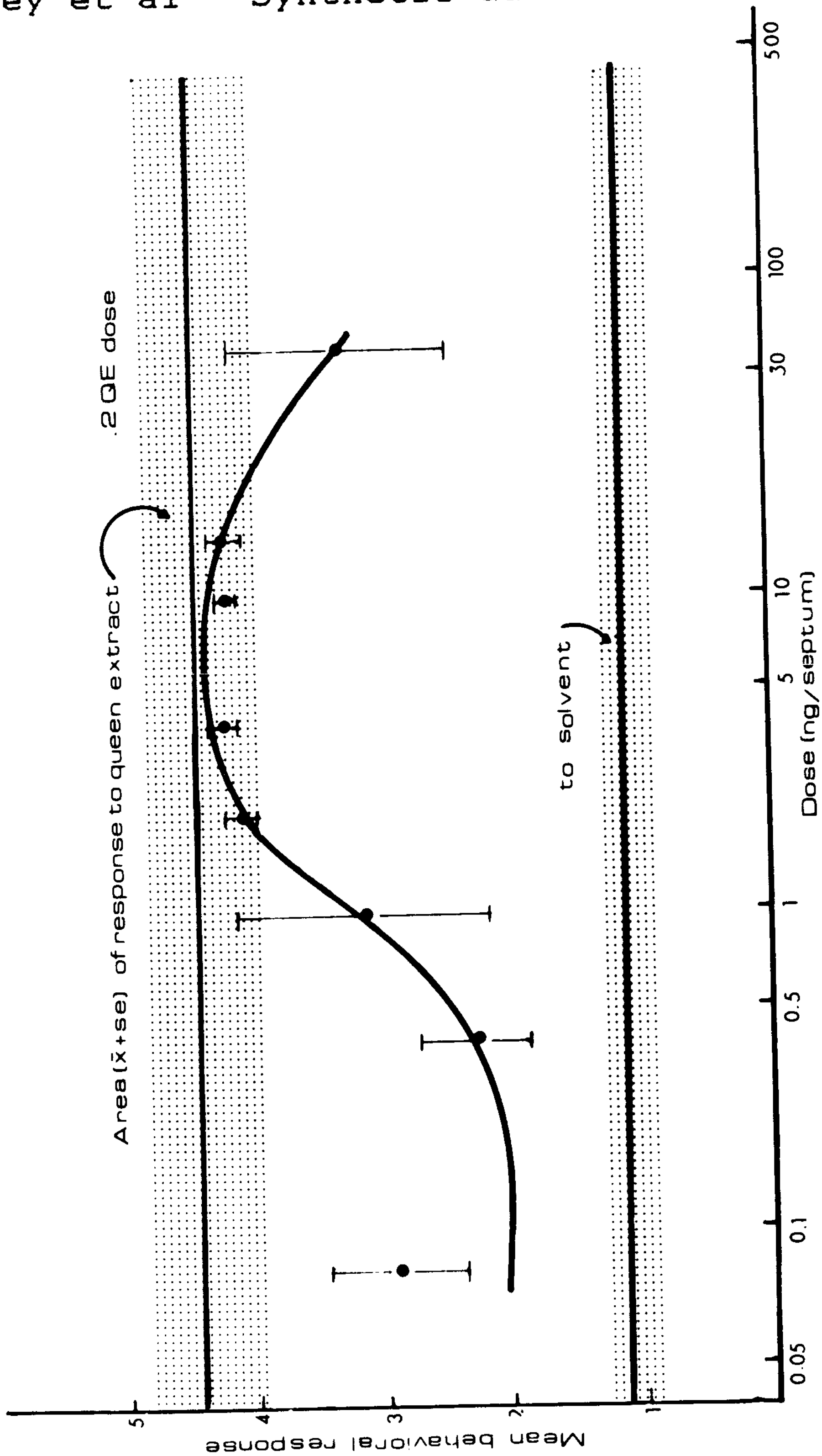



Fig. 3. Response of worker ants from field disrupted colonies to varying doses of a combination of pheromone components AB and C on surrogate queens.

