DIVERSITY, ABUNDANCE, SEASONALITY AND INTERACTIONS OF ANTS (HYMENOPTERA: FORMICIDAE) IN PECANS IN MUMFORD, ROBERTSON CO., TEXAS

A Thesis

by

ALEJANDRO CALIXTO SANCHEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2004

Major Subject: Entomology

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Approved as to style and content by:

Marvin K. Harris (Chair of Committee)	Allen Knutson (Member)
Charles Barr (Member)	Kirk Winemiller (Member)

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ABSTRACT

Diversity, Abundance, Seasonality and Interactions of Ants (Hymenoptera: Formicidae) in Pecans in Mumford, Robertson Co., Texas.

(December 2004)

Alejandro Calixto Sánchez, B.S., Universidad de los Andes

Chair of Advisory Committee: Dr. Marvin K. Harris

Alpha diversity, population dynamics and interactions of ant assemblages were studied in a pecan orchard located in Mumford, Texas. The assemblages included the invasive species *Solenopsis invicta* Buren, known as the red imported fire ant (RIFA). The study addressed the major question of what is the response of the ant assemblage to the reduction of RIFA following insecticide applications (bait and contact insecticide) and the impact of these on individual species. To address this question three treatments were established in a 16 hectare area in the orchard. The treatments were randomly assigned in 1.33 hectare blocks with four replications and periodically monitored. Treatments were: 1) insect growth regulator (IGR) bait treatment (Extinguish^{TM}, active compound is 0.5% s-methoprene) applied twice in 2000 and once in 2001; 2) the contact insecticide chlorpyrifos (Lorsban^{TM}) applied on tree trunks four times in 2000 and once in 2001; and 3) untreated Control. Blocks were sampled using pitfall traps, baited vials, direct sampling, and colony counts. Data were analyzed by using ANOVA-GLM with the LSD multiple comparison test to compare the effect of treatment on the ant assemblage (using the Shannon index) and the effect on individual species. Additionally, data obtained from Control plots were used to compare sampling techniques and to determine what method is most efficient for collecting ants in this agroecosystem. Shannon indices were estimated for each method and compared. The ant assemblage consisted of 16 ant species. *S. invicta* was the most abundant followed by *Paratrechina sp.* and *Monomorium minimum*. The IGR treatment consistently reduced RIFA (77%). Native ants were found to coexist with RIFA in the Control and chlorpyrifos plots at lower densities and maintained higher densities in IGR plots. Chlorpyrifos trunk treatment did not have a significant impact on RIFA or native ant densities. The native ant, *Dorymyrmex flavus*, was greater in IGR plots following RIFA reduction and higher densities were found to persist for more than two years after the last IGR treatment. During this period, *D. flavus* was observed carrying large numbers of dead RIFA, some taken inside the nest, and some disarticulated RIFA taken out of the nest. RIFA remains were accumulated in *D. flavus* middens, further indication of an important interaction between these two species. These results indicate *D. flavus* resisted reinvasion by RIFA.

To Mom and Dad.

To Lina.

To Marvin, Charles, Allen and
Bill; for these last wonderful years...

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I especially would like to thank Dr. Marvin Harris, chair of the committee, who accepted me into his program a few years ago and for believing in what I could do. Since then has taught me how to be a better scientist and a better person. His patience, kindness and scholarship are highly appreciated; his program has really inspired me and is continuing to inspire young entomologists that, like me, one day, expect to give the best to this society and their careers. Thanks to Dr. Charles Barr, from whom I have learned so much about life and who has taught me also how to be a good scientist. He has been an inspiration during these wonderful years and believing and putting me in this enviable position. Thanks to Dr. Allen Knutson for giving me the chance to be part of this team, for supporting me throughout these years and for his guidance and support. Many thanks go to Dr. Kirk Winemiller, who has supported me and added invaluable comments and help for the conclusion of this project. Thank you to Allen Dean, for his unconditional friendship, guidance, kindness and support throughout these years, and for always being there for me and putting me on the "radar screen" with this department a few years ago. My appreciation goes to Bill Ree, whose love for pecans and knowledge have been an inspiration and support for this project.

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Lab), Geoffrey Denny and Juan Serrano provided support with data processing and analysis. Many thanks to Robert Kwiatkowski, the orchard manager of the Holmes Pecan Orchard for his cooperation and for allowing us to conduct this research in this orchard. And finally, many thanks go to all the people I forgot to mention but who assisted me throughout these years, my colleagues at the Pecan Insect Lab, and the Fire Ant Lab. This project was funded by the Texas Fire Ant Research and Management Program and the United States Department of Agriculture, Areawide Suppression of Fire Ants program in Texas.

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CHAPTER I

INTRODUCTION

Ants play an important role in natural and managed ecosystems because of their high diversity, abundance and their interactions with fauna, flora and physical components of their environment. They also are important in trophic systems, serving as herbivores, predators, scavengers and prey. They are also important in pollinating plants, dispersing seeds, aerating soil, cycling nutrients and often have mutualistic associations with plants and other insects (Risch and Carrol 1982, Holldobler and Wilson 1990). The general importance of selected ant species in natural and managed systems is documented, however the role of ant assemblages in agricultural systems is not well understood (Risch and Carrol 1982). This study describes the ant fauna residing in a pecan orchard in Mumford, TX, and explores the interactions of the native ant assemblages with the invasive ant species, *Solenopsis invicta*, an important pest that affects pecan production in Texas. In addition, the impact of the insect growth regulator, (IGR) Extinguish™, and the trunk treatment, Chlorpyrifos, Lorsban®, on *S. invicta* populations and native ant assemblages in this crop were evaluated.

The Pecan Agroecosystem

…these wonderful walnuts
- Cabeza de Vaca (1536)

The pecan *Carya illinoinensis* (Wangenh.) K. Koch is autochthonous from central Illinois to Oaxaca, Mexico. The earliest written records of pecan trees and their

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This thesis conforms to the format of Environmental Entomology.

culture are found in old book passages that describe how North American Indians built their lives around the pecan tree and how it was used as a life sustaining nut (Brison 1986, Hancock 1997). The first writings by Spanish conquistadores about this tree dated from 1519 when Hernando Cortez first came to North America and visited areas where native pecans grew and walked among them (Brison 1986, Hancock 1997). The Spanish nobleman, Cabeza de Vaca described them as "…these wonderful walnuts," and further recorded that "… it is the subsistence of the people for two months in the year without any other thing." He was the first explorer to document the extensive use of pecans in North America by native tribes (Brison 1986, Hancock 1997).

