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Study conducted in cooperation with the U.S. Department of Transportation, Federal Highway Administration.
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16. Abstract

The Texas Department of Transportation (TxDOT) is concerned that maintenance activities along highway rights-of-way have encouraged the spread and establishment of the red imported fire ant throughout Texas. The objective of this study was to determine if highway rights-of-way harbor larger fire ant populations than do adjacent properties. The methodology was to collect data on ant populations from three transects along highways within fire ant-infested areas of the state. One north-to-south transect, a southern east-to-west transect, and a northern east-to-west transect were established. Ant populations in rights-of-way were compared to populations in pastures immediately adjacent to highways. Twenty-eight transect sites were studied as well as five refuge sites and seven rest areas.

No significant differences in number or vitality of fire ant mounds in highway rights-of-way compared to adjacent properties were detected. However, significantly more ant colonies were found on roadbeds ($\leq$ 1.0 m from paved surfaces) than were expected in rights-of-way. Roadbed colonies were also significantly smaller, which suggests that colonies were younger and more recently founded than were colonies in the remainder of rights-of-way. Mound population densities and numbers of ants in bait cups were not well correlated with the vegetational characteristics measured in this study.

Highway rights-of-way should not be considered important conduits of fire ant invasion into uninfested regions of Texas, when compared to other modes of movement such as mating flights and transport of horticultural and agricultural products. Nonetheless, fire ant colony establishment along roadbeds and later movement into rights-of-way could be of concern in fringe areas of the infestation front.

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Implementation Statement

The objective of the research reported herein was to determine if highway rights-of-way harbored significantly more red imported fire ants than did adjacent properties not maintained by the Texas Department of Transportation. The implied objective was to determine if TxDOT-maintained areas encouraged the spread fire ants from infested to uninfested regions in Texas.

We believe that fire ant populations in Texas highway rights-of-way have reached a dynamic equilibrium with populations in adjoining properties. Mowing, as a major disruptive force in rights-of-way, has not adversely impacted fire ant populations. No evidence exists that the height nor cover of grasses in rights-of-way have affected fire ants.

Without benefit of a long-term study nor a historical view, we believe that circumstantial but compelling evidence exists that, within infested areas, TxDOT activities do not aid fire ant dispersal. However at infestation fronts throughout Texas, fire ants may gain important footholds just off paved surfaces, along highway roadbeds. New colonies develop as newly mated queens alight, survive, and produce brood in the relative warmth and moisture on roadbeds. Later, colonies may contribute to localized dispersal.

With these considerations in mind, we recommend the following items for continuance or implementation:

1. Mowing regimes of the department need not be changed.
2. Thoroughly and frequently inspect maintenance equipment for "hitch-hiking" fire ant colonies.
3. Do not move soil, in any form, unless it is fire ant-free.
4. Use bait cups for fire ant surveys in rights-of-way. Bait cups are probably most sensitive to identification of small fire ant infestations.
5. Protect the safety and convenience of rest area patrons by reducing fire ant populations in core areas of activity. Several integrated pest management tactics are available.
6. Do not transplant horticultural materials from containers nor plant trees with soil balls without first treating soil with insecticides to kill ants.
7. Inspect and protect electrical equipment from fire ant infestation. Fire ants are attracted to electrical current in enclosed cabinets. Dead ants and debris may cause short circuits and promote fires.
8. Continue establishment of habitat refugia for threatened animals and plants.
9. Consider pesticidal applications on roadbeds along fringe areas of infestation fronts.

Increased safety to patrons of rest areas and other TxDOT areas is an obvious benefit to amelioration of fire ant populations. Tourism industries will especially benefit from less frequent contact by visitors to fire ants. Citizens of Texas are concerned with the spread of fire ants to their regions and attendant economic losses. Losses to agricultural, sporting, and recreational industries affect all citizens.

Research on the red imported fire ant is as dynamic as are the pest populations. Additional work recommended for the TxDOT include (a) protecting electrical equipment from fire ant invasion, (b) intense study of roadways at fringes of fire ant infestation fronts, and (c) population management of fire ants in TxDOT areas with high human contact.

Author's Disclaimer

The contents of this report reflect the views of the authors, who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official view or policies of the U.S. Department of Transportation, Federal Highway Commission, or the Texas Department of Transportation. This report does not constitute a standard, specification, or regulation.

Patent Disclaimer

There is not invention or discovery conceived or first actually reduced to practice in the course of or under this contract, including any art, method, process, machine, manufacture, design or composition of matter, or any new useful improvement thereof, or any variety of plant, which is or may be patentable under the patent laws of the United States of America or any foreign country.

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Table of Contents

List of Tables

CHAPTER 1. INTRODUCTION AND BACKGROUND.

1.1 Introduction of the Red Imported Fire Ant into North America.

The genus *Solenopsis* (Hymenoptera: Formicidae) originated in the New World tropics during the Tertiary period (Wilson 1986). *Solenopsis invicta* Buren, the red imported fire ant (RIFA), is native to the floodplains of the Paraguay River and its tributaries in Brazil and Paraguay and ranges across Uruguay, Argentina, and Bolivia (Lofgren 1986, Wojcik 1986). RIFA has been an important economic pest in North America since its accidental introduction near Mobile, Alabama, between 1933 and 1945 (Buren et al. 1974, Vinson and Sorensen 1986), possibly in soil used as ballast for ships from South America (Vinson and Sorensen 1986, Thompson 1990). Multiple introductions have probably occurred, and RIFA has rapidly invaded the southern and southeastern states of the USA and has extended its range westward into Texas (Lofgren 1986).

1.2 RIFA Spread Into Texas

Since its introduction into Texas in 1953, RIFA has spread quickly across the state by natural dispersal and by human transport. In 1989, RIFA infestation in Texas reached approximately 198,983 sq. km which represented 29% of the state (Cokendolpher and Phillips 1989). By 1991, this pest infested 159 counties in Texas, representing approximately the eastern third of the state (Porter et al. 1991). Currently about 40% of Texas is infested (Vinson 1997). The rate of spread has decreased somewhat as RIFA has encountered the arid regions of western Texas and colder winter temperatures. However, the ant is expected to move into irrigated areas, urban environments, and land along rivers and lakes. The western edge of the Texas infestation also represents this species' most western extent in the USA. Spread into the rest of the southwestern USA and into Pacific Coast states may depend, in part, upon the diligence of Texans.

1.3 RIFA Dispersal

Fire ant populations disperse by mating flights, floating on flood waters, accidental transport on vehicles and farm products, and infesting nursery stock. Winged, virgin females fly into the air to become inseminated by males and may be blown or drift many miles from original infestation sites before settling to the ground to begin new colonies (Markin et al 1971). However, human transport is most significant in long-distance movement (Cokendolpher and Phillips 1989). For example, many isolated infestations can be traced to nursery stock and grass sod shipment from ant-infested to uninfested areas (Summerlin and Green 1977). Multiple queen (polygyne) colonies, rather than monogyne (single-queen colonies), are more likely spread in sod or nursery stock because colony fragments and satellite colonies are much more likely to contain fertile queens (Porter et al. 1991). RIFA has been spread by earth-moving equipment, as witnessed at a construction site in Junction, Texas (M. Trostle, Texas Dept. of Agriculture, personal communication). Infested oil field equipment from Texas was the mode of RIFA invasion of Puerto Rico (HGT, personal observation). Furthermore, newly mated females often have been transported unknowingly hundreds of miles in beds of open pickup trucks, railroad cars, and trailers (Vinson 1997).

1.4 RIFA Colonies

Colonies of RIFA vary greatly in size from several hundred to hundreds of thousands of individuals (Vinson 1997). Two types of colonies have been reported in the USA, single queen (monogyne) and multiple queen (polygyne). Monogyne colonies are very territorial and vigorously protect their mounds and foraging areas, resulting in relatively low mound population densities ($\leq$ a few hundred mounds per acre). Polygyne colonies contain multiple queens. These colonies are much more tolerant of unrelated RIFAs and, therefore, may reach mound population densities up to ten times that of monogyne colonies (Vinson 1997).

Reproductive ants (alates) of both sexes fly into the air under favorable weather conditions and mate while in flight. These mating flights usually occur when the relative humidity is above 80% and the ambient temperature is 24-32° C (Rhoades and Davis 1967, Markin et al. 1971). The months of May through July are the most favorable for these events. Male reproductives die shortly after mating, whereas newly mated females (queens) seek out suitable nesting sites and borrow into the ground. A new queen removes her wings and prepares to lay eggs. A new queen is vulnerable, and few survive to actually produce a viable colony (Whitcomb et al. 1973, Vinson 1997). If a queen does survive, the colony may number into the thousands and contain reproductive ants in as few as six months.

Workers of RIFA respond quickly to disturbance and vigorously defend colonies. Hundreds of ants may cover and sting an intruder, and each ant can deliver multiple stings. Stings produce painful pustules that when scratched may lead to secondary infections. The venom consists mainly of alkaloids and allergic antigens in the protein portion, to which humans may be hypersensitive (Thompson 1990). Multiple stings may lead to death of a victim (Vinson and Sorensen 1986).

1.5 Pest Status

RIFA causes human medical problems (Adams and Lofgren 1982, Clemmer and Serfling 1975, Rhoades et al. 1977), threatens domestic animals (Hunt 1976, Wilson and Eads 1949), endangers wildlife (Mount 1981, Sikes and Arnold 1986, Allen et al. 1994), and damages agricultural crops such as maize, soybeans, potatoes, cabbage, and citrus (Adams et al. 1983, 1988, Apperson and Powell 1983, Banks et al. 1991, Eden and Arant 1949, Lyle and Fortune 1948).

Glancey et al. (1979) reported a 63.4% loss in corn yield potential due to RIFA feeding on corn plants. Approximately 14.5% of soybean yields have been lost to RIFA infestations (Lofgren and Adams 1981), and 50% of eggplant production has been lost (Adams 1983). Smittle et al. (1988) estimated loss to RIFA feeding on young citrus trees in controlled experiments at $750 per ha. RIFA tends aphids and harvests honeydew, thereby increasing aphid populations and disease transmission to plants (Nielson et al. 1971, Reilly and Sterling 1983). In contrast, RIFA is considered a beneficial insect by reducing populations of the sugar cane borer (Reagan et al. 1972), the lone star tick (Harris and Burns 1972), whiteflies (Morrill 1977), alfalfa weevils, and green pea aphids (Morrill 1978).

1.6 RIFA Invasion of Electrical Equipment

RIFA causes economic damage by invading electrical circuitry and equipment. The earliest reports of RIFAs accumulating in and damaging such equipment came from Southwestern Bell Telephone in Galveston, Texas. In September 1939 alone, 83 of 446 subscriber's residential telephone failures were caused by ants (Eagleson 1940). Surveys from June 1985 to August 1988 in Bryan and College Station, Texas, revealed RIFA presence in 75% of Texas Highway Department's signal cabinets (Vinson and MacKay 1990) and damage to 20% of the cabinets (MacKay and Vinson 1990). In the same Texas area, air-conditioner service companies reported that nearly 33% of their repair calls were due to RIFAs "shorting" residential and commercial units (Vinson and MacKay 1990). A 1986 survey of state departments of transportation in Alabama, Florida, Georgia, Louisiana, Mississippi, and Texas confirmed RIFA's status as an economic and maintenance nuisance (MacKay et al. 1989).

Ants accumulate in such large numbers in electrical devices that proper movement of the mechanical portions is prevented in telephone ringers (Eagleson 1940, Little 1984, MacKay et al. 1989, Vinson and MacKay 1990) and in contact connections in signal cabinets (Vinson and MacKay 1990). Ants remove insulative material from wires (Eagleson 1940, Galli and Fernandes 1988, MacKay et al. 1990, MacKay and Vinson

1990) and short apparatuses by physically bridging electrical contacts with their bodies, resulting in electrification by excessive internal current flow (Little 1984, MacKay et al. 1989, Vinson and MacKay 1990). The electrocution of ants during a short-circuit creates large numbers of ant corpses that remain in and around circuitry. Ants often nest inside electrical equipment (MacKay et al. 1989, Vinson and MacKay 1990) and introduce large quantities of soil, food particles, and other debris that may cause damage to equipment through increased humidity and corrosion (Eagelson 1940). In addition to highway and electrical company equipment, household light sockets, televisions, electric fences, well pumps, and airport landing light systems have been destroyed (Jolivet 1986, Little 1984, Vinson and MacKay 1990).

1.7 Economic Status

Diffe et al. (1991) reported monetary impact to an average Georgia household as $35 per year, and total annual impact in Georgia was calculated at $35 million (Bass et al. 1992). Thompson et al. (1994) estimated losses over the entire rural southern states to be $451.8 million. Annual damage (medical, agricultural, wildlife, electrical, and recreational) caused by RIFA in Texas has been estimated at $52.8 million. Using this level of damage, Ervin et al. (1990) performed a benefit-cost analysis for controlling RIFA populations in Texas and found that the ant was an economically damaging pest. Current losses may be significantly higher; in urban costs alone (structural repairs, human medical expenses, etc.) may be $93.2 million annually (Frisbie 1997). Losses to Texas beef cattle industry may be $67.0 million annually (Barr and Drees 1995). Therefore, a conservative estimate of current annual economic losses caused by RIFA in Texas is $300 million (Frisbie 1997).