Table 4. Response of field colonies of *S. invicta* and *S. geminata* from Live Oak, FL to surrogate queens treated with natural and synthetic *S. invicta* queen recognition pheromones.

| Sample | Dose[1/] | $\bar{X}$ *S. inv.* | $\bar{X}$ *S. gem.* | Reps | SE *S.i.* | SE *S. g.* |
|---|---|---|---|---|---|---|
| Queen extract | 0.2QE | 3.62[a] | 1.0 | 12 | 0.32 | 0 |
| ABC | 17:45:17ng | 3.35[a] | 1.0 | 12 | 0.38 | 0 |
| AB | 17:45ng | 3.33[a] | 1.0 | 3 | 0.85 | 0 |
| AC | 17:17ng | 1.60[b] | 1.0 | 3 | 0.31 | 0 |
| BC | 45:17ng | 1.73[b] | 1.0 | 3 | 0.14 | 0 |
| SBO | 0.5mg | 1.0[b] | 1.0 | 3 | 0 | 0 |
| Solvent | 25ul | 1.11[b] | 1.0 | 1 | 0.08 | 0 |

1/ Quantity of each component per surrogate. Means within a column followed by the same letter are not significantly different. Duncan's multiple range test.

can be seen in Table 3 (Sanford) in which the response to AC is statistically better than the solvent (zero response), but not as effective as AB or ABC. In both areas, BC did not elicit a positive response.

Tables 4-7 show the results of tests in which the same dosage of material was given to a *S. invicta* colony and a nearby colony of a different *Solenopsis* species. The Live Oak, Florida colonies of *S. invicta* (Table 4) showed a positive response only to the queen extract and the synthetic combinations of AB and ABC. The foreign species, *S. geminata*, gave an absolute zero response to all test surrogates. The Georgia colonies of *S. invicta* (Table 5) gave a wide range of responses. The queen extract gave a response that was significantly better than AB and ABC. Strangely, the BC combination showed some activity at this location, while the AC showed none. The other species tested, *S. xyloni*, did not respond at all to any of the materials offered. In Mississippi, the *S. invicta* colonies responded exceptionally well to all the combinations (Table 6). In fact, the AC combination was as effective as the queen extract or the AB or ABC combination. Additionally, the BC combinations were still significantly, though not as effective as the other combinations, better than the solvent. The other species tested, the black imported fire ant, *S. richteri* gave minimal response to the surrogate queens which were not significantly different from the responses to the solvent. When the responses of *S. invicta* are compared to those for *S. richteri* (Table 7) it can be seen that these are negligible.

Table 5. Response of field colonies of *S. invicta* and *S. xyloni* from Monticello and Athens, GA. respectively to surrogate queens treated with natural and synthetic *S. invicta* queen recognition pheromones.

| Sample | Dose[1/] | $\bar{x}$ *S. inv.* | $\bar{x}$ *S. xyl.* | Reps | SE *S.i.* | SE *S.x.* |
|---|---|---|---|---|---|---|
| Queen extract | 0.2QE | 4.26[a] | 1.0 | 12 | 0.03 | 0 |
| ABC | 33:45:33ng | 3.81[b] | 1.0 | 12 | 0.18 | 0 |
| AB | 33:45ng | 3.83[b] | 1.0 | 3 | 0.03 | 0 |
| AC | 33:33ng | 1.27[d] | 1.0 | 3 | 0.27 | 0 |
| BC | 45:33ng | 1.9[c] | 1.0 | 3 | 0 | 0 |
| SBO | 0.5mg | 1.3[d] | 1.0 | 3 | 0.31 | 0 |
| Solvent | 25ul | 1.07[d] | 1.0 | 12 | 0.08 | 0 |

1/ Quantity of each component per surrogate. Means within a column followed by the same letter are not significantly different. Duncan's multiple range test.

In an ANOVA, the significant differences due to locations (P=.003) cause us to reject the hypothesis that there are no population differences in ant responses to the pheromone. Finally, ant response to a food material (soybean oil, 0.5 ng per septum) did not differ significantly from the response to the solvent.

## DISCUSSION

The results from all the field tests show that the synthetic queen recognition pheromone components of *S. invicta* induce queen recognition by workers of this species. Two of the components, A and B in combination are vital to the induction of responses. The role of C, the third component is not clear. Overall, C in combination with A or B, is statistically different from the controls, but is not as effective as AB or ABC. Only in one case, that of the Mississippi tests, does C show activity equivalent to AB or ABC. The responses to C lead us to conclude that the differences are due to colony variation or location.