Pecan is the most valuable native North American nut crop (Brison 1974, Brison 1986, Hancock 1997) and it has been proclaimed as the state tree of Texas. The pecan belongs to the family Juglandaceae that includes walnuts and hickories (Brison 1974, Brison 1986). The pecan is a perennial capable of bearing nuts within 6 to 10 years after planting and continues to produce annually for a decade or two depending on moisture and nutrients available (Brison 1986). Trees can live for 250+ years, become more than 30 m in height with a trunk of 1.5 m in diameter. The pecan successfully grows in climates varying from humid to very arid, and native trees are found predominantly in deep well drained alluvial soil adjacent to rivers (Hancock 199, Wolstenholme 1997). Vegetatively propagated varieties are grown on about 70,820 hectares throughout most of Texas. Texas has more than 330.000 hectares of autochthonous pecans, with about 10 percent of this land being deliberately managed (Knutson and Ree 2002).

This system provides a diversity of microhabitats for arthropods (Harris et al. 1998, Liao et al. 1984). Hundreds of phytophagous insect species occur on pecan trees and many can cause damage (McWhorter et al. 1976, Harris 1983, Ree 2004). Key pests impact nut production, such as the pecan nut casebearer *Acrobasis nuxvorella* Neunzig, the hickory shuckworm *Cydia caryana* (Fitch) and the pecan weevil *Curculio caryae* (Horn) and numerous stink bugs; secondary pests attack the foliage, such as pecan aphids *Monellia caryella* (Fitch), *Melanocallis caryaefoliae* (Davis), *Monelliopsis pecanis* Bissell, the pecan phyloxera *Phylloxera sp.*, and walnut caterpillar *Datana integerrima* Grote & Robinson (Harris 1983, Ree and Knutson 1997, Knutson and Ree 2002).

The red imported fire ant, *Solenopsis invicta* Buren, interferes with pecan production by damaging drip and sprinkler irrigation systems and purportedly impacts densities of different species of arthropods in this agroecosystem (Tedders et al. 1990, Dutcher et al. 1999, Calixto et al. 2001, Harris et al. 2003). RIFA are widely distributed and occur in high densities in most of Texas, including the pecan agroecosystem. Most other ant species are considered innocuous, but leaf cutter ants, *Atta texana* (Buckley), and carpenter ants, *Camponotus sp*., may have some impact in isolated situations (Ree 2004).

The pecan agroecosystem is biologically interesting, economically important, and underinvestigated. The preservation of biodiversity and conservation of natural resources are high priorities among biologists, policy makers and the public. However, the impact of human activities on biodiversity has been little studied in natural and agricultural systems and the role of biodiversity in maintaining the productivity of agricultural ecosystems is also not well understood (Paoletti and Pimentel 1992). Studying ant assemblages in pecans will provide helpful insights into these issues.

Ants in Agroecosystems

…It is sufficient to disturb the composure of an entomologist's mind,
to look forward to the future dimensions of a complete catalogue.
- Darwin, The Voyage of the Beagle

Previous studies of ants have focused on comparing undisturbed vs. managed ecosystems (e.g., Wilson and Johns 1982, Johns 1985, Roth et al. 1994). With these few exceptions, little attention has been given biodiversity in agroecosystems, even though there is some indication that certain disturbed or managed ecosystems, including agroecosystems, can maintain a high degree of biodiversity (Pimentel et al. 1992, Perfecto and Snelling 1995).

Ants in agroecosystems may meet the requirements of a good biological indicator: they 1) are easy to sample; 2) are relatively diverse; 3) correlating density with that of other arthropods; 4) respond to environmental change; 5) can be inventoried more rapidly than many other organisms and, 6) taxonomy is relatively well known (Oliver and Beattie 1996, Alonso and Agosti 2000). In addition ants possess several attributes associated with the potential to act as effective biological control agents, 1) they are extremely responsive to spatial variations in the density of their food and exploit high food concentrations via a behaviorally complex system of chemical communication and recruitment (Risch and Carrol 1982, Holldobler and Wilson 1990); 2) they can persist as viable predators in spite of temporal fluctuations in food supply, in some cases self regulating by cannibalism of the colony brood (Carrol 1974); 3) they can store food in their colonies so that predator satiation is less likely to limit their effectiveness as biological control agents (Risch and Carrol 1982); 4) they can have a negative impact on their prey beyond that represented by the simple number of individuals consumed (Janzen 1966, Bentley 1977, Risch and Carrol 1982); and 5) the foraging patterns of ants can be manipulated and managed in order to maximize their impact on pests (Brown and Wilson 1959, Leston 1973, Room 1975, Majer 1976, Leston 1978).

Ant assemblages have been studied in different agroecosystems. Bestelmeyer and Wiens (1996) studied ground foraging ants and the effect of land use in grazing areas of northern Argentina. Risch (1981) and Risch and Carrol (1982) studied the role of two species of ants in agroecosystems in Mexico as important predators of rootworm eggs. Room (1975) sampled the diversity and distribution of the ant fauna in Papua, New Guinea, where he compared the diversity of ants in three different habitats; forest, grassland and tree crops, and not finding marked differences among habitats in terms of species richness, but finding differences in ant composition. Leston (1978) studied ants in cocoa farms in Brazil and introduced for the first time the concept of the "ant mosaic", where he tried to explain how ant assemblages may exploit and coexist in agroecosystems. He explained how several species exhibited changes in their behavioral patterns, and placed their nests surrounding the dominant species, therefore they were capable to exploit the environment more efficiently, avoid competition, and coexist. This "mosaic" may enhance pest and disease control in agroecosystems. Majer (1972, 1975, 1976a, 1976b, 1976c, 1983) and Majer and Camer-Pesci (1991) largely supported Leston's "ant mosaic" role in New Guinea cocoa farms. Also Room (1975) and Room and Smith (1975) studied ants in cocoa plantations looking at their distribution comparing pests and other components present in the cocoa ecosystem in Papua, New Guinea. Majer at al (1994) studied the arboreal ant fauna associated with cocoa farms.