CHAPTER 2. REVIEW OF PREVIOUS WORK AND STATEMENT OF OBJECTIVES

2.1 Review of Previous Studies

Vinson and Sorensen (1986) speculated that the clearing of highway rights-of-way (ROWs) and then continued disturbance by mowing and maintenance would provide an invitation for increased establishment of RIFAs. ROWs also provide abundant food matter such as dead insects and other animals caused by vehicular traffic.

A survey in Texas for distribution and density of monogyne and polygyne fire ant colonies was made by selecting approximately 700 sites along ROWs of public roads (Porter et al. 1991). The survey included 122 counties within the fire ant quarantine area and 46 counties outside the quarantined area. At each pre-selected site, mound densities were determined from four belt transects, two on each side of the road. One transect on each side was along the outer border of the mowed area; the other was along the inner border adjacent to the road. Each transect was 49.0 m long and 2.6 m wide (area = 127.4 m^2). To measure ant foraging activity, eight baits (sliced hot-dogs) were positioned at 10-pace intervals on each side of the road, and fire ant and other ant numbers were counted. Also, vegetation was categorized at each site.

Monogyne and polygyne colonies were found in a mosaic pattern across the eastern one-third of Texas. Polygyne sites averaged 680 mounds per ha; whereas, monogyne sites averaged 295 mounds per ha, and mean numbers were significantly different. In addition, fire ant populations were probably dense along roadsides because of an edge effect. Mound population densities along edges of roads were not significantly different from mounds along the outer borders of mowed areas. Patterns of mounds in infested areas were generally unrelated to habitat and environmental conditions. For example, mound population densities were not well correlated with heights of grass along ROWs (Porter et al. 1991). However, polygyne colonies were 50% less common than expected along roadsides surrounded by forests. Direction of roads did not affect total numbers of mounds at sites nor how many mounds were on each side of roads. Porter et al. (1991) concluded that gross differences in solar exposure resulting from changes in road direction did not affect population densities of fire ant mounds along Texas roadsides.

Roadside sites were surveyed in the southeastern USA states of Florida, Georgia, Alabama, Mississippi, and Louisiana and were compared to RIFA populations in central Brazil (Porter et al. 1992) using the same procedures of Porter et al. (1991). Fire ant mound population densities in the USA were nearly six times those in Brazil. In the USA, polygyne sites averaged 544 mounds per ha, and monogyne sites averaged 170 mounds per ha. Roadsides in Brazil were generally not mowed; consequently, grass at sites in Brazil were about twice as high as in the USA. However, no correlations were found between height of grass and mound population density among sites in either country. No significant relationships were found in several other environmental variables as well.

Previous work dealing with the widespread distribution of RIFA used the highway ROWs (Porter et al. 1991; 1992) but did not include adjacent land. Correlations with habitat information previously have been limited to height of grass in ROWs, presence of nearby trees, grade of roadway (slope), soil type, and temperature.

2.2 Texas Department of Transportation Concerns

The Texas Department of Transportation (TxDOT) has been concerned that maintenance activities along highway ROWs has encouraged spread and establishment of RIFA in Texas. Clearing of ROWs, continued disturbance by mowing and maintenance, and bountiful food materials (litter, dead insects, and other animals) may predispose ROW sites to RIFA invasion (Vinson and Sorensen (1986). ROWs then could become conduits from which RIFAs would invade nearby habitats and disperse across the state.

2.3 Study Objective

The objective of this study was to determine if highway ROWs harbored populations densities of RIFA that were significantly different from those populations densities in adjacent lands. Significantly greater population densities of ROWs would indicate more suitable habitats for RIFA establishment and, consequently, a source from which adjacent properties could be infested. Conversely, significantly larger populations in adjacent areas would suggest: 1) more suitable habitats with well established populations, 2) sources of infestation for TxDOT properties, and/or 3) ineffective RIFA population control tactics.

CHAPTER 3. TRANSECT STUDY

RIFA populations were evaluated by an extensive sampling program along Texas highways and adjacent lands. Transect methodology was used to compare infestations across various habitat, geographic, and environmental gradients. Transects crossed through areas where RIFAs had been established for varying time periods in Texas. One north-south transect and two east-west transects through various Texas landscapes were sampled for RIFA populations and for ecological factors that may have impacted ants. Location of sites surveyed, date and temperatures are give in Appendix A.

3.1 Research Approach and Procedures

Transects were established during May and June 1996. Transects were chosen by following state-maintained highways which transversed diverse vegetational areas of Texas (Hatch and Pluher 1993). Highways were of similar size, traffic intensity, and maintenance regimes. Along each transect, and at approximately 80 km (50 mile) intervals, sites were evaluated. The north-south transect extended from north of Paris, Texas, on Highway 271 southward along Highways 19, 69, 59, and 288 to north of Lake Jackson, Texas. The southern east-west transect stretched from China, Texas, on Highway 90 westward along Highway 290 through Brenham, Giddings, Elgin, Austin, and Fredericksburg, and south to Junction, Texas, on Highway 377. The northern east-west transect ran from west of Texarkana on Highway 67, westward through Omaha, Mount Vernon, and Greenville, and along Highway 380 through Denton, Decator, and Runaway Bay.

Sites were selected on the basis of similarity in habitat between ROWs and adjacent lands to provide uniformity for statistical analysis. Adjacent lands were of various usage types, but were consistently similar in that cultivated lands were avoided as were parking lots, and recreational areas. Adjacent lands were typically grassland pastures, and cultivated fields, parking lots, and recreational areas were avoided. Adjacent, therefore, refers to a tract of land immediately adjacent to ROWs, "across the fence", and outside the maintenance regime of the Texas Department of Transportation. Sites were identified (Appendix A) using both mileage details and global positioning (Geo Explorer, Trimble Navigation Limited, Sunnyvale, California).

At each site, numbers of RIFA mounds were determined in three 0.10 ha plots in ROWs and in the adjacent property. Mounds were rated for vitality following Harlan et al. (1981); then for statistical purposes, ratings were converted to the weighted system of Lofgren and Williams (1982). If a ROW site was too narrow to use a 17.85 m. rope-and-stake, circular plot method, the width of the site was measured, and 0.10 ha was divided by the width to determine the length needed to equal the standardized area of 0.10 ha. Notations were made of the number of RIFA mounds in ROWs which were in contact with the roadbed ($\leq$ 1.0 m of pavement). Typically, widths of ROWs were 10.0 meters; therefore, numbers of roadbed mounds to the remainder of ROWs mounds were expected to be in the ratio of 1:9. This hypothesis was tested using Chi-square analysis. Chi-square test results are tabulated in Appendix A (Table A.1, A.2, A.3).

Each site had two treatments. The highway ROW was one treatment, and the adjacent land was the second treatment. Mean number of RIFA mounds in each treatment at each site was calculated and used in statistical analysis, and mean vitality ratings were calculated in each of the two treatment areas. Comparisons between the two areas were made using the Student's t-test (critical value; $P \leq 0.05$). Statistical analyses was done using StatWorks: Statistics with Graphics for Macintosh (Rafferty et al. 1985) computer software. Numbers for each transect were also pooled and evaluated by treatment (between location) and ANOVA was used to test for differences among transects. All 28 sites were pooled together and Student's t-test used to test for statistical differences between locations. Data were tested for normality using Kolmogorov-Smirnov one-sample test and were found to be normally distributed.

Bait cups, containing a protein bait (vienna sausage, Armour, Dial Corp., Phoenix, AZ), were placed in the field for approximately one hour. Cups were arrayed in three transects of 10 cups each, with 3.0 m between each cup and 10 m between each transect. These transects crossed through 0.10 ha. circles that were surveyed for RIFA mound population density at each transect site. Both ROWs and the adjacent land area were sampled at each site.

Upon collection, bait cups were capped and stored on ice until specimens were identified and counted in the laboratory. Specimens were identified as either RIFA or other species of ants and were sorted and counted. Data represented recruitment of ants to the food source and provided an index of relative abundance of ants.

Bait cup numbers provided mean numbers of RIFA per cup, per site, and per transect. Mean numbers of other ants were also calculated. For each site the Student's t-test was used to compare ROWs with the adjacent land (critical value; $P \leq 0.05$). In the same manner as were the mound densities, the transect sites were pooled for comparisons, and all 28 sites were pooled for further evaluation.

Vegetation at each site was analyzed using the line-transect method for percent cover and was cataloged by physiognomic and structural descriptions (Kent and Coker 1992). Percent cover was recorded to the nearest five percent in each of the following classifications: bare ground, leaf litter, grass (vegetation 0.0-0.8 m in height), field (vegetation 0.8-2.0m in height), scrub (vegetation 2.0-8.0 m in height), and canopy (vegetation over 8.0 m in height). Line transects ran through the 0.10 ha circles. Six vegetation transects were surveyed at each location, each 20 m in length; three in the ROW and three in the adjacent land. Regression analysis was used to determine correlations between the numbers of RIFA mounds and various vegetational characteristics measured at each site. Cricket Graph (version 1.3.2) computer software was used in the regression analysis (Rafferty and Norling 1986).

Temperature at the soil surface and air temperature were measured at each site. Survey work at each site took approximately two hours to complete.

3.2 Transect Findings

Data at each transect varied with no obvious pattern emerging. RIFA mound densities varied from 0.0 mounds per 0.1 ha at some sites to 93.0 mounds per 0.1 ha at one site in northeastern Texas. Recruitment of foragers to bait cups varied similarly. Vegetation was more consistent because of the research approach used to select study sites.

3.2.1 North-to-South Transect

a. Mound Population Densities

The population density of RIFA mounds at 11 sites ranged from 0.0 to a high of 74.3 mounds per 0.10 ha in the ROW at site 4. In the adjacent land area the density of mounds ranged from 2.7 (site #11) to 65.7 (site #6) mounds per 0.10 ha. Using Student's t-test, significant differences between mean densities were detected at four sites (Table 1). At sites 3 and 4, significantly more RIFA mounds were found in the ROW than in the adjacent land. However, at sites 8 and 11 significantly more RIFA mounds were found in the adjacent land.

Numbers of RIFA mounds in ROWs were apportioned into roadbed ($\leq$ 1.0 m from edge of paved roadway) and into remainder (> 1.0 m from edge of paved roadway; Table 3.1.). Significantly more mounds than expected were located within the roadbed at seven of the eleven sites along this transect (Chi-square analysis:expected 1:9 ratio; critical value $P \leq 0.05$; Table 1).

b. Vitality Ratings of Mounds

All mounds found along this transect contained dealated reproductives, eggs, and brood; therefore, all mounds rated 5 or above (Lofgren and Williams 1982). The mean mound vitality ratings ranged from 0.0 at locations where no RIFA mounds were located

Table 3.1. RIFA mound densities along the north-to-south transect through Texas (May 1996).

	Mean number of mounds [ab]		Total number of mounds [cd]	
Site[e]	Right-of-way	Adjacent	Roadbed	Remainder
1	37.7a	11.7a	26.0c	87.0d
2	19.7a	40.7a	30.0c	29.0d
3	54.3a	34.3b	69.0c	94.0d
4	74.3a	28.7b	80.0c	143.0d
5	52.0a	31.0a	36.0c	120.0d
6	49.7a	65.7a	17.0c	132.0c
7	5.3a	12.3a	7.0c	9.0d
8	25.0a	58.3b	23.0c	52.0d
9	11.7a	16.7a	4.0c	31.0c
10	2.3a	7.7a	0.0c	7.0c
11	0.0a	2.7b	0.0c	0.0c

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW $\leq$ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Numbers within site followed by different letters are significantly different from the expected 1:9 ratio (Chi-square test; critical value $P \leq 0.05$).

[e] Site 1 = northern-most site; site 11 = southern-most site.

Table 3.2. RIFA mound vitality ratings along the north-to-south transect through Texas (May 1996).

	Mean mound vitality ratings[ab]		Mean ROW mound vitality ratings [bc]	
Site[d]	Right-of-way	Adjacent	Roadbed	Remainder
1	14.0a	13.4a	11.6c	14.9d
2	14.5a	20.3b	12.8c	17.2c
3	16.5a	16.4a	15.1c	17.7c
4	17.8a	15.6a	16.1c	18.9d
5	17.6a	19.0a	15.2c	18.1d
6	16.1a	16.8a	12.7c	16.6d
7	20.1a	22.8a	15.2c	18.8c
8	17.3a	20.7b	15.4c	18.1c
9	16.3a	18.3a	12.5c	16.5c
10	10.5a	21.3a	0.0c	10.5c
11	0.0a	17.9b	0.0c	0.0c

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Site 1 = northernmost site; site 11 = southernmost site.