The dosage response curves indicate that the synthetic materials are active over a range of 5-50 ng per septum. There is a definite reduction in responses at higher dosage rates. It is possible that at very high concentrations the pheromone may over-load the ants' sensory mecha-

Table 6. Response of field colonies of *S. invicta* and *S. richteri* from Newton and Starkville, MS, respectively to surrogate queens treated with natural and synthetic *S. invicta* queen recognition pheromones.

| Sample | Dose[1/] | $\bar{x}$ *S. inv.* | $\bar{x}$ *S. ric.* | Reps | SE *S.i.* | SE *S.r.* |
|---|---|---|---|---|---|---|
| Queen extract | 0.2QE | 4.06[a] | 1.51[a] | 12 | .11 | .10 |
| ABC | 33:45:33ng | 4.08[a] | 1.42[a] | 12 | .21 | .22 |
| AB | 33:45ng | 4.25[a] | 1.3[a] | 3 | .10 | .30 |
| AC | 33:33ng | 3.70[a] | 1.27[a] | 3 | .05 | .27 |
| BC | 45:33ng | 2.7[b] | 1.27[a] | 3 | .46 | .27 |
| SBO | 0.5mg | 1[c] | 1.3[a] | 3 | 0 | .30 |
| Solvent | 25ul | 1.18[c] | 1.03[a] | 12 | .15 | .07 |

1/ Quantity of each component per surrogate. Means within a column followed by the same letter are not significantly different. Duncan's multiple range test.

nism and confuse or repel them. The synthetic material is about as attractive as the queen extract, but in the dose-response tests attraction did not exceed the mean response for the extract. The synthetic and natural pheromones are attractive to 5 different *s. invicta* populations maximally separated by over a distance of 500 miles. The large standard errors obtained with the Live Oak and Gainesville populations of RIFA could be due in part to environmental factors; however, even though these two populations gave an overall lower response, their individual responses to the synthetic AB and ABC and the natural queen extract were significantly different from the other pheromone combinations and the controls. Finally, field tests show conclusively that other species of fire ants do not respond to either the natural queen extract or the synthetic components of the RIFA pheromone.

The isolation, identification and synthesis of the three synthetic components of the queen recognition pheromone and the verification of their biological activity with field colonies represent a breakthrough in information on the nature of the chemical control of sociality in the fire ant. Currently, we are investigating the relationship of the queen recognition pheromone to other pheromonal systems in the ant colony and their potential for integrated pest control strategies for *S. invicta*.

Table 7. Analysis of differences in response by the red and black fire ants to the same chemical, data from Table 6.

| Sample | F. Value | PR>F |
|---|---|---|
| Queen extract | 252.83 | 0.0001 |
| ABC | 113.69 | 0.0001 |
| AB | 87.02 | 0.0007 |
| AC | 80.44 | 0.0009 |
| BC | 6.26 | 0.066 |
| SBO | 1.00 | 0.3739 |
| Solvent | 0.94 | 0.3416 |

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## REFERENCES

Glancey, B. Michael. 1980. Biological studies on the queen pheromone of the red imported fire ant. *In* Ecological Animal Control by Habitat Management. The Fire Ant Problem No. 7, Tall Timbers Research Station, Tallahassee, Fl. 1980. pp. 149-159.

Glancey, B. M., A. Glover, and C. S. Lofgren. 1981. Pheromone production by virgin queens of *Solenopsis invicta* Buren. Sociobiology 6: 119-127.

Glancey, B. Michael, C. S. Lofgren, J. R. Rocca and J. H. Tumlinson. 1983. Behavior of disrupted colonies of *Solenopsis invicta* towards queens and pheromone-treated surrogate queens placed outside the nest. Sociobiology 7: 283-288.

Jouvenaz, D. P., W. A. Banks and C. S. Lofgren. 1974. Fire ants: attraction of workers to queen secretions. Ann. Entomol. Soc. Am. 67: 442-444.

Lofgren, C. S., B. M. Glancey, A. Glover, J. Rocca and J. Tumlinson. 1983. Behavior of workers of *Solenopsis invicta* (Hymenoptera: Formicidae) to the queen recognition pheromone: Laboratory studies with an olfactometer and surrogate queens. Ann. Entomol. Soc. Am. 76: 44-50.

Rocca, J. R., J. H. Tumlinson, B. M. Glancey and C. S. Lofgren. 1983a. The queen recognition pheromone of *Solenopsis invicta*, preparation of (*E*)-6-(1-penenyl)-2H-pyran-2-one. Tetrahedron Lett. 24: (18): 1889-1892.

__________. 1983b. Synthesis and sterochemistry of tetrahydro-3,5-dimethyl-6-(1-methylbutyl)-2H-pyran-2-one, a component of the queen recognition pheromone of *Solenopsis invicta*. Tetrahedron Lett. 24:(18): 1893-1896.

SAS Institute. 1982. SAS User's Guide: Statistics, 1982 Edition. Cary, NC: SAS Institute Inc., 1982. 584 pp.

Vander Meer, R. K., B. M. Glancey, C. S. Lofgren, A. Glover, J. H. Tumlinson and J. Rocca. 1980. The poison sac of red imported fire ant queens: Source of a pheromone attractant. Ann. Entomol. Soc. Am. 73: 609-612.