Ants also have been studied in banana and cacao plantations. In Costa Rica, Roth et al (1994) investigated the effects of management on ground foraging ants and considered them as potential indicators to examine the conservation potential in a mosaic of differently disturbed areas (Roth et al. 1994); on citrus in Florida where ants were reported, with the appropriate management, be an important predator of the root weevil *Diaprepes abbreviatus* (L), an important pest in this agroecosystem (Buren and Whitcomb 1977); in banana plantations where the impact of insecticides and herbicides on ant diversity was evaluated and compared with other crops (citrus, macadamia and palms) finding that in banana plantations the effect of management on richness and ant structure was greater compared with the others (Matlock and De La Cruz 2003);in maize in Nicaragua Perfecto (1990) studied the direct and indirect effects of insecticides on ant community finding that several systemic insecticides, had a negative effect on the ant assemblage and that an increase of the fall armyworm, an important pest in this agroecosystem, was observed as a result of this decrease; the community structure of ants in habitats associated with citrus plantations in South Africa has been also studied, in this study Pheidole, the big headed ant, was reported the dominant species accounting for almost 96% of the collections, indicating some instability of the system due to the inappropriate methods of management (Samways 1983); ants were inventoried in ornamental bushes in suburban areas in Raleigh, North Carolina finding that the densities of ants were high even in human modified habitats (Nuhn and Wright 1979); in Nigeria, ant species composition in forested areas and fallows were studied and compared, the results demonstrated that forested areas support less ants than unforested areas as a consequence of the differences in physical habitat characteristics, food availability, nesting habits, predation and other factors (Ewuim et al. 1997); and competition among four ant species in coconut plantations in the Solomon Islands, describing frequency changes in activity patterns so this species avoid competition,, optimizing the habitat and enhancing biological control of *Amblypelta cocophaga* China, an important pest in coconuts (Greenslade 1971). Perfecto and Snelling (1995) examined the patterns of alpha and beta diversity in the ant community, in particular, they examined the differences in measures of ant species diversity correlated with changes in vegetational complexity associated with the improvement of the coffee agroecosystem. They found that diversity decreased on the surface of coffee bushes, there was a high degree of similarity among ant assemblages in monoculture plantations, but low degree among farms with high vegetational diversity. Whitcomb et al. (1972) inventoried soybean plantations in Florida and pointed out the role that some ant species may have on controlling major pests in this. These studies have primarily been conducted in the tropics, with limited attention to temperate habitats

Field studies are needed to census the ant species present in pecan orchards, determine their phenologies and relative densities under a variety of conditions and to assess their interactions within the ant species complex and with other fauna. This database is expected to provide valuable insights into development of management strategies to conserve human valued resources.

The Red Imported Fire Ant and Pecan Orchards

> A fire ant'll bite everything in sight without so much as by-your-leave,
> they'll scare your kids and make your dogs uptight and make you question
> all that you be-*leeve*.
>
> \- Miss Fire Ant Beauty competition theme song, Georgia

The role of the red imported fire ant (RIFA), *Solenopsis invicta* Buren, in pecan orchards and their effects on pests and natural enemies has been studied (Tedders et al. 1990, Dutcher et al. 1999, Calixto et al. 2001, 2002, 2003, Harris et al.. 2003), but their interactions with native ant fauna has not been reported. Invasion of the polygyne form of the red imported fire ant reportedly reduced the native ant fauna as well other as arthropods in Florida (Porter and Savignano 1990). Wojcik (1994) also studied in Florida the impact of fire ants on native ant fauna and came to the same conclusions. However Morrison and Porter (2003) later reported a positive association between RIFA and some native ant species as well other arthropods. So better management strategies can be developed, long term studies are needed to better understand the biologies of these ants and their interactions.

RIFA is indigenous to the subtropical savannas of Brazil and Paraguay and was originally introduced through ship ballast into Alabama. RIFA initially spread to Texas about 45 yrs ago and is considered a "weed" species (Tschinkel 1987). This aggressive invasive species was initially confronted with massive eradication efforts using broad spectrum insecticides (Summerlin et al. 1977). These efforts were unsuccessful and infestations continue to spread today. The ant now occurs in the eastern 2/3 of Texas and is expected to expand its range in future years (Camilo and Phillips 1990, Logfren 1986, Porter et al. 1988). Adverse effects of this ant on agriculture (Bhatkar 1973, Claborn and Phillips 1986), human health, and a wide range of human valued resources have been widely documented, although thorough investigations of many areas remain to be conducted, including effects on endangered species (Porter and Savignano 1990, Williams et al. 2003). Current management efforts typically rely acute treatment with one or more insecticides to reduce and maintain densities at tolerable levels. Effects on non-target organisms are largely unknown, particularly with regard to other ant species whose presence may mitigate noxious effects of *S. invicta* (Williams et al. 2003). Currently, the United States Department of Agriculture (USDA) has implemented an areawide program for controlling fire ants in pastures by using baits and biological control (Pereira 2004). However, the role of native ant species in buffering reinvasion of this invasive species and in enhancing biological control of RIFA by parasitic flies and natural diseases is relatively unknown.

This study examines the diversity, abundance and population dynamics of RIFA and related native ant species in pecans in Mumford, Robertson, Co. Texas. This information is needed to develop and implement RIFA management strategies that capitalize on natural forces as well as insecticides and classical biocontrol to achieve desired effects.

Research Objectives

Four mayor objectives were proposed for this study: 1) determine the impact on diversity, population dynamics and interactions of ant assemblages of a broadcast insect growth regulator (IGR) bait treatment and a contact insecticide trunk treatment to control RIFA; 2) assess the response of these assemblages to the presumed RIFA reduction by both treatments; 3) closely investigate the interactions between RIFA and the pyramid ant, *D. flavus* (Lockley), and evaluate potential of the latter as a buffer species, and, 4) determine what sampling technique is most efficient for collecting ants in this agroecosystem.