(site 11, ROW) to 22.8 at site 7 in the adjacent area (Table 3.2). Vitality ratings were significantly greater in these sites adjacent to ROWs. At site 11, mounds were not found along the highway ROW, and no mounds were found on the roadbed at site 10. Within ROW sites, mounds along roadbeds were significantly smaller at four separate sites but were not different from other ROW mounds at seven sites (Table 3.2.).

c. Bait Cup Collections

Mean numbers of RIFAs per cup varied from 0.9 ants to 283.0 ants. Significantly more RIFA were collected in bait cups in adjacent areas at sites 2, 4, 5, and 10 (Table 3.3.). Considering the numbers of other species of ants, only at site 10 was a significant difference detected between ROW and adjacent locations. A trend in bait cup collections was apparent. Where few RIFAs were collected, greater numbers of ants of other species were collected.

d. Vegetational Analysis

The north-to-south transect along highways crossed through four different vegetational regions of Texas (Hatch and Pluher 1993)which provided diversified examination of RIFA habitat. At the most northern point, the transect was initiated in the post oak-savannah region. The transect also crossed through pineywood, blackland prairie, and gulf prairie vegetational regions. Obviously, highway ROW sites differed from vegetational region characteristics because of highway construction and maintenance. Also, sites were chosen where grassland pastures were found at immediately adjacent areas. Therefore, vegetation along the transect was dominated by grasses less than 0.8 m in height, in both the ROW and the adjacent land. At all eleven sites, ROWs and adjacent lands were of similar composition in the amounts of cover. Only one site in the ROWs contained less than 75% grass (site #4, Table 3.4). No ROW site contained more than 5% bare ground, and only four sites had this characteristic.

In adjacent land areas, five sites contained less than 75% grass (sites #1, 4, 5, 8, 11, Table 3.4). Two of the adjacent sites contained more than 5% bare ground (sites 4 and 5), while two contained 5% bare ground (sites 7 and 9).

Table 3.3. RIFA and non-RIFA species in bait cups along the north-to-south through Texas (May 1996).

Site[c]	Mean number of RIFA[ab]		Mean number of non-RIFA species[ab]	
	Right-of-way	Adjacent	Right-of-way	Adjacent
1	3.4a	0.9a	53.8a	62.8a
2	92.3a	170.3b	0.8a	0.2a
3	184.2a	192.7a	3.6a	0.0a
4	216.1a	283.4b	0.0a	0.0a
5	29.4a	78.5b	17.6a	3.5a
6	10.7a	26.0a	0.0a	0.0a
7	236.2a	217.0a	10.9a	8.2a
8	123.4a	159.5a	0.0a	0.0a
9	44.2a	41.2a	0.9a	0.0a
10	1.5a	205.3b	67.6a	25.5b
11	101.2a	98.3a	72.3a	2.7a

[a]Mean of three transects of ten cups each (n=30); adjacent = land adjacent to ROW.

[b]Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=29).

[c] Site 1 = northernmost site; site 11 = southernmost site.

Table 3.4. Vegetational characteristics along the north-to-south transect through Texas (May 1996).

	Percent cover in ROW[a]				Percent cover in adjacent[a]			
Site[b]	Bare	Litter	Grass	Field	Bare	Litter	Grass	Field
1	0	15	85	0	0	30	70	0
2	0	0	100	0	0	0	100	0
3	0	0	90	10	0	0	90	10
4	5	10	40	45	30	10	55	5
5	5	20	75	0	30	5	65	0
6	0	0	100	0	0	0	100	0
7	0	0	90	10	5	5	90	0
8	0	0	95	5	0	95	0	5
9	5	5	90	0	5	5	80	10
10	5	5	95	0	0	0	100	0
11	0	10	80	10	0	0	35	65

[a] Mean of three 20.0 m transects in each location (ROW and adjacent; adjacent = land adjacent to ROW) per site. Bare = no ground cover present; Litter = leaf litter present; Grass = vegetation 0.0-0.8 m in height; Field = vegetation 0.8-2.0 m in height.

[b] Site 1 = northernmost site; site 11 = southernmost site.

3.2.2 Southern East-to-West Transect

a. Mound Population Densities

RIFA mound population densities along the southern east-to-west transect ranged from 0.0 to 54.0 mounds in individual 0.10 ha circles. Five sites had significant differences between mean numbers of mounds (Student's t-test; $P \leq 0.05$; df=4) in ROW and the adjacent land (Table 3.5). Four of these sites contained significantly greater mound densities in the ROW (sites 1, 3, 7, and 8), whereas only one site (site 4) contained a greater density of mounds in the adjacent area.

Within ROWs mound densities in roadbeds ranged from 0.0 to 8.3 mounds per ha, and 0.0 to 32.3 mounds per ha occurred in the remainder (Table 3.5). Within ROWs, four sites (2, 6, 8, 9) had more than the expected numbers of mounds in roadbeds, and two sites (4, 5) had fewer than expected mounds in roadbeds (Chi-square test; $P \leq 0.05$; expected ratio 1:9).

b. Vitality Ratings of Mounds

Mean mound ratings along the southern east-to-west transect varied from 0.0 (no mounds present) to 23.2 (Table 3.6). Two sites had significantly different vitality ratings between the ROW and the adjacent area (Student's t-test; $P \leq 0.05$). Both of these sites had greater vitality ratings in the adjacent areas than in ROWs. Within ROWs areas, only one location (site 2) had a significantly higher mean mound vitality rating than the remainder of the ROW.

c. Bait Cup Collections

Significantly more RIFAs were collected in bait cups at sites 2 and 4 (Student's t-test; $P \leq 0.05$). More ants of other species were collected in ROWs at sites 4 and 10 (Table 3.7). At site 10, no RIFAs were collected, and other ant species were very active in the highway ROW.

d. Vegetational Analysis

The southern east-to-west transect crossed through four vegetational zones (Hatch and Pluher 1993: Gulf Prairies zone, Post Oak savannah, Blackland Prairies, and into the Edwards Plateau (east to west). Highway transect sites were dominated by the grass layer (vegetation 0.0 m-0.8 m in height) with all but two of the ROW areas having at least 70% cover as grass (Table 3.8). In the adjacent areas, the cover also was dominated by a grass layer with eight sites containing at least 65% grass cover.

Site 10 had less than 5% ground cover, and more than 95% was bare ground in the adjacent area (Table 3.8) at the site near Junction, Texas. Along this transect, the grass layer was plentiful at most sites. One site contained less than 70% grass in the ROW (site 5), and three sites consisted of 100% grass in ROWs.

Table 3.5. RIFA mound densities along the southern east-to-west transect through Texas (May 1996).

	Mean number of mounds[ab]		Total numbers of mounds[cd]	
Site[e]	Right-of-way	Adjacent	Roadbed	Remainder
1	10.0a	5.7b	3.0c	27.0c
2	27.0a	37.3a	25.0c	56.0d
3	10.0a	3.0b	6.0c	24.0c
4	15.0a	40.7b	2.0c	43.0c
5	24.7a	30.0a	2.0c	72.0c
6	40.3a	18.3a	24.0c	97.0d
7	9.7a	1.7b	3.0c	26.0c
8	32.0a	16.7b	21.0c	75.0d
9	7.3a	1.7a	11.0c	11.0d
10	0.0a	0.0a	0.0c	0.0c

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Numbers within site followed by different letters are significantly different from the expected 1:9 ratio (Chi-square test; critical value $P \leq 0.05$).

[e] Site 1 = easternmost site; site 10 = westernmost site.

Table 3.6. RIFA mound vitality ratings along the southern east-to-west transect through Texas (May 1996).

	Mean mound vitality ratings[ab]		Mean ROW mound vitality ratings[bc]	
Site[d]	Right-of-way	Adjacent	Roadbed	Remainder
1	18.6a	21.2a	6.1c	18.5c
2	18.8a	19.8b	16.9c	19.6d
3	18.5a	23.2b	9.2c	19.6c
4	18.2a	15.8a	5.8c	0.0c
5	17.7a	16.2a	6.7c	17.9c
6	19.0a	21.0a	17.4c	19.1c
7	14.0a	4.0a	8.3c	14.2c
8	14.9a	16.0a	9.6c	15.2c
9	12.3a	13.8a	12.1c	12.0c
10	0.0[e]	0.0[e]	0.0[e]	0.0[e]

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Site 1 = easternmost site; site 10 = westernmost site.

Table 3.7. RIFA and non-RIFA species in bait cups along the southern east-to-west transect through Texas (May 1996).

Site[c]	Mean number of RIFA[ab]		Mean number of non-RIFA species[ab]	
	Right-of-way	Adjacent	Right-of-way	Adjacent
1	2.4a	1.0a	14.6c	0.0c
2	52.4a	160.2b	0.0c	0.0c
3	12.6a	7.1a	6.7c	10.7c
4	3.7a	92.5b	14.2c	0.1d
5	0.2a	0.0a	0.0c	0.0c
6	10.3a	0.3a	0.0c	9.5c
7	0.8a	0.0a	0.0c	0.0c
8	5.3a	0.0a	0.0c	0.0c
9	0.0a	0.0a	14.0c	25.2c
10	0.0a	0.0a	158.5c	78.8d

[a]Mean of three transects of ten cups each (n=30); adjacent = land adjacent to ROW.

[b]Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=29).

[c] Site 1 = easternmost site; site 10 = westernmost site.

Table 3.8. Vegetational characteristics along the southern east-to-west transect across Texas (May 1996).

	Percent cover in ROW[a]					Percent cover in adjacent[a]				
Site[b c]	Bare	Litter	Grass	Field	Canopy	Bare	Litter	Grass	Field	Canopy
1	0	5	0	95	0	0	10	90	0	0
2	5	25	70	0	0	0	100	0	0	0
3	0	0	100	0	0	15	20	65	0	0
4	0	0	100	0	0	0	0	100	0	0
5	0	5	45	50	0	5	5	70	20	0
6	5	5	85	5	0	5	5	80	10	0
7	10	0	80	10	0	35	0	65	0	0
8	0	0	100	0	5	0	0	100	0	0
9	-	-	-	-	-	-	-	-	-	-
10	20	0	80	0	0	95	0	5	0	0

[a] Mean of three 20.0 m transects in each location (ROW and adjacent; adjacent = land adjacent to ROW) per site. Bare = no ground cover present; Litter = leaf litter present; Grass = vegetation 0.0-0.8 m in height; Field = vegetation 0.8-2.0 m in height; Canopy = vegetation greater than 8.0 m in height. Because the canopy layer may overlap a shorter layer, sites with canopy cover may have more than 100% cover in this table.

[b] Site 1 = easternmost site; site 10 = westernmost site

[c] Transects of vegetation were not calculated at site 9 (see text).

3.2.3 Northern East-to-West Transect

a. Mound Population Densities

At all sites along the northern east-to-west transect, RIFA mounds were present in at least one treatment (ROW or adjacent). RIFA mound densities ranged from 0.0 to 93.0 mounds per individual, 0.10 ha circles across this region of Texas. Mean mound densities were significantly different between ROWs and adjacent areas at three sites (Table 3.9). Adjacent areas in sites 1, 2, and 4 each had significantly more RIFA mounds than in ROW plots (Student's t-test; $P \leq 0.05$). Within ROWs, roadbeds had significantly more mounds than expected at four (Chi-squared test; $P \leq 0.05$).

b. Vitality Ratings of Mounds

All mounds along this transect contained dealated reproductives, eggs, and brood; therefore, individually these mounds all rated at least a five on the weighted scale of Lofgren and Williams (1982). Mean mound ratings per location ranged from 5.7 in one roadbed area to 22.2 in an adjacent area. The first three sites contained significantly more RIFA mounds in adjacent areas than did ROW areas (Student's t-test; $P \leq 0.05$; Table 3.10). Vitality ratings of ROW mounds in roadbeds and remaining areas were not significantly different.

c. Bait Cup Collections

Five sites along the northern east-to-west transect had significantly different mean numbers of RIFAs in bait cup collections between ROWs and the adjacent areas (Student's t-test; $P \leq 0.05$; Table 3.11). Three sites contained significantly more RIFA per cup in ROWs; whereas, two sites contained more in adjacent locations. Differences among other ant species per cup varied. Site 3 had significantly more in the adjacent area, and site 4 had more other ant species in the ROW. Again, data indicate that ants of other species foraged more actively when RIFA foragers were in fewer number.

d. Vegetational Analysis

The northern east-to-west transect crossed through four vegetational zones. The transect began in the northeastern piney woods, continued westerly through the post oak savannah and the blackland prairies, and ended in the cross timbers and prairies zone. Along this transect the adjacent sites all contained 100% vegetative cover as the grass layer (Table 3.12). Two sites in the adjacent areas also included 5% canopy layer. The ROWs had varying amounts of ground cover consisting of a grass layer and the taller field layer. At no site was bare ground or litter recorded in the ROW nor the adjacent.

Table 3.9. RIFA mound densities along the northern east-to-west transect through (May 1996).

	Mean number of mounds[ab]		Total number of mounds[cd]	
Site[e]	Right-of-way	Adjacent	Roadbed	Remainder
1	17.0a	39.0b	22.0c	29.0d
2	32.2a	85.0b	24.0c	65.0d
3	2.0a	0.0a	1.0c	5.0c
4	11.3a	25.7b	13.0c	29.0d
5	4.7a	2.7a	7.0c	5.0d
6	9.0a	5.3a	7.0c	20.0d
7	11.3a	21.7a	7.0c	27.0c

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Numbers within site followed by different letters are significantly different from the expected 1:9 ratio (Chi-square test; critical value $P \leq 0.05$).

[e] Site 1 = easternmost site; site 7 = westernmost site.

Table 3.10. RIFA mound vitality ratings along the northern east-to-west transect through Texas (May 1996).

	Mean mound vitality ratings[ab]		Mean ROW mound vitality ratings[bc]	
Site[d]	ROW	Adjacent	Roadbed	Remainder
1	17.8a	20.4b	16.0c	19.4c
2	17.8a	15.0b	16.7c	18.1c
3	17.9a	0.0b	8.3c	6.3c
4	18.3a	17.3a	5.7c	18.2c
5	18.1a	22.2a	17.1c	19.4c
6	20.5a	18.4a	20.4c	20.6c
7	14.3a	15.0a	17.1c	18.0c

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Means within site followed by different letters are significantly different (Student's t-test, $P \leq 0.05$; df=4).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Site 1 = easternmost site; site 7 = westernmost site.

Table 3.11. RIFA and non-RIFA species in bait cup along the northern east-to-west transect through Texas (May 1996).

Site[c]	Mean number of RIFA[ab]		Mean number of non-RIFA species[ab]	
	Right-of-way	Adjacent	Right-of-way	Adjacent
1	77.0a	155.9b	1.4c	0.0c
2	181.0a	202.5a	8.8c	1.7c
3	189.9a	8.0b	0.0c	116.2d
4	155.8a	252.2b	20.7c	0.1d
5	38.8a	5.3b	49.2c	18.7c
6	134.0a	92.8b	17.7c	16.3c
7	230.0a	213.4a	0.0c	0.0c

[a]Mean of three transects of ten cups each (n=30); right-of-way = the area from the pavement edge to the outside margin of the TxDOT maintained roadsie; adjacent = land adjacent to highway right-of-way.

[b]Means within site followed by different letters are significantly different (Student's t-test, P $\leq$0.05; df=29).

[c] Site 1 = easternmost site; site 7 = westernmost site.

Table 3.12. Vegetational characteristics along the northern east-to-west transect through Texas (May 1996).

	Percent cover in ROW[ab]					Mean percent cover in adjacent[ac]				
Site[b]	Bare	Litter	Grass	Field	Canopy	Bare	Litter	Grass	Field	Canopy
1	0	0	90	10	0	0	0	100	0	0
2	0	0	5	95	0	0	0	100	0	0
3	0	0	95	5	5	0	0	100	0	5
4	0	0	0	100	0	0	0	100	0	0
5	0	0	100	0	0	0	0	100	0	0
6	0	0	95	5	5	0	0	100	0	0
7	0	0	100	0	0	0	0	100	0	5

[a] Mean of three 20.0 m transects in each location (ROW and adjacent) per site. Bare = no ground cover present; Litter = leaf litter present; Grass = vegetation 0.0-0.8 m in height; Field = vegetation 0.8-2.0 m in height; Canopy = vegetation greater than 8.0 m in height. . Because the canopy layer may overlap a shorter layer, sites with canopy cover may have more than 100% cover in this table.

[b] Right-of-way = the area from the pavement edge to the outside margin of the TxDOT maintained roadsie.

[c] Adjacent = land adjacent to highway right-of-way.

[d] Site 1 = easternmost site; site 7 = westernmost site.

3.3 Pooled Transect Data

Although data were pooled by transect and among transects, analyses did not reveal significant differences in mound densities (Table 3.13), there were numerical differences. The highest numerical mound density for ROW areas was that of the north-to-south transect which was 31.3 mounds per 0.1 ha (Table 3.13). The highest mean mound density in adjacent areas was also along the north-to-south transect (28.8 mounds per 0.1 ha). ANOVA revealed no significant differences among transects in mound densities in either ROWs or adjacent areas. Pooling all 28 sites did not detect a significant difference in mound densities between locations (Student's t-test, $P \leq 0.05$; Table 3.13).

Significant differences between roadbeds and remainder of ROWs were detected in each transect. Along all three transects, significantly more mounds than expected (Chi-square test, critical value $P \leq 0.05$, expected ratio 1:9, roadbed:remainder) were found in the roadbeds. Analysis of pooled data from all 28 sites revealed significantly more mounds than expected in roadbeds (Chi-square test, critical value $P \leq 0.05$, expected ratio 1:9, roadbed:remainder; Table 3.13). Among transects, numbers of mounds in roadbeds and in the remainder of ROWs were not significantly different.

Student's t-test detected significantly more mounds in roadbeds than in the adjacent areas ($P \leq 0.05$). In the adjacent areas, the mean number of mounds per 0.1 ha was 23.0 (±21.7 mounds; n=28); whereas in the roadbeds, the mean was 55.9 mounds (±64.9 mounds; n=28; Table 3.14). Simple linear regression was used in an effort to determine if RIFA mound population density in the roadbed was predictive of RIFA mound population densities in the adjacent areas. In the regression model, the coefficient of determination (r^2) was 0.2, indicating little of the change in mound density in the adjacent areas could be explained by the changes in mound density in the roadbed. A positive relationship between the roadbed mound density and the mound density in the adjacent area existed (slope of model = 0.13; y-intercept at 15.7).

The mean vitality ratings for each transect were not significantly different between locations (ROW vs. adjacent or roadbed vs. remainder of ROW (Table 3.15). When data from all 28 sites were pooled together, the reminder of ROWs had a significantly higher mean vitality rating than did the roadbed (Student's t-test, $P \leq 0.05$). Mean vitality ratings ranged from 14.6 to 18.4 in ROWs and adjacent areas. In roadbeds, vitality ratings were numerically lower than the remainder of ROWs, ranging from 9.2 to 14.5.

Table 3.13. Mound densities for all transects through Texas (May-June 1996).

	Mean number of mounds[ab]		Total number of mounds[cd]	
Transect	Right-of-way	Adjacent	Roadbed	Remainder
North-to-South	31.3Aa	28.2Aa	292.0Cc	704.0Cd
Southern east-to-west	17.6Aa	15.5Aa	97.0Cc	431.0Cd
Northern east-to-west	12.5Aa	25.6Aa	81.0Cc	180.0Cd
All sites pooled[e]	20.5a	23.1a	470.0c	1315.0d

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Different small case letters within rows are significantly different (Student's t-test, $P \leq 0.05$, df=4). Different upper case letters within columns are significantly different (ANOVA, LSD, $P \leq 0.05$).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Numbers within site followed by different letters are significantly different from the expected 1:9 ratio (Chi-square test; critical value $P \leq 0.05$).

[e] Means for each site (28) from all transects (3) were pooled.

Table 3.14. Pooled mound densities (RIFA mounds per 0.1 ha) for roadbed and adjacent areas (May-June 1996).

	Mean number of mounds[a]	
Transect	Roadbed[b]	Adjacent
North-to-south	88.5Aa	28.2Ab
Southern east-to-west	32.2Aa	15.5Aa
Northern east-to-west	38.5Aa	25.6Ab
All sites pooled[c]	55.9a	23.0b

[a] Different small case letters within rows are significantly different (Student's t-test, $P \leq 0.05$, df=4). Different upper case letters within columns are significantly different (ANOVA, LSD, $P \leq 0.05$).

[b] The mean mound density of three roadbed grids (number of mounds per 0.01 ha) at each site was multiplied by 10 to compare with the adjacent density (number of mounds per 0.1 ha); roadbed = ROW≤1.0 m from edge of pavement; adjacent = land adjacent to the ROW.

[c] Means for each site (n=28) from all transects (3) were pooled.

Table 3.15. Vitality ratings of mounds for all transects through Texas (May - June 1996).

	Mean mound vitality ratings[ab]		Mean ROW mound vitality ratings[cd]	
Transect	Right-of-way[a]	Adjacent	Roadbed	Remainder
North-to-South	14.6Aa	18.4Aa	11.5Cc	15.2Cc
Southern east-to-west	15.2Aa	15.1Aa	9.2Cc	13.6Cc
Northern east-to-west	17.8Aa	15.5Aa	14.5Cc	17.1Cc
All sites pooled	15.6a	16.5a	10.8c	15.0d

[a] Mean of three 0.1 ha grids at each site; adjacent = land adjacent to ROW.

[b] Different small case letters within rows are significantly different (Student's t-test, $P \leq 0.05$, df=4). Different upper case letters within columns are significantly different (ANOVA, LSD, $P \leq 0.05$).

[c] Roadbed = ROW ≤ 1.0 m from edge of pavement; Remainder = remaining portion of ROW > 1.0 meter from edge of pavement.

[d] Numbers within site followed by different letters are significantly different from the expected 1:9 ratio (Chi-square test; critical value $P \leq 0.05$).

Table 3.16. Number of RIFA and non-RIFA species in bait cups by (May-June 1996).

	Mean number of RIFA[abc]		Mean number of non-RIFA species[abc]	
Transect	Right-of-way	Adjacent	Right-of-way	Adjacent
North-to-South	95.0Aa	133.9Aa	20.7Cc	9.3Cc
Southern east-to-west	8.8Ba	26.1Ba	20.8Cc	12.4Cc
Northern east-to-west	143.8Aa	132.9Aa	14.0Cc	21.9Cc
All sites pooled	82.3a	97.6a	18.5c	14.6c

[a] Right-of-way= the area from the pavement edge to the outside margin of the TxDOT maintained roadsie; Adjacent= land immediately adjacent to the highway right-of-way.

[b] Different small case letters within rows are significantly different (Student's t-test, $P \leq 0.05$, df=4).

[c] Different upper case latters within columns are significantly different (ANOVA, LSD, $P \leq 0.05$).

Analyses of bait cup collections within each transect did not detect any significant differences between locations on transects (Student's t-test, $P \leq 0.05$). The southern east-to-west transect did have significantly fewer ants per cup than did the other two transects (ANOVA, $P \leq 0.05$, Table 3.16).

Scatter plots were constructed to compare vegetational with RIFA indices (mound densities and RIFAs per bait cup), and a liner regression model was fit to each scatter plot. Coefficients of determination measured the proportion RIFA indices (mound density or RIFA/cup) as determined by the variation in vegetation. This analysis was done for mounds in ROWs and also in the adjacent, versus each layer of vegetation. Data for bare ground, leaf litter, scrub and canopy layers is not reported here due to the limited number of data points. Not all sites contained all of these layers, because grass and field were the dominant layers they are reported here. This regression analysis was also done for mean numbers of RIFA per bait cup in each location (ROW and adjacent).

The best fitting line for the regression model of RIFA mounds and grass produced a coefficient of determination (r^2) value of 0.111 for ROWs areas (Table 3.17). In the adjacent areas the best fitting line (mound density versus grass) produced a coefficient of determination value of 0.056. Coefficients of determination for the layer of vegetation up to 2.0 m in height (grass + field layers) produced equally weak models. Plotting the mean numbers of RIFA per cup versus the various vegetational layers also failed to provide further understanding. Plotting the mean number of RIFAs per bait cup against the combination of the grass layer and the field layer (all vegetation up to 2.0 m) provided a coefficient of 0.483 (Table 3.17). Bait cup mean numbers plotted against the grass layer produced a coefficient of determination of 0.020 in ROWs and 0.012 in the adjacent areas (Table 3.17). There seemed to be poor coefficients of determination between RIFA indices and vegetation.

3.4 Discussion

Along Texas highway ROW, red imported fire ants are not more numerous than in the adjacent land areas. Highway ROW are not considered conduits of invasion in RIFA infested areas of Texas. However, establishment of newly mated queens along roadbeds is a problem. Attraction of flying queens to reflective surfaces (Vinson 1997) may direct them to paved road surfaces. Queens will crawl to adjacent roadbeds and find shelter beneath rocks and highway litter such as crushed beverage cans, paper, wood, cardboard, etc. The relative warmth of roadbed thermal mass and moisture availability from condensation and or runoff provide adequate habitat for founding queens. Mortality rates of founding queens is great, perhaps 99%, much of which is caused by foraging ants from established RIFA colonies that are very territorial. Small colonies that do survive find adequate food resources by scavenging animal materials on roadbeds (dead insects and larger animals) and by foraging in nearby mowed ROWs. Porter et al. (1991) reported no differences between the ROW along edges of roads and the outer boarder of the ROW.

Roadbeds may act as barriers for RIFA colonies relocate within ROW. A colony may butt against a roadbed, be prevent moving across paving, and move along the roadbed. As colony are flooded from ROW ditches during rain, they may drift against roadbeds and re-establish on drier ground. This re-establishment may account for high numbers of RIFA mounds on roadbeds.

Table 3.17. Coeffients of determination (r^2) for regression analyses of vegetation and RIFAs from three transects through Texas (May and June 1996).

	Coefficients of Determination			
	RIFA mound density		RIFA per bait cup	
Vegetation	Right-of-way	Adjacent	Right-of-way	Adjacent
Grass[a]	0.111	0.056	0.020	0.012
Field[b]	0.000	0.084	0.049	0.483
Grass + Field[c]	0.020	0.065	0.002	0.483

[a] Grass= vegetational layer 0.0-0.8 m in height; regression model based on all sites in the transect study which contained this layer of vegetation.

[b] Field= vegetational layer 0.8-2.0 m in height; regression model based on all sites in the transect study which contained this layer of vegetation.

[c] Grass + Field= all vegetation 0.0-2.0 m in height; regression model based on all sites in the transect study which contained this layer of vegetation.

CHAPTER 4. MOWING REGIME STUDY

4.1 Research Approach and Procedures

Observations made during the transect study indicated that mowing regimes along Texas highways could affect RIFA population density and dispersal. It was noted that RIFA may be less abundant in areas of tall vegetation than in highly maintained areas.