CHAPTER II

INTERACTIONS OF ASSEMBLAGES OF ANTS WITH THE RED IMPORTED FIRE ANT

> The legions of these Myrmidons covered all the hills and vales in my woodyard, and the ground was already strewn with the dead and dying, both red and black. It was the only battle which I have ever witnessed, the only battle-field I ever trod while the battle was raging; internecine war; the red republicans on the one hand, and the black imperialist on the other
>
> - Thoreau, Walden and Other Writings

Investigation of the ant fauna inhabiting pecan typically focused on the dynamics of the invasive species *S. invicta*, and the impact it may have on pecan production and trophic systems (Tedders et al. 1990, Dutcher et al. 1999, Harris et al. 2003). However, these studies do not describe the overall ant fauna resident in this important agroecosystem. Until recently, efforts to preserve biodiversity have focused on natural ecosystems despite the fact these areas make up only about 5% of the terrestrial environment (Western and Pearl 1989). In contrast, approximately 50% of land is currently under agricultural production (Western and Pearl 1989). There is an increasing recognition that most species interact with agricultural systems, even if their primary habitat is in a natural area. A large proportion of the total species of a region are likely to be found in agroecosystems (Pimentel et al. 1992). Understanding the ant assemblages in this agricultural system can dramatically improve the understanding of the levels of biodiversity, as well as the biology and ecology of particular species.

The red imported fire ant is an aggressive invader that competitively displaces previously dominant species (Shower 1985, Porter and Savignano 1990). The hierarchy that emerges is dominated by RIFA and a reordering of the remaining ant assemblage, favorably affecting some and disfavoring others, compared to their original condition (Wilson 1971, Morrison and Porter 2003). Cohabitation of an area with the aggressive and competitive RIFA appears to favor ant species that avoid confrontation thereby increasing their reproductive fitness (Markin et al. 1974). Other authors note that remaining species can coexist and survive by: 1) exploitation of non-limited resources, or alternative resources not used by RIFA; 2) chemical repellency and marked antagonistic behaviors; 3) high mobility maintaining close proximity to resources; and, 4) being small and opportunistic and acting as a cryptic species (Buren et al. 1974, Baroni Urbani and Kannowski 1974, Phillips et al. 1986, Helms and Vinson 2001). Understanding the interactions within the ant assemblage in pecan can provide a better understanding of the biology of RIFA in relation to the rest of the ant assemblage. This should aid development of management strategies that disfavor RIFA, while favoring or keeping neutral, effects on other ant species.

This study approached some of these interactions comparing treated areas with baits and contact insecticides versus untreated areas. Baits are a combination of an active ingredient, in this case s-methoprene, and food material (for fire ants, food attractant is vegetable oil) that is attractive to RIFA. The RIFA workers are attracted to the bait and its picked up and carried back to the nest in less than two hours, here it is incorporated into the food chain and fed to the queen and developing young. The s-methoprene works by sterilizing the queen and by preventing immature ants from maturing. Bait not picked up by the ants loses potency quickly when exposed to water and sunlight. (Klotz et al 2003). Little is known regarding the effect of this baits in non-targeted ant species as well the mechanics underlying this process. The primary objective is to document RIFA interaction with other species in the ant assemblage in pecan following bait and contact insecticide treatments and the impact these two may have on the ant assemblage to determine which, if any, might be exploited further to reduce the carrying capacity of the environment for RIFA.

Materials and Methods

Study Site. The study was conducted in a 125 hectares commercial orchard (Holmes Pecan Orchard) located in Mumford, Robertson County (30º44’54’’N; 96º33’19’’W) and 25 miles NW of College Station (Fig. 1). The surrounding areas of this orchard are represented mainly by small patches of oak trees, some hickories and prairies associated with the valleys of the Brazos River, and several cotton, corn and soybean fields (Fig. 1). Total annual precipitation ranged from 914.4 millimeters in 2000, to 1,168.4 millimeters in 2001 and 1,084.5 millimeters in 2002, and temperatures oscillating from 1.1°C to 43.3°C across the year (Appendix). The orchard was planted in 1985 with two varieties: “Cheyenne”, a medium size precocious (six years) pecan variety with good nut production and kernel quality. This variety is highly susceptible to aphid infestation accompanied by high honeydew production and “Choctaw” which develops into a larger tree that produces good quality nuts and kernels. This variety comes into production in approximately eight years. “Cheyenne” constituted the primary variety examined in this study. The site received standard management consisting of herbicide, pesticide and fertilizer applications (Table 1). Ground cover vegetation consists mainly of perennial grasses, and some seasonal forbs, maintained by a combination of mowing and selective application of herbicide.

Experimental Design and Treatment Methods. The ant assemblage was studied inside experimental blocks, each consisting of eight by nine tree rows (each plots of 1.33 hectare, trees are spaced 13.716 m by 13.411 m each), in a complete randomized block design with four replications (Fig. 2). The treatments were: 1) Extinguish™ Bait treatment (0.5% gm s-methoprene) using one pound per acre broadcast applied twice in 2000 (19 May, 12 October), and once in 2001 (12 June); 2) Lorsban® 4E (44.9% chlorpyrifos) trunk treatment following trunk spray method described by Barr and Best (2002), 0.0295 liters was applied to each trunk from soil level to a height of 1 m three times during 2000 (12 May, 24 July, 12 October) and once in 2001 (12 June).

Sampling Methods. Four techniques were used in the study site to determine the diversity and the population dynamics of ants on the ground and in the trees during 2000 (May - December), 2001 (January - December) and 2002 (January - June).

Pitfall Traps. Pitfall traps were used to estimate the abundance and species composition of ground active ants in the study area. Four traps were used per treatment. Each trap was located in the center of each plot. Samples were collected on a weekly basis for two years (from 28 April 2002 through 3 June 2002). Traps consisted of a 591 ml plastic cup filled with propylene glycol (commercial antifreeze) and a funnel that prevented escape of insects captured inside the trap. Traps with lids were set in the field before collecting the first samples and opened 48 hours later to minimize "digging in" effects (Greenslade 1973).

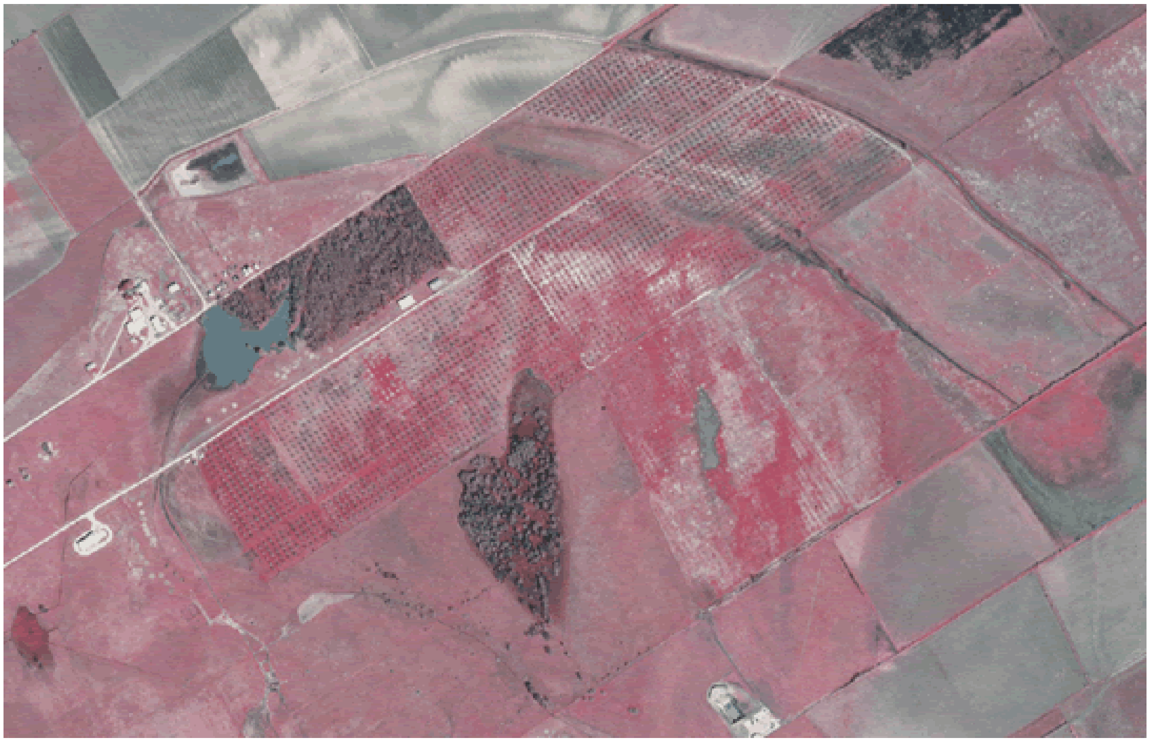

Figure 1. Location of the Holmes Pecan Orchard at Mumford, Robertson County, Texas (30º44’54’’N; 96º33’19’’W).

Table 1. Holmes pecan orchard (Mumford, TX) pesticide and nutrient application for years 2000, 2001 and 2002.[1]

Year	Date of application	Product and amount applied
2000	March 27 – April 4	8 oz Roundup
	April 5-6	Fertilizer 100# urea
	May 4	1 oz Dimilin
	May 6	80 oz Confirm
	May 14	1 qt Lorsban
	June 3-10	8 oz Roundup
2001	April 18-26	8 oz Roundup
	May 7	Aerial application 10 oz Confirm
		24 oz/100 gl Contact
		Zn application
	June 25-27	10 oz Confirm
		1 qt Lorsban
	Sept. 15	8 oz Roundup
2000	April 24-28	8 oz Roundup
	May 9	20 oz Confirm
	Sep. 11	8 oz Roundup

[1] Information obtained directly from the grower.

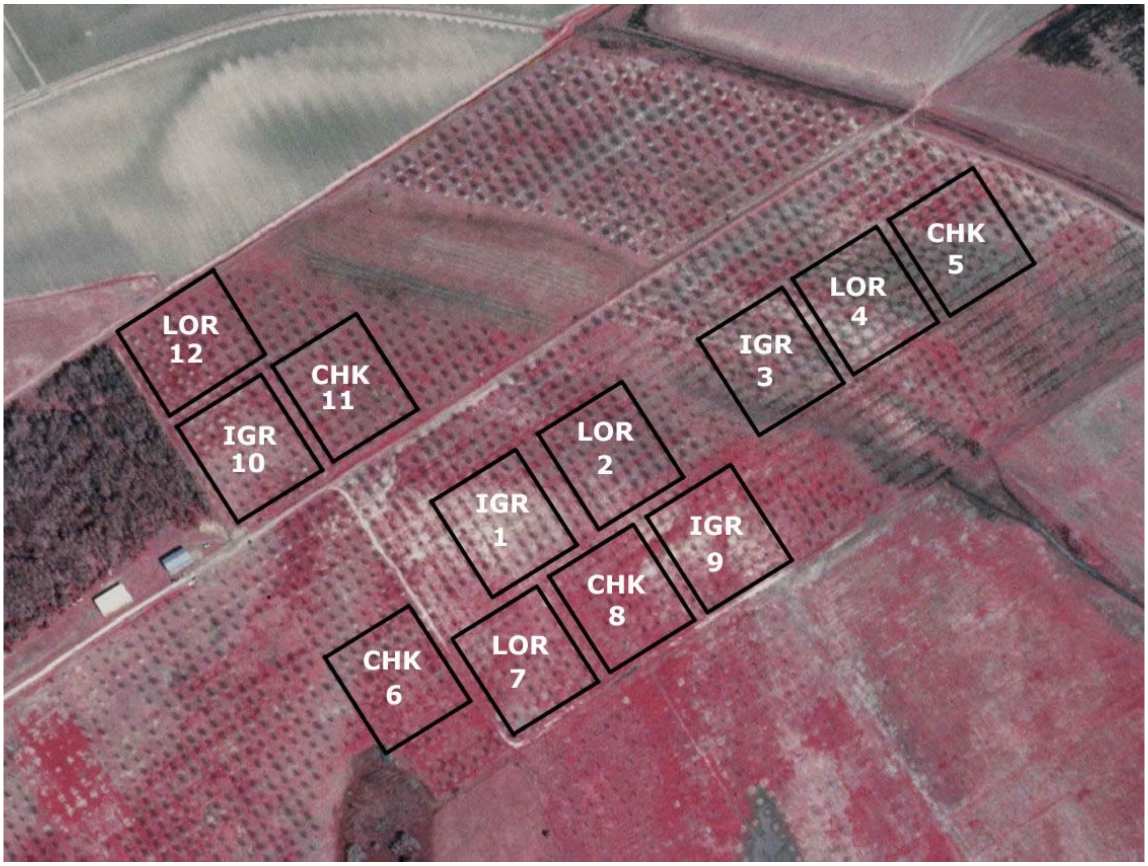

Figure 2. Location and distribution of experimental plots within the orchard (IGR= Insect Growth Regulator; LOR= Chlorpyrifos; CON= control).