In numerous areas across the state, TxDOT personnel have established plant and animal refugia that are non-mowed and are set aside as long-term remediation of native habitats that were elsewhere lost to highway development. Personnel from TxDOT identified five sites where non-mowed and adjacent mowed areas could be found (Appendix C.1).

At each site, numbers of RIFA mounds were determined in five 0.10 ha (0.25 acre) plots in the non-mowed and in the adjacent, mowed area. Mounds were rated for vitality following Harlan et al. (1981). For statistical purposes, ratings were converted to the weighted system of Lofgren and Williams (1982). If a ROW site was too narrow to use a 17.85 m rope-and-stake, circular plot method, the width of the site was measured, and 0.10 ha was divided by the width to determine the length needed to equal the standardized area of 0.10 ha.

Each site consisted of two treatments. The non-mowed area was one treatment, and the adjacent mowed ROW was the second treatment. Mean number of RIFA mounds in each treatment at each site was calculated and used in statistical analyses, and mean vitality ratings were calculated in each of the two treatment areas. Comparisons between the two areas were made using the Student's t-test (critical value; $P \leq 0.05$). Statistical analyses was performed using StatWorks: Statistics with Graphics for Macintosh (Rafferty et al. 1985) computer software. Numbers for each transect were also pooled and evaluated by treatment (between location). Data were tested for normality using Kolmogorov-Smirnov one-sample test and were found to be normally distributed.

Bait cups containing a protein bait (vienna sausage, Armour, Dial Corp., Phoenix, AZ), were placed in the field for approximately one hour. Cups were arrayed in three transects of 10 cups each, with 3.0 m between each cup and 10 m between each transect. These transects crossed through 0.10 ha. circles that were surveyed for RIFA mound population density at each transect site. Both the non-mowed and the adjacent mowed area were sampled at each site.

Upon collection, bait cups were capped and stored on ice until specimens were identified and counted in the laboratory. Specimens were identified as either RIFA or other species of ants. Data represented recruitment of ants to the food source and provided an index of relative ant abundance.

Bait cup numbers provided mean numbers of RIFA per cup per site. Mean numbers of other ants were also calculated. For each site the Student's t-test was used to compare the non-mowed with the adjacent mowed area (critical value; $P \leq 0.05$). In the same manner as were the mound densities, the sites were pooled for comparisons.

Vegetation at each site was analyzed using the line-transect method for percent cover and was cataloged by physiognomic and structural descriptions (Kent and Coker 1992). Percent cover was recorded to the nearest five percent in each of the following classifications: bare ground; leaf litter, grass (vegetation 0.0-0.8 m in height), field (vegetation 0.8-2.0m in height), scrub (vegetation 2.0-8.0 m in height), and canopy (vegetation over 8.0 m in height). Line transects ran through the 0.10 ha circles. Six vegetation transects were surveyed at each location, each 20 m in length; three in the non-mowed area and three in the adjacent mowed land. Regression analysis was used to detect correlations between the numbers of RIFA and various vegetational characteristics measured at each site. Cricket Graph (version 1.3.2) computer soft was used in the regression analysis (Rafferty and Norling 1986).

Temperature at the soil surface and air temperature were measured at each site. Survey work at each site took approximately two hours to complete. Field sampling to compare mowed and non-mowed ROW areas was completed in October 1996 (site #1-4) and March 1997 (site #5).

4.2 Findings of Mowing Regime Study

Non-mowed areas had taller vegetation, sometimes a canopy cover, and less ecological disturbance than in the mowed areas. One site had not been mowed for 14 years. Other sites in this study had not been mowed for 2-4 years. In general, the mowed ROWs were similar to ROWs in the transect study, having regular mowing schedules and the associated disturbances typical of highly maintained ROWs.

4.2.1 Mound Densities

Mound densities of the individual circles varied from zero mounds per 0.1 ha circle grid (a mowed location) to as many as 94 mounds per 0.1 ha circle (a non-mowed location). Mean mound densities ranged from 5.6 to 47.2 in the mowed areas (Table 4.1) whereas the non-mowed areas had means between 16.3 and 47.2 mounds per 0.1 ha. Mean mound densities differed significantly (Student's t-test, $P \leq 0.05$) at sites 1 and 2. Site 2 contained more mounds per 0.1 ha in the mowed area, and site 1 contained more mounds in the non-mowed areas.

Table 4.1. RIFA mound densities and vitality ratings at five mowed and non-mowed sites in Texas (May 1996).

	Mean number of mounds[a]		Mean mound vitality ratings[a]	
Site[b]	Mowed	Non-mowed	Mowed	Non-mowed
1	18.2a	40.8b	10.2c	13.6d
2[c]	46.2a	16.3b	13.5c	15.8c
3[d]	35.5a	32.3a	10.9c	11.2c
4	47.2a	27.0a	16.5c	13.0d
5	26.8a	46.4a	15.2c	13.8c
Pooled	34.8a	32.6a	13.3a	13.5a

[a] Mean of five 0.1 ha circle grids at each site (n=5), means followed by different letters within sites are significantly different (Student's t-test, P≤0.0, df=8).

[b] For exact location of each site see Appendix; Figure A.4.

[c] Only four circle grids in the non-mowed due to space limitations; n=4 in non-mowed.

[d] Only four circle grids in each treatment; n=4.

The mean vitality ratings varied from 10.3 to 15.8 with two sites having significant differences between the mowed and non-mowed areas (Student's t-test, $P \leq 0.05$, df=4; Table 4.1). At site 1 mound vitality ratings were significantly greater in the mowed area, whereas at site 4 mound vitality ratings were significantly greater in the mowed area.

4.2.2 Bait Cup Collections

RIFA was collected in bait cups at all five sites. Other ant species were collected at only sites 3 and 5 and these were less than 15 ants per cup. Individual bait cup collections ranged from 0.0 to > 600 RIFA per cup. At sites 3 and 4, significant differences (Table 4.2) in the numbers of RIFA collected in bait cups were detected, site 3 being greater in the mowed and site 4 being greater in the non-mowed area.

4.2.3 Vegetational Analysis

Vegetative cover varied in ROW sites from 90% leaf litter with 10% bare ground (Table 4.3) to sites of 100% grass. A mixture of grass, field, and scrub layers occurred in the non-mowed areas. Four sites in this study contained varying amounts of canopy cover. Site 1 was a highway median which had been unmowed for fourteen years and was dominated by the field layer with some scrub layer present (mean 90% field and 10% scrub; Table 4.3). The non-mowed area of site 3 was characterized by early successional tree species and contained the most canopy cover (mean 45%; Table 4.3).

4.3 Pooled Mowing Regime Data

Mean number of mounds in mowed areas was 34.8 mounds per 0.1 ha, which was not significantly different from the non-mowed mean of 32.6 mounds per 0.1 ha (Student's t-test, $P \leq 0.05$, df=8; Table 4.1). Mean vitality ratings were not significantly different in the areas of study. Pooled bait cup collections were very similar in both mowed and non-mowed areas, showing no significant difference (Student's t-test, $P \leq 0.05$, df=8; Table 4.2). This trend was also found in the counts of ants of other species (no significant differences detected). Regression analysis of RIFA indices compared to vegetational layers did not provide any understanding into RIFA colonization patterns. Regression models produced insignificant coefficients of determination values, possibly due to clumping of data regarding vegetation (Table 4.3).

4.4 Discussion

Mowing activities do not significantly impact RIFA populations. However, the movement of equipment from infested areas to uninfested areas for maintenance activities may facilitate RIFA dispersal.

Table 4.2. Comparison of RIFA numbers collected from bait cups in mowed and non-mowed ROW sites in Texas (October 1996; March 1997).

	Mean number of RIFA[a]		Mean number non-RIFA species[a]	
Site	Mowed	Non-mowed	Mowed	Non-mowed
1	293.0a	281.3a	0.0a	0.0a
2	202.0a	182.4a	0.0a	0.0a
3	186.0a	77.3b	0.6a	14.2a
4	206.0a	257.1b	0.0a	0.0a
5	103.2a	110.4a	0.3a	0.0a
Pooled	198.0a	181.7a	0.2a	2.8a

[a] Mean RIFA and non-RIFA numbers collected from cups baited with sausage at each site (n=30). Means followed by different letters are significantly different (Student's t-test, $P \leq 0.05$, df=58).

Table 4.3. Vegetational characteristics at mowed and non-mowed ROW sites in Texas (October 1996, March 1997).

	Mean percent cover in mowed ROW[a]						Mean percent cover in non-mowed ROW[a]					
Site	Bare	Litter	Grass	Field	Shrub	Canopy	Bare	Litter	Grass	Field	Shrub	Canopy
1	20	0	80	0	0	20	0	0	0	90	10	0
2	0	0	100	0	0	0	0	0	0	100	0	0
3	0	0	0	100	0	0	0	0	40	40	20	45
4	0	0	0	100	0	10	0	0	0	85	15	0
5	10	90	0	0	0	40	0	0	0	100	0	0

[a] Three 20.0 m transects in each location (mowed and non-mowed) per site. Vegetation was classified by stuctural descriptions: Bare = no ground cover; Litter = leaf litter; Grass = vegetation 0.0-0.8 min height; field = vegetation 0.8-2.0 m in height; scrub = vegetation 2.0-8.0 m in height; canopy = vegetation great than 8.0 m in height. Because canopy layer may overlap shorter layers, sites with canopy may have more than 100% cover in this table.

Care should be taken when transporting equipment such as bush hogs, earth movers, etc. to prevent the random spread of RIFA. Equipment should be clean, free of soil buildup and grass debris which could provide temporary cover to RIFA queens or colonies.

In areas of established RIFA infestations, vegetative cover was not found to make a significant difference in RIFA population densities. The areas surveyed in this study may not have provided the variability in vegetation needed to make generalized conclusions regarding RIFA infestations and vegetative cover. The lack of correlation between vegetation and RIFA corroborates the findings of Porter et al. (1991, 1992) in Texas and across the southeastern United States.

CHAPTER 5. TxDOT REST AREA EVALUATION

5.1 Research Approach and Procedures

Evaluation of RIFA infestations at TxDOT rest areas was conducted in March 1997 around the San Antonio, Texas, area. Seven rest areas along interstate highways were evaluated. Rest rooms were considered sites of the most intense human activity at rest areas; therefore, RIFA populations were evaluated in a pattern radiating outward from this center. These highly maintained rest-stop areas were considered the normal maintenance treatment. Adjacent, less used and less maintained areas were considered the control for statistical comparisons.

The sampling program at each rest area consisted of three transects, each consisting of five 9.1 m x 15.2 m quadrats. Two transects radiated in opposite directions from rest room facilities and defined "distance zones" from the rest rooms. The third transect was established in an adjacent, less visited, mowed but less maintained area that was frequently alongside entrance or exit roads from the rest area. Within each transect, RIFA mound population densities were measured and recorded. In addition, three, 10-bait cup transects were established parallel to each mound transect. Cups contained a piece of vienna sausage and were placed three paces apart, and cup transects were ten paces apart. Bait cups were gathered after approximately 45 minutes, labeled, and stored on ice in a cooler for the return trip to the laboratory for identification. Vegetative characteristics, canopy cover, and ground cover were recorded along each transect. Pesticide histories and maintenance records were obtained whenever possible.

Picnic tables, water dispensers, vending, and informational areas were also noted for RIFA activity. RIFA populations in ornamental plant displays and landscaping features were recorded.

5.2 Findings of TxDOT Rest Area Evaluation

Significantly more RIFA mounds were found in relatively less maintained areas in three of the seven TxDOT rest areas surveyed (Table 5.1). For the pooled data, less maintained areas had significantly more mounds per quadrat than did the area immediately surrounding the rest rooms. Insecticide applications to ameliorate RIFA populations were made in rest areas by TxDOT personnel. These treatments were mound type treatments using various insecticides and were apparently effective. No differences in the mean numbers of RIFA mounds per quadrat were detected among the transect quadrats which radiated outward from the rest room facilities (Student's t-test, $P \leq 0.05$). Vitality ratings of mounds in these quadrats were not significantly different (Table 5.1). Numbers of RIFA foragers collected in bait cups were greater in five, less

Table 5.1. RIFA mound densities and vitality ratings at Texas Department of Transportation Rest Areas in Texas (March 1997).

	Mean mound number[ab]		Mean Mound Vitality Ratings[ab]	
Site	Rest Area	Adjacent	Rest Area	Adjacent
1	4.4a	10.8a	11.6c	15.2c
2	13.8a	12.4a	15.5c	16.1c
3	4.9a	11.0b	16.3c	14.4c
4	1.4a	9.6b	15.5c	16.3c
5	0.5a	7.2b	19.0c	14.3c
6	1.4a	2.4a	11.4c	14.2c
7	2.1a	4.4a	10.7c	13.4c
Pooled	3.9a	8.3 b	14.3c	14.8c

[a] Each quadrate was 0.01 ha in size and the data are the mean of 10 quadrats. Adjacent area data are the mean of 5 quadrats. At site 1 only five quadrats were evaluated in the rest area.

[b] Means within rows followed by different numbers are significantly different, (Student's t-test, $P \leq 0.05$, df=68).

maintained areas (Table 5.2). As expected, bait cup collections paralleled mound population densities. For the pooled data analysis, significantly more RIFAs per cup occurred in the adjacent areas. No differences in numbers of other ant species were detected. No significant differences were detected among the different quadrats within transects in bait cup counts.