Baited Vials. Baited vials were used to estimate the composition and richness of active foraging ant species patterns. Ecological and behavioral dominance and a general measure of ant foraging efficiency were also assessed with this method. Ant foragers were sampled by using 7.4 milliliter glass vials separately baited with cat food (Purina Tender Vittles®) as a protein source and sugar candy (Skittles® bite size candy) as a carbohydrate. Sixty-four vials were used per treatment. Vials were placed in four trees on each plot, with two vials (one with protein and one with carbohydrate) placed about 1.5 meters above the ground in the tree crotch to collect foliage dwelling foragers, and the other two vials (one with protein and one with carbohydrate) were placed on the ground within 0.5 m of the trunk to target ground dwelling foragers. The four trees were selected in each plot within the same area where colony counts were made and pitfalls were placed. Vials were left for 24 hours, collected by quickly capping them to trap ants inside, and returned to the laboratory where ants were sorted and counted. Samples were taken on a weekly basis from 7 May 2000 through 18 January 2002.

Direct Sampling. Ants were searched for and collected directly from eight tree trunks and canopies per treatment by using a buccal aspirator in two trees per plot beginning on 7 June 2000 and continuing on a weekly basis through 26 January 2001. Two trees were intensively sampled for two minutes in each plot every week. Ants found on the trunk and in the canopy of the tree were collected and brought to the lab for identification.

Survey of Ant Colonies. Ant colonies were surveyed in four transects per treatment each 2.5 m wide by 15.25 m long in the center of each plot, four times during

2000 (26 March, 27 June, 25 July and 7 December), twice during 2001 (12 April, 4 October) and once in 2002 (31 July). Colonies within transects were carefully inspected. Inspections typically were done “on knees” (Coddington et al. 1991) where the collector searches along a transect below knee height, and includes searches on soil, leaf litter, forest floor debris, etc. for any indication of ant nests. Surveys usually were done during the morning, and completed within thirty minutes in each transect. Twenty individuals from each of the different colonies found were collected using an aspirator, and samples were brought back to the laboratory for species identification.

Ant Identification. Collected ants were sorted and curated in separate labeled vials containing 70% ethanol. All ants were identified to genus and in some cases to species using keys and descriptions in Bolton’s (1994, 1995) and Creighton (1950), as well as additional publications as applicable (Smith 1972, Holldobler and Wilson 1990, O’Keefe et al. 2002).

Voucher Specimens. Twenty representatives of each species or morphospecies were deposited in the insect collection of the Department of Entomology, Texas A&M University at College Station, TX. Specimens were labeled for locality, sampling technique, date, collector information and comments. Duplicates of the specimens also were deposited in the insect collection of the Pecan Insect Lab, Department of Entomology at Texas A&M University, Voucher specimen #650 (Mr. Ed Riley, Collection Manager).

Environmental Data. Environmental factors were monitored using a data logger (HOBO™), to record daily rainfall and temperature data for the study site.

Analysis of Data. The data collected were analyzed in several ways.

Impact of Treatments on Ant Assemblage and Individual Species. Relative densities of each species were compared using the different sampling techniques to determine the impact of the different treatments on ant assemblages. The diversity of ants was compared among treatments by sampling technique using the diversity index of Shannon (H') (Shannon 1949, Magurran 2004). Indices were estimated per trap and then the averages were compared using ANOVA-GLM with the LSD multiple comparison test. Diversity estimations including Shannon, Evenness (E_H), Simpson (D) and Chao 1 (Chao 1984), on the pooled data for the entire study and by year and by technique were also estimated and reported.

Shannon is commonly used to characterize species diversity in a community, it accounts for both abundance and evenness of the species richness present, the proportion of species i relative to the total number of species (p_i) is calculated and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species and multiplied by -1 and is expressed with the formula $H' = \sum pi \ln pi$.

The Shannon-Evenness is a heterogeneity measure that takes into account the degree of evenness in species abundance, it is expressed with the formula $E_H = H'/\ln(S)$, (H') is the diversity index of Shannon for that site or sample; (S) the species richness or the total number of species collected within the samples (Magurran 2004).

Simpson's diversity index (D) (Simpson 1949) estimates the proportion of species i relative to the number of species ($p^2{}_i$) which is calculated and squared, and then summed and the reciprocal is taken and expressed with the formula $D = \sum p^2 i$. As D increases, diversity decreases and thus is usually expressed as 1-D or 1/D (Magurran 2004).

The Chao 1 index (Chao 1984) is a simple estimator of the absolute number of species in ecological assemblages and is based on the number of rare species in a sample. It accounts for the number of observed species represented by a single individual called singletons (F1) and the number of observed species represented by two individuals called doubletons (F2), then the ratio between singletons and doubletons is calculated as expressed in the formula $S_{Chao1} = S_{obs} + F_1^2/2(F_2+1)$.

Treatments were compared by method and by year using ANOVA-GLM with the LSD multiple comparison test (values were significantly different when $P<0.05$). A multivariate analysis (Correspondence analysis–CA) was used with the data obtained from pitfall traps since it comprised all the species in this environment, to determine and corroborate the effect of treatment on ant assemblages and the response of native ant assemblage to fire ant reduction based on species abundance by treatment matrices for 2000, 2001 and 2002. CA is an indirect gradient technique that simultaneously ordinates sample and species scores obtained by reciprocal averaging (Quinn and Keough 2002). In this analysis, rare species were down weighted and selected Hill's scaling option (scaling the eigenvectors so that dissimilarities between points are chi-squared distances (Quinn and Keough 2002).

Comparison of Sampling Methods. Data obtained from the Control plots were used to compare sampling methods and to determine what method is the most efficient when sampling ants in this agroecosystem. The diversity of ants was compared among techniques by using the diversity index of Shannon (H') (Shannon 1949, Magurran 2004). Indices were estimated per individual sample (pitfall trap, vial, tree and transect), and then the mean was estimated using the entire dataset obtained in 2000, 2001 and 2002 for each technique. Means were compared by using ANOVA-GLM with the LSD multiple comparison test. Additionally, the diversity indices of Evenness (E_H), Simpson (D) and the Chao 1 (Chao 1984) were estimated, not for each sample, but for the pooled data for each technique and reported for future references.

The statistical package SPSS 11.5 (SPSS Inc.) was used to conduct all the tests. The program Estimates 6 (Colwell 1997) was used to calculate the statistical values of species diversity, evenness and richness. The program CANOCO 4 (Microcomputer Power) was used to conduct the multivariate analysis.

Results and Discussion

A total of 57,386 ants were collected during the entire study and among treatments using pitfall traps, baited vials and direct sampling (22,052 for year 2000, 31,951 for 2001 and 3,383 for 2002), and a total of 1236 mounds were registered using mound surveys (589 for year 2000, 539 during 2001 and 108 during 2002). Numbers do not indicate abundance through time because sampling intensity varied across years. Seasonality was related with the patterns of temperature and rainfall for each year (Appendix). The assemblage in pecans consisted of sixteen ant species, they were distributed in five subfamilies. Myrmecinae contributed the most species (9), followed by Dolichoderinae (3), Formicinae (2) and Ponerinae (1) and Pseudomyrmecinae (1)

(Table 2).

Pitfall Traps. Fifteen species were recorded in IGR treatments, 14 in Chlorpyrifos treatment and 14 in the controls in 2000 (Fig. 3). *Solenopsis invicta* predominated them all; however, density increases were observed for other species in IGR plots. Sixteen species were observed in IGR plots, 15 in chlorpyrifos and 14 in the controls in 2001 (Fig. 4). *S. invicta* was again the dominant species and native ant assemblages increased in the IGR compared to other treatments. Twelve species were observed in IGR plots, three in chlorpyrifos and four in the controls in 2002 (Fig. 5). *S. invicta* interaction with native ants was consistent with trends observed in previous years. When comparing the areas where fire ants were reduced versus infested areas the diversity and abundance of species remained similar and showed evidence of coexistence between native ant species and RIFA.

Tests comparing the effect of treatments on ant diversity showed they were not significantly different in 2000 (ANOVA, 2 df; F= 52.46; P=0.571) but diversity had a significant increase in 2001 in IGR plots (ANOVA, 2 df; F= 52.46; P=0.156), but differences were found in the diversity composition for the year 2002 when comparing all treatments, IGR plots had higher diversity values compared to the Control (ANOVA, 2 df; F= 52.46; P<0.05). Bait treatment was not applied in 2002. Diversity of ant assemblages remained stable in IGR plots while decreasing dramatically in other treatments in 2002 (Tables 3, 4, 5, 6). The trunk treatment (chlorpyrifos) did not affect RIFA density compared to the control based on pitfall data (Figs. 3, 4, 5). If RIFA density is impacting density of remaining species in the ant assemblage, then diversity

Table 2. List of ant species recorded in the treatments during 2000, 2001, and 2002 using all sampling methods.

Family Formicidae	Treatment		
	IGR	LOR	CHK
Subfamily Formicinae			
Brachymyrmex sp.	X	X	X
Paratrechina sp.	X	X	X
Subfamily Dolichoderinae			
Dorymyrmex flavus	X	X	X
Forelius pruinosus	X	X	X
Tapinoma sessile	X	X	X
Subfamily Pseudomymecinae			
Pseudomyrmex sp.			X
Subfamily Ponerinae			
Hypoponera opacior	X	X	X
Subfamily Myrmecinae			
Cyphomyrmex wheeleri	X	X	X
Monomorium minimum	X	X	X
Myrmecina sp.	X	X	X
Pheidole sp.	X	X	X
Pogonomyrmex barbatus	X	X	X
Smithistruma sp.	X	X	X
Solenopsis (=Diplorhoptrum)	X	X	X
Solenopsis invicta	X	X	X
Strumigenys sp.	X	X	X
Total	15	15	16