5.3 Discussion

Rest areas contained lower mound densities and fewer RIFA per bait cup than did the adjacent, non-treated areas. Current pest management practices do appear to be reducing RIFA infestations in TxDOT rest areas. The continued infestation in rest areas can be attributed to the higher mound densities in these adjacent, non-treated areas. By their nature, rest areas are a favorable habitat for RIFA because of abundant water from irrigation, food left by visitors, and increased thermal radiation from concrete and brick structures. These factors not only influence re-infestation after treatment but increase the ability of RIFA to overwinter.

Table 5.2. RIFA and non-RIFA numbers collected in bait cups at Texas Department of Transportation Rest Areas in Texas (March 1997).

	Mean number of RIFA[ab]		Mean number of non-RIFA species[ab]	
Site	Rest Area	Adjacent	Rest Area	Adjacent
1	-	-	-	-
2	18.5a	18.7a	0.0c	0.0c
3	30.1a	84.1b	0.5c	0.0c
4	14.0a	39.3b	5.0c	0.7c
5	7.1a	22.9b	5.2c	15.5c
6	0.7a	8.5b	0.0c	0.0c
7	1.1a	21.2b	1.5c	0.0c
Pooled	11.9a	32.9b	1.2c	1.6c

[a] Means in the rest areas are from 60 cups; means in the adjacent areas are from 30 cups. Bait cup collections were not made at site 1. [a a a]

[b] Means within rows followed by different letters are signififcantly different (Student's t-test, $P \leq 0.05$).

CHAPTER 6. LEACHATE AND REPELLENCY TESTS

6.1 Research Approach and Procedures

6.1.1 Leachate Tests

During the field work, fewer RIFA mounds were noted in areas of dense oak and pine leaf litter. At rest areas, RIFA mounds were not observed in landscaped areas where sage was present. Based on these observations, laboratory tests were initiated to determine if certain mulches or other natural products in landscaping were repellent to RIFA.

Leachate compounds were prepared from five mulch materials and tested for repelling RIFA. Pine needles (dead), oak leaves (dead), and sage (live) were collected from local plants. Pine bark mulch and cypress bark mulch were purchased at a local garden center. Cedar shavings (often used in pet bedding) were purchased. Green sage branches were placed in the drying oven at 80°C for seven hours.

One thousand grams of each material was mixed with one liter of deionized water, and allowed to remain together for 48 hours for substances to leach out as they might in the field. These mixtures were randomly shaken every two hours. The liquid was filtered off using 2 mm mesh screen and placed into foil wrapped, sterile bottles for refrigerator storage during testing. A control solution of one liter of deionized water was similarly prepared.

Twenty-one RIFA colonies were collected near Abilene, Texas on 24 April 1997 and maintained in 38 x 52 x 7.6-cm trays. The top portion of trays was coated with Fluon (Northern Products, Woonsocket, Rhode Island) to prevent ant escape. Each colony was provided with liberal amounts of water and food. Each tray contained a smaller, covered plastic box (11 x 11 x 3.5 cm) the bottom of which was covered with dental plaster to a depth of 1.3 cm. This smaller box provided a favorable high humidity micro-habitat to prevent desiccation of workers, queens, eggs, and immatures. All colonies were maintained in the Fire Ant Research Laboratory at Texas Tech University at room temperature (21-27°C) and a relative humidity between 60 and 75%.

Colony trays were cleared of debris (dead ants, uneaten food, soil, etc.) and numbered for identification purposes before treatments were initiated. Each colony tray received two treatments, one a control (DI water) and the other, one of the six leachates which were presented in the following manner: two 9.0-cm, tagboard circles were placed into each tray, equal distance from the water source and the brood box. On each tagboard was placed a 6.0-cm, inverted, disposable petri dish lid containing one 5.5-cm dia. filter paper (Whatman catalogue #1001 055). One ml of one leachate solution was placed on the filter paper of one dish, and 1.0 ml of DI water (control) was placed on the paper of the other dish. The control was labeled using a pencil mark to distinguish it from the treated dish. Ants were counted in zones around the dishes at regular time intervals. The zones consisted of: Zone A, the tagboard circle; 1.5-cm radius around the dish: Zone B, the edge of the petri dish; and Zone C, inside the dish, in contact with the filter paper (treated with leachate). Additional solution was added at various intervals with all colonies having the appropriate solutions added at the same time and in the same amounts.

Tests were conducted three times using three replications of each treatment. Statistical analysis consisted of paired t-tests ($P \leq 0.10$) that compared numbers of ants in a treated dish with a control dish in the same colony.

6.1.2 Repellency Tests

A second series of lab tests validated the methodology of the leachate experiments. In this series, five known repellents and one attractant were used, along with DI water as a control. These tests were conducted on the same colonies after three weeks of no testing or other disturbances.

Neem (0.09% solution, Natural Guard, VGA Gardening Group, Bonham, Texas) cedar wood oil (Fisher Scientific Company, Fair Lawn, New Jersey), Deet, pyrethrin (0.02%, Green Light Company, San Antonio, Texas), and naphthalene were used as known repellents, and apple was used as the attractant. This testing followed the same methodology as the leachate tests using one ml of the liquids and 1.0 g of the solids (apple and naphthalene). Counts in the three zones were done as above. These tests were also conducted three times with three replications each time. Statistical analysis was the same as in the leachate tests.

6.2 Findings

6.21 Findings of Leachate Tests

After one hour of exposure there were significant differences found in five of the tests (Table 6.1). In the zone of contact with the leachate, dried sage, pine needle, oak leaf, and cedar shaving solutions all contained significantly fewer ants than did the paired control dish (Student's t-test, $P \leq 0.05$, df=7). Cedar shaving solution continued to contain significantly fewer ants in contact with the leachate after 4 hours, others did not contain significantly different numbers of ants between the control and the treatment. After 24 hours significant differences existed in two treatments, dried sage leachate and deionized water (control). At the end of 48 hours differences were again present in the dried sage and the deionized water. There appears a pattern, in that contact with the treated area is important.

Table 6.1. Leachate test results after one hour exposure (April - March 1997).

Leachate Suspension	Zone[b]	Mean number of RIFA[a] Treated	Control
Dried Sage	A	22.7a	27.2a
	B	13.7a	13.4a
	C	1.6a	13.3b
Pine Bark Mulch	A	43.2a	41.0a
	B	28.2a	20.8a
	C	30.7a	20.9a
Pine Needles	A	45.0a	53.9a
	B	24.6a	32.6a
	C	21.3a	45.1b
Oak Leaves	A	29.8a	42.2a
	B	11.9a	22.8a
	C	8.6a	23.0a
Cedar Shavings	A	41.9a	31.3a
	B	24.4a	26.9a
	C	10.8a	17.3b
Cypres Mulch	A	40.2a	38.1a
	B	32.1a	26.2a
	C	18.7a	24.7a
D. I. Water	A	27.8a	35.6b
	B	19.1a	18.3a
	C	20.4a	14.4a

[a] Mean of nine replications (n=9); means followed by different letters within rows are significantly different (Paired t=test, $P \leq 0.05$, df=8).

[b] Zone A = 1.5-cm radius around dish; Zone B = the edge of the petri dish; Zone C = inside the dish, in contact with the filter paper treated with leachate.

6.2.2 Findings of Repellency Tests

The known repellents used in the repellence testing resulted in numbers similar to what would be expected when RIFA encounter these products. After one hour of exposure four products produced significantly different results than did the corresponding control. Neem, DEET and Naphthalene each had significantly fewer RIFA around the dish than the control (Student's t-test, $P \leq 0.05$, Table 6.2). Deet and Naphthalene also accumulated significantly fewer ants on the edge of the dish than the control. Apple, as expected, attracted significantly more ants to all three areas around it than did the control (Student's t-test, $P \leq 0.05$, Table 6.2). At 24 hours Neem and DEET had significantly fewer ants than the control. Pyrethrin, Naphthalene and apple all had more ants present than did the controls. After 48 hours other significant differences did exist but these also were rather inconsistent.

6.3 Discussion

Laboratory testing of various materials produced mixed results. The repellant ability of some leachates after one hour may be related to the presence of moisture; whereas, after that time period, the filter papers became dried. Contact with the substance seemed to be an important factor, as the zone of contact was most often lower in RIFA numbers. Observations in the field of the absence of RIFA in certain leaf litters and plants may be related to other factors not tested in the laboratory (soil type, soil compaction, aeration, etc.). Researchers have reported an abundance of RIFA mounds in association with specific plants and the absence of RIFA mounds in association with other plants (Bart Drees, Texas Agricultural Extension Service, personal communication).

Table 6.2. Repellent and attractant test results after one hour exposure (April - March 1997).

		Mean Number of RIFA[a]	
Treatment	Zone[b]	Treated	Control
Neem	A	11.9a	16.6b
	B	5.8a	9.9a
	C	7.2a	14.2a
Cedar Oil	A	8.6a	20.2a
	B	2.9a	14.3a
	C	0.7a	22.8a
DEET	A	7.7a	15.4b
	B	1.1a	11.4b
	C	2.1a	13.6a
Pyrethrin	A	25.0a	29.1a
	B	8.8a	13.6a
	C	7.3a	11.1a
Naphthalene	A	9.4a	16.1b
	B	1.8a	13.9b
	C	20.4a	13.8a
Apple	A	33.4a	14.3b
	B	36.1a	12.7b
	C	105.2a	13.2b
D. I.	A	11.9a	13.8a
Water	B	14.6a	10.6a
	C	15.8a	17.7a

[a] Mean of nine replications (n=9); means followed by different letters within rows are significantly different (Paired t=test, $P \leq 0.05$, df=8).

[b] Zone A = 1.5-cm radius around dish; Zone B = the edge of the petri dish; Zone C = inside the dish, in contact with the filter paper treated with leachate.

Chapter 7. DISCUSSION AND RECOMMENDATIONS

7.1 TRANSECT STUDY

Data of all 28 transect sites were pooled and statistically analyzed to better understand trends in RIFA colonization of Texas ROWs.

- **Fire ant population densities in highway ROWs were not different from those in adjacent areas.** No significant differences were detected in the number of fire ant mounds in ROWs compared to adjacent properties. Also, vitality ratings of ROW mounds were not significantly different compared to those of adjacent properties.

- **Roadbed colonies were more numerous and smaller than other ROW mounds.** Significantly more mounds were found in roadbeds (≤1.0 m from paved surface) than were expected compared to the remainder of ROWs, and roadbed colonies were significantly smaller. This trend was very apparent at some sites along the western fringe of RIFA infestation in Texas.

- **RIFA populations were not well correlated to grass cover.** Neither ROW and adjacent area mound densities nor numbers of ants in bait cups were well correlated with vegetational characteristics measured in this study. This finding was not intuitive because numbers of mounds were thought to be negatively correlated with ground cover.

Discussion

Highway ROWs should not be considered as conduits of invasion in RIFA-infested areas of Texas. In fact at many highway sites, RIFA populations were greater in adjacent properties and may be sources of infestion of ROWs. Within infested habitats, ant populations have reached a mature, dynamic equilibrium. Resources of natural communities have been allocated, and only climatic, seasonal, and man-induced disturbances affect ant populations.

However, establishment of newly mated queens along roadbeds is a problem. Newly fertilized queens are attracted to shiny surfaces, including paved road surfaces. Queens crawl to adjacent roadbeds and find shelter beneath rocks and highway litter such as crushed beverage cans, paper, wood, and cardboard. Roadbeds provide thermal mass and, hence, relatively warm microhabitats. Rain water runs off pavement to roadside shoulders, and water gathers by condensation. Roadbeds provide adequate microhabitats for founding queens to survive and raise first broods. Surviving, small colonies may find adequate food resources by scavenging dead animal materials, e.g. insects, along roadbeds and by foraging in nearby grassy ROWs. Small colonies along roadbeds are relatively young, recently founded, and will likely move to nearby ROWs as they grow and require more nutrients.

The limiting factor of RIFA invasion into western Texas is aridity. However, irrigated urban and wetland areas may harbor infestations. Cold winter temperatures limit RIFA in the northern areas of establishment.

Recommendations

1. Focus inspections of RIFA populations on roadbeds, especially in "fringe" or "frontier" areas of the advancing, RIFA infestion front in Texas. The southern and western movement of fire ants in Texas is illustrated on the latest RIFA quarantine map produced by the Texas Department of Agriculture (Fig. D.1). "Fringe" areas are the boundaries between infested and uninfested counties. Bait cups placed along roadbeds may be the most sensitive indicators of RIFA presence.

2. Pest management efforts should be focused along roadbeds. Granular applications of insecticides spread along roadbeds will best target vulnerable, young RIFA colonies. Machinery designed to apply insecticides along highway rights-of-way should especially target roadbeds ($\leq$ 1.0 m from paved surfaces). Applications along the fringe areas may slow the advance of RIFA infestation.

3. TxDOT must be concerned about "hitch-hiking" RIFA colonies on mowers, graders, other contruction equipment, and in soil so as not to transport ants long distances. Close inspection of equipment and construction materials such as soil, gravel, and other fill will be necessary. Appropriate cleaning of equipment to remove fire ant colonies before transport to other construction sites is imperative.

7.2 HIGHWAY RIGHTS-OF-WAY AND REFUGE AREAS

Various ROW sites have been contructed or preserved to conserve habitats (refuges) for threatened and endangered animal or plant life. Five refuge sites that received little if any regular mowing were compared to adjacent site that had regular TxDOT maintenance.

- **No significant differences in number or vitality of fire ant mounds was detected between non-mowed, refuge sites and adjacent mowed sites.**

- **No differences in bait cup collections of ants in mowed and refuges sites were detected.**

Recommendations

1. The concept and practice of providing refuge habitats for threatened and endangered animal and plant life is applauded and encouraged.

2. Unfortunately, red imported fire ant populations have well colonized refuge sites as they have other ROW sites. Disruption by scheduled mowing of sites has not deterred fire ant establishment. It is probably impractical to attempt fire ant population management in refuges. Nature may have to take its course.

7.3 REST AREAS

Vending areas, rest rooms, information booths, parking lots, and access sidewalks were centers of human activity at Texas highway rest areas. Secondary zones of importance included picnic areas and landscaped lawns. Measurements of fire ant populations within core areas of human activity were made and compared to the relatively less maintained, less visited, but still adjacent ROW properties.

- **No differences in RIFA populations were found in distance-zones radiating from toilet facilities.**
- **In three of seven rest areas, more RIFA mounds were found in adjacent areas than in rest area cores of activity.**

Recommendations

1. Concentrate pest management applications to the human activity core of rest areas; that is, toilet facilities, vending areas, information booths, and parking. Elimination of RIFAs from core areas will reduce human contact and, therefore, protect the health and safety of patrons.

2. Inspect picnic areas, trash cans, and areas with accessible water (faucets, irrigation equipment, any leaking pipes). Apply bait-formulated, granular, broadcast

treatments of a growth regulator or another toxicant in the autumn to place stress upon colonies entering the winter season. Later, apply insecticide drenches to each RIFA mound. Toxicant granules applied directly to mounds and drenched are effective. Two applications per season may be required (late spring and mid-autumn). Consider population suppression treatments in adjacent areas to reduce movement to core facilities. Follow label directions and most recent recommendations from the Texas Agricultural Extension Service, such as the "Texas two-step" program. Assign specific, well-trained employees to RIFA control within maintenance districts.

3. Consider landscaping features. Use plastic underlayment below mulches and gravel around core facilities. Direct rain downspouts away from buildings, prevent pooling of water near foundations and walls, and maintain relatively dry barriers around buildings to discourage establishment of RIFA colony mounds. Keep a 1.0 m gravel barrier around buildings for ease of inspection and chemical application. Do not plant ground covers, flowers, and shrubbery within 1.0 m of buildings. Further research may reveal appropriate vegetative plantings and natural mulches that may repel RIFA colonization.

4. As far as possible, do not butt sidewalks and other concrete pads directly against walls of buildings. Concentrate inspections along curbs, walls, and edges of sidewalks where fire ants benefit from solar gain and moisture condensation. Carefully clean spilled food and beverages from near vending machine and picnic areas.

5. Carefully inspect all electrical equipment for presence of RIFA colonies. Fire ants aggregate within or near any active electrical equipment, including switches, contact points, transformers, air-conditioners/refrigeration units, electric motors, water pumps, soft-drink machines, irrigation and lighting timers, etc. Ants become electrified, spray alarm pheromones, move debris, and eventually short-circuit and shut-down equipment. Shorts may lead to overheating and fire. Be especially diligent in inspection of fuse boxes and circuit-breaker boxes. Texas Tech researchers are working on prevention tactics, including electrical devices that repel ants from sensitive electrical equipment (patent application pending).

7.4 LEACHATE TRIALS

Several natural products hold promise for RIFA repellency in the laboratory. Water suspensions of sage, pine needles, oak leaves, and cedar shavings had repellency effects on fire ants after one hour. Further studies are necessary before horticultural plantings and mulches could be recommended for fire ant amelioration. Unfortunately, we did not have the time nor the resources to fully explore this research avenue under the current contract. However, this approach has many possible beneficial aspects, and we recommend that a proposal be invited for this topic.

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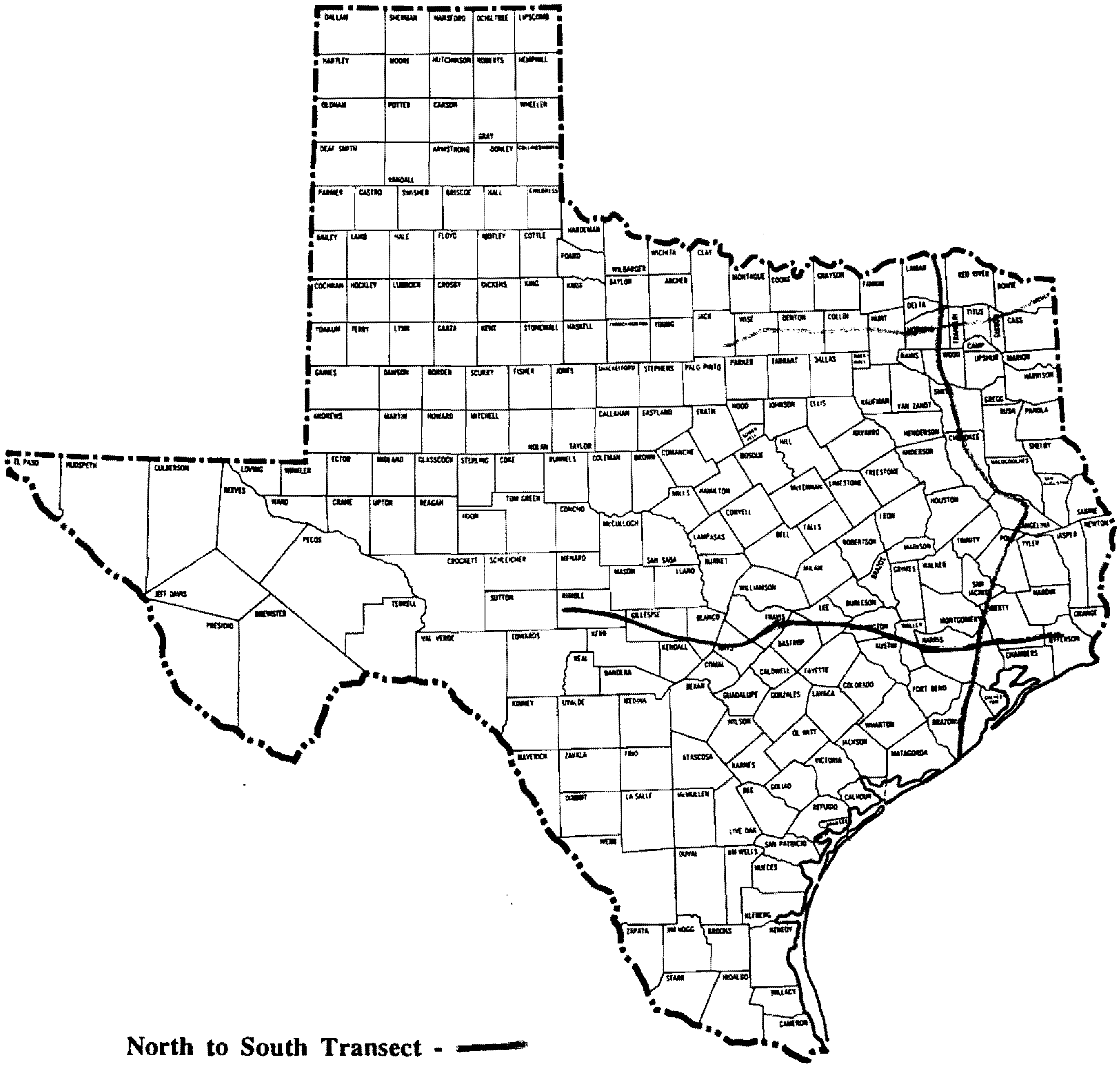
Appendix A

Location of transect sites

Figure A.1 Map depicting transect locations across Texas.



North to South Transect -

Southern east-to-west Transect-

Northern east-to-west Transect-

Figure A.2

North-to-south Transect; from north of Paris Texas to north of Lake Jackson, Texas (May 1996). Date of evaluation and temperatures.

Site	Highway Location	Global Positioning System
#1	Hwy 271, 0.8 km south of Pat Mayse Lake turn off.	N-33° 50' 16.37"
	14 May, 1996; Temperature: Soil 20.6°C, Air 28.1°C	W-95° 30' 52.05"
#2	Hwy 19, 4.8 km south of Hwy 24.	N-33° 24' 19.5"
	14 May, 1996; Temperature: Soil NA ,Air NA	W-95° 36' 6.83"
#3	Hwy 154, 1.76 km south of Yantis.	N-32° 54' 47.44"
	14 May, 1996; Temperature: Soil 23.1°C, Air 30.0°C	W-95° 34' 6.88"
#4	Hwy 69, north of Lindale.	N-32° 33' 18.73"
	15 May, 1996; Temperature: Soil 23.7°C Air 24.3°C	W-95° 25' 32.46"
#5	Hwy 69, 0.96 km north of Mount Selman.	N-32° 04' 56.25"
	15 May, 1996; Temperature: Soil 28.2°C Air 31.0°C	W-95° 17' 8.64"
#6	Hwy 69, north of Alto.	N-31° 41' 38.44"
	15 May, 1996; Temperature: Soil 32.3°C Air 33.6°C	W-95° 05' 9.13"
#7	Hwy 69, 9.6 km north of Lufkin.	N-31° 26' 19.27"
	16 May, 1996; Temperature: Soil 26.0°C Air 27.6°C	W-94° 50' 25.67"
#8	FM 942, 2.4 km west of Hwy 59.	N-30° 49' 25.57"
	16 May, 1996; Temperature: Soil 32.5°C Air 27.6°c	W-94° 53' 37.18"
#9	Hwy 59, 3.84 km north of FM 2090.	N-30° 15' 34.13"
	16 May, 1996; Temperature: Soil 33.4°C Air 31.0°C	W-95° 08' 46.36"
#10	Hwy 288, 0.32 km south of FM 59.	N-29° 32' 12.29"
	16 May, 1996: Temperature: Soil 31.6°C Air 30.6°C	W-95° 23' 13.79"
#11	Hwy 288, 2.4 km south of CR 220.	N-29° 06' 16.22"
	17 May, 1996; Temperature: Soil 24.9°C Air 27.1°C	W-95° 27' 12.16"

Figure A.3

Southern east to west transect; from west of Beaumont, Texas to south of Junction, Texas (May 1996). Date of evaluation and temperatures.

Site	Highway Location	Global Positioning System
#1	Hwy 90, 3.2 km west of China. 17 May, 1996; Temperature: Soil 27.2°C Air 33.0°C	N-30° 03' 28.5" W-94° 15' 23.87"
#2	Hwy 90, 2.24 km east of Dayton. 17 May, 1996; Temperature: Soil 32.5°C Air 30.0°C	N-30° 00' 42.9" W-94° 56' 03.1"
#3	Hwy 290, west of Houston. 18 May 1996; Temperature: Soil 33.7°C Air 31.0°C	N-30° 00' 39.9" W-95° 48' 03.5"
#4	Hwy 290, 2.08 km west of Hwy 39. 19 May, 1996; Temperature: Soil 26.6°C Air 29.1°C	N-30° 10' 14.7" W-96° 26' 41.69"
#5	Hwy 290, 3.2 km west of Giddings. 19 May, 1996; Temperature: Soil 26.0°C Air 32.0°C	N-30° 10' 57.0" W-97° 01' 18.26"
#6	Hwy 290, 12.8 km west of Elgin. 19 May, 1996; Temperature: Soil 30.9°C Air 36.0°C	N-30° 21' 03.15" W-97° 30' 14.00"
#7	Hwy 290, west of Oak Hill. 22 May, 1996; Temperature: Soil 29.2°C Air 30.7°C	N-30° 11' 47.54" W-98° 00' 03.59"
#8	Hwy 290, west of Stonewall. 22May, 1996; Temperature: Soil 24.4°C Air 33.8°C	N-30° 13' 17.2" W-98° 42' 47.5"
#9	Hwy 290, 16 km west of Harper. 22 May, 1996; Temperature: Soil 30.0°C Air 26.8°C	N-30° 17' 47.6" W-99° 24' 22.7"
#10	Hwy 377, 4.8 km south of Junction. 23 May, 1996; Temperature: Soil 29.3°C Air 26.8°C	N-30° 27' 18.3" W-99° 48' 17.5"

Figure A.4

Northern east to west transect; from west of Texarkana, Texas to Runaway Bay east of Jacksboro, Texas (May- June 1996). Date of evaluation and temperatures.

Site	Highway Location	Global Positioning System
#1	Hwy 67, 3.36 km west of FM 2148. 30 May, 1996; Temperature: Soil 26.5°C Air 26.8°C	N-33° 23' 06" W-94° 12' 16"
#2	Hwy 67, west of Omaha. 30 May, 1996; Temperature: Soil 38.4°C Air 29.1°C	N-33° 11' 16" W-94° 47' 00"
#3	Hwy 67, 1.12 km west of FM 900. 31 May, 1996; Temperature: Soil 26.4°C Air 28.2°C	N-33° 10' 52.2" W-95° 20' 42.8"
#4	Hwy 380, west of Greenville. 31 May, 1996; temperature: Soil 25.4°C Air 27.8°C	N-33° 09' 0.6" W-96° 15' 30.9"
#5	Hwy 380, 0.48 km west of Navo. 31 May, 1996; Temperature: Soil 25.1°C Air 29.9°C	N-33° 13' 13.8" W-96° 55' 25.6"
#6	Hwy 380, west of Decatur. 31 May, 1996; Temperature: Soil 29.5°C Air 30.8°C	N-33° 13' 52.15" W-97° 36' 57.8"
#7	Hwy 380, west edge of Runaway Bay. 1 June, 1996; Temperature: Soil 24.6°C Air 27.0°C	N-33° 09' 51.2" W-97° 53' 07.9"

Table A.1. Results of Chi-square Testing on Mean number of mounds in contact with the roadbed compared to the remainder of the right-of-way. Expected ratio 1 Roadbed mound to 9 Remainder mounds. North-to-south Transect. (May 1996).

Site	Mean Roadbed	Mean Remainder	Chi-square Value	(P) of Chi-square Value
1	8.7	29.0	7.16	0.01*
2	10.0	9.7	36.36	≤ 0.001*
3	23.0	31.3	63.17	<0.001*
4	26.7	47.7	55.47	<0.001*
5	12.0	40.0	9.88	0.001*
6	5.7	44.0	0.12	0.90
7	2.3	3.0	6.57	0.05*
8	7.7	17.3	12.02	<0.001*
9	1.3	10.3	0.02	0.90
10	0.0	2.3	0.26	0.10
11	0.0	0.0	0.0	1.0[a]

*= Reject 1:9 ratio at these sites based on P≤0.05; more than expected RIFA mounds present in the Rdbd.

[a]= No RIFA mounds found at this site.

Table A.2. Results of Chi-square Testing on Mean number of mounds in contact with the roadbed compared to the remainder of the right-of-way. Expected ratio 1 Roadbed mound to 9 Remainder mounds. Southern east-to-west Transect. (May 1996).

Site	Mean Roadbed	Mean Remainder	Chi-square Value	(P) of Chi-square Value
1	1.0	9.0	0.0	1.0
2	8.3	18.7	12.905	<0.001*
3	2.0	8.0	0.625	0.50*
4	0.67	14.3	0.507	0.50*
5	0.67	24.0	4.965	<0.05*
6	8.0	32.3	4.345	<0.05*
7	1.0	8.7	0.001	0.95
8	7.0	25.0	5.014	<0.05*
9	3.7	3.7	13.337	0.001*
10	0.0	0.0	0.0	1.0[a]

*= Reject 1:9 ratio at these sites based on $P \leq 0.05$; more than expected RIFA mounds present in the Rdbd.

[a]= No RIFA mounds found at this site.

Table A.3. Results of Chi-square Testing on Mean number of mounds in contact with the roadbed compared to the remainder of the right-of-way. Expected ratio 1 Roadbed mound to 9 Remainder mounds. Northern east-to-west Transect. (May- June 1996).

Site	Mean Roadbed	Mean Remainder	Chi-square Value	(P) of Chi-square Value
1	7.3	9.7	20.497	0.001*
2	10.7	21.7	19.144	0.001*
3	0.3	1.7	0.1	<0.90
4	1.7	9.7	0.309	0.50
5	3.0	1.7	15.132	0.001*
6	2.3	6.7	2.419	0.10
7	2.3	9.0	1,346.0	0.10

*= Reject 1:9 ratio at these sites based on $P \leq 0.05$; more than expected RIFA mounds present in the Rdbd.

[a]= No RIFA mounds found at this site.

Appendix B

Location of mowing regime sites

Figure B.1

Non-mow versus Mowed Areas: Surveyed October 1996, March 1997.

Site	Highway Location	Global Positioning System
Mowed #1	I-45, south of Fairfield, southbound rest area.	N-31°33' 6.9" W-96°07' 53.7"
	3 October, 1996; Temperature: Soil 28.7°C Air 27.3°C	
Nonmowed #1	I-45, south of Fairfield, median	N-31°33' 6.9" W-96°07' 53.7"
Mowed #2	I-45, north of Fairfield, CR 833 interchange, median.	N-31°48' 22.3" W-96°15' 42.6"
	4 October, 1996; Temperature: Soil 21.0°C Air 20.9°C	
Nonmowed #2	I-45, north of Fairfield, CR 833 interchange along northbound ROW.	N-31°48' 22.3" W-96°15' 42.6"
Mowed #3	I-20, east of Tyler, ROW between I-20 and access road (westbound).	N-32° 26' 18.5" W-95°01' 06.9"
	5 October, 1996; Temperature: Soil 21.0°C Air 20.9°C	
Nonmowed #3	I-20, east of Tyler, ROW along highway.	N-32° 26' 18.5" W-95° 01' 06.9"
Mowed #4	I-20, interchange with FM 314, at Van. Median of interchange.	N-32° 30' 21.1" W-95°38' 23.3"
	6 October, 1996; Temperature: Soil 22.0°C Air 25.1°C	
Nonmowed #4	I-20, interchange with FM 314, at Van. East bound ROW.	N-32° 30' 21.1" W-95°38' 23.3"
Mowed #5	I-27,South of San Antonio (33 miles); southbound picnic area.	
Nonmowed #5	I-27,South of San Antonio (33 miles); adjacent nonmowed right-of-way. (Surveyed March 19997)	No GPS Available
	17 March, 1997; Temperature: Soil 22.0°C Air 21.6°C	

Appendix C

Location of rest area sites

Figure C.1

Location of Rest Areas: Surveyed March 1997.

Site	Highway Location
Rest Area 1	I-35 southbound, north of San Marcos (approx. 10 miles) 17 March, 1997; Temperature: Soil NA Air NA (≤15.5°C, rainy)
Rest Area 2	I-10 eastbound, exit 621; east of Seguin 17 March, 1997, Temperature: Soil 18.1°C Air 22.2°C
Rest Area 3	I-10 westbound, exit 621, east of Seguin 17 March, 1997; Temperature: Soil 23.1°C Air 22.9°C
Rest Area 4	I-35 southbound, 30 miles south of San Antonio 18 March, 1997; Temperature: Soil 23.2°C Air 23.3°C
Rest Area 5	I-35 northbound, 30 miles south of San Antonio 18 March 1997; Temperature: Soil 23.0°C Air 22.0°C
Rest Area 6	I-10 eastbound, exit 514, 5 miles east of Kerrville 19 March, 1997: Temperature: Soil 13.1°C Air 10.9°C
Rest Area 7	I-10 westbound, exit 514, 5 miles east of Kerrville 18 March, 1997: Temperature: Soil 17.6°C Air 19.0°C

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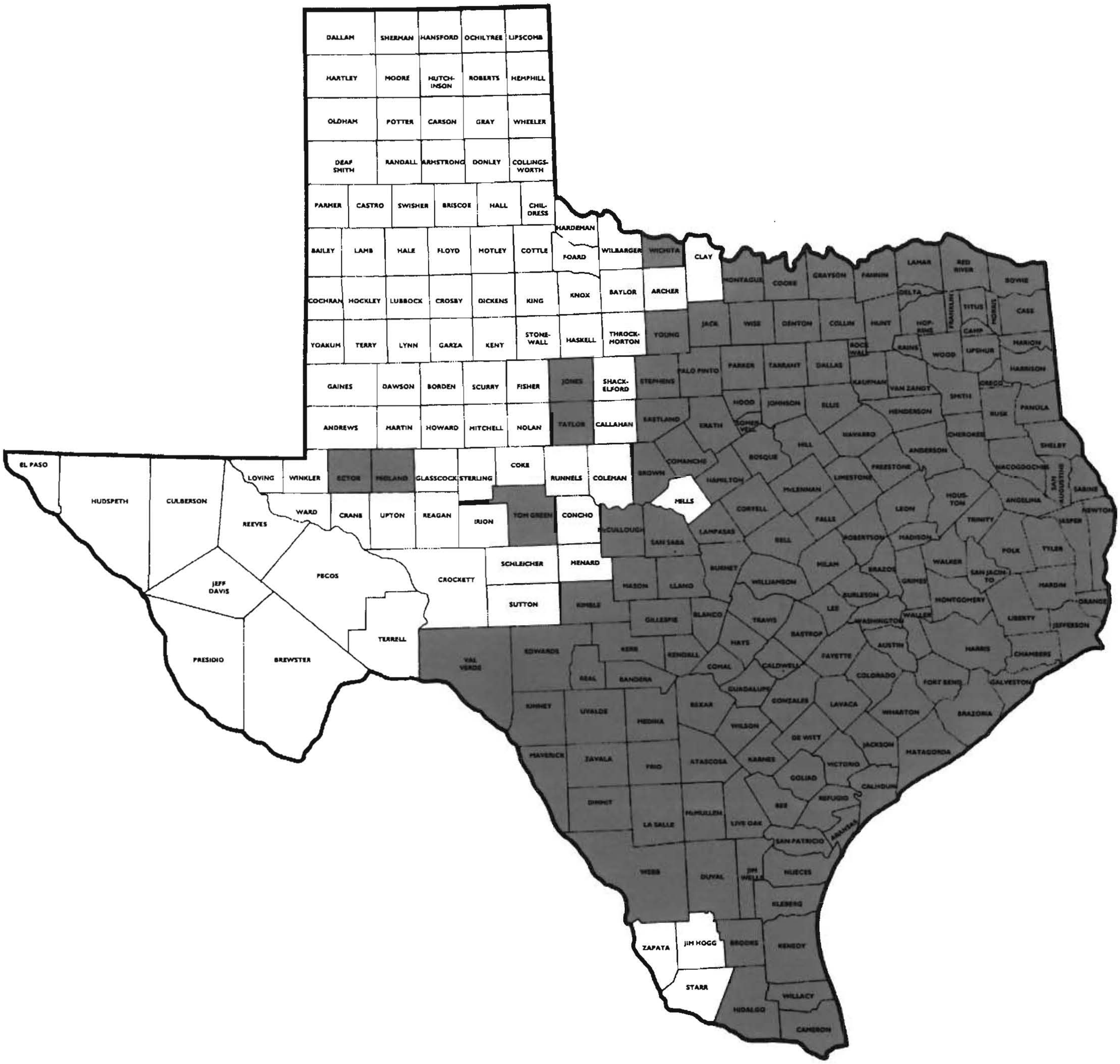
TEXAS DEPARTMENT OF AGRICULTURE

Rick Perry, Commissioner

IMPORTED FIRE ANT QUARANTINE



Imported Fire Ant Quarantined Areas
April 1998

Counties that are designated as Imported Fire Ant Regulated Areas within the state of Texas:

Anderson
Angelina
Aransas
Atascosa
Austin
Bandera
Bastrop
Bee
Bell
Bexar
Blanco
Bosque
Bowie
Brazoria
Brazos
Brooks
Brown
Burleson
Burnet
Caldwell
Calhoun
Cameron
Camp
Cass
Chambers
Cherokee
Collin
Colorado
Comal
Comanche
Cooke
Coryell
Dallas
Delta
Denton
Dewitt
Dimmitt
Duval
Eastland
Ector
Edwards
Ellis
Erath
Falls
Fannin
Fayette
Fort Bend
Franklin
Freestone
Frio
Galveston
Gillespie
Goliad
Gonzales
Grayson
Gregg
Grimes
Guadalupe
Hamilton
Hardin
Harris
Harrison
Hays
Henderson
Hidalgo
Hill
Hood
Hopkins
Houston
Hunt
Jack
Jackson
Jasper
Jefferson
Jim Wells
Johnson
Jones
Karnes
Kaufman
Kendall
Kenedy
Kerr
Kimble
Kinney
Kleberg
Lamar
Lampasas
LaSalle
Lavaca
Lee
Leon
Liberty
Limestone
Live Oak
Llano
Madison
Marion
Mason
Matagorda
Maverick
McCullough
McLennan
McMullen
Medina
Midland
Milam
Montague
Montgomery
Morris
Nacogdoches
Navarro
Newton
Nueces
Orange
Palo Pinto
Panola
Parker
Polk
Rains
Real
Red River
Refugio
Robertson
Rockwall
Rusk
Sabine
San Augustine
San Jacinto
San Patricio
San Saba
Shelby
Smith
Somervell
Stephens
Tarrant
Taylor
Titus
Tom Green
Travis
Trinity
Tyler
Upshur
Uvalde
Val Verde
Van Zandt
Victoria
Walker
Waller
Washington
Webb
Wharton
Wichita
Willacy
Williamson
Wilson
Wise
Wood
Young
Zavala

The following quarantined articles require a certificate or permit prior to movement from a quarantined area into or through a non-quarantined area.

1. Soil, separately or with other things, except soil samples shipped to approved laboratories. **
   Potting soil is exempt, if commercially prepared, packaged and shipped in original containers.
2. Plants with roots with soil attached, except houseplants maintained indoors and not for sale.
3. Grass sod.
4. Baled hay and straw that have been stored in contact with soil. ***
5. Used soil-moving equipment.
6. Logs, pulpwood and stumps. ****
7. Any other products, articles, or means of conveyance of any character whatsoever not covered by the above, when it is determined by an inspector that they present a hazard of spread of the imported fire ant.

** Information as to approved laboratories may be obtained from an inspector.
*** Not eligible for movement outside the quarantined area.
**** Exemptions from permit requirements apply for treated railroad loading sites.