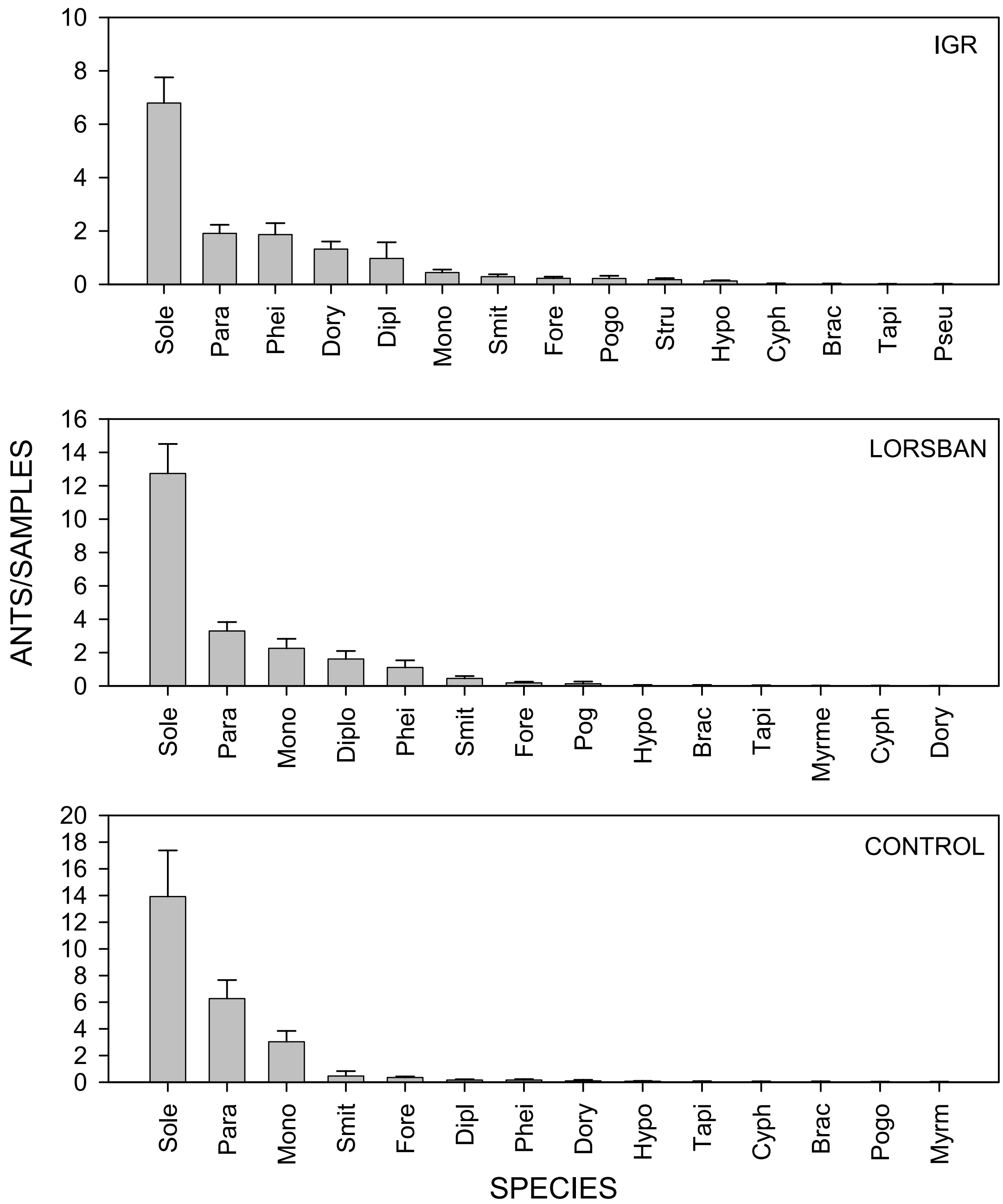

Figure 3. Ant species collected in pitfall traps in all treatments in 2000. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrm= *Myrmecina*; Pseu= *Pseudomyrmex*).

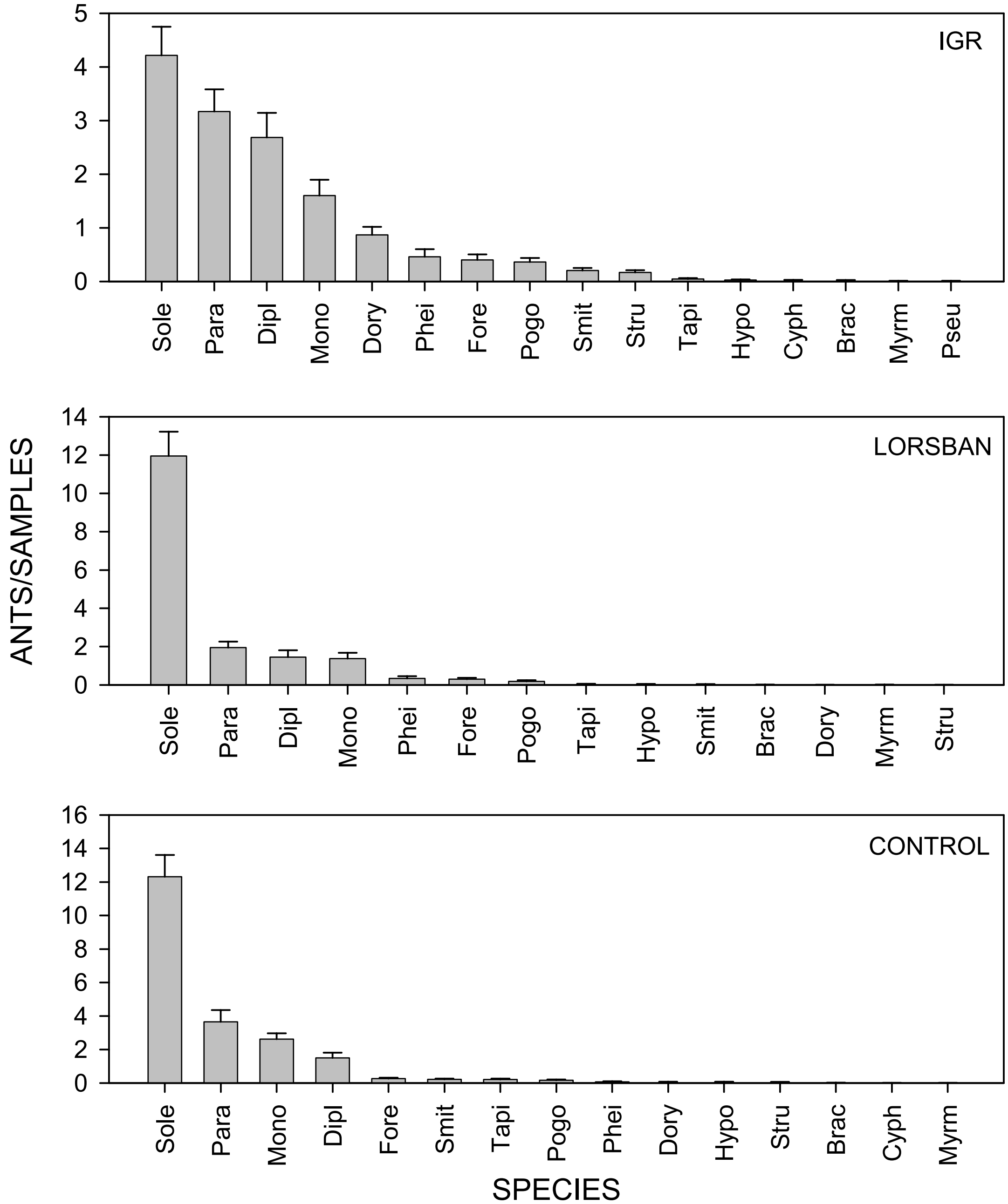

Figure 4. Ant species collected in pitfall traps in all treatments in 2001. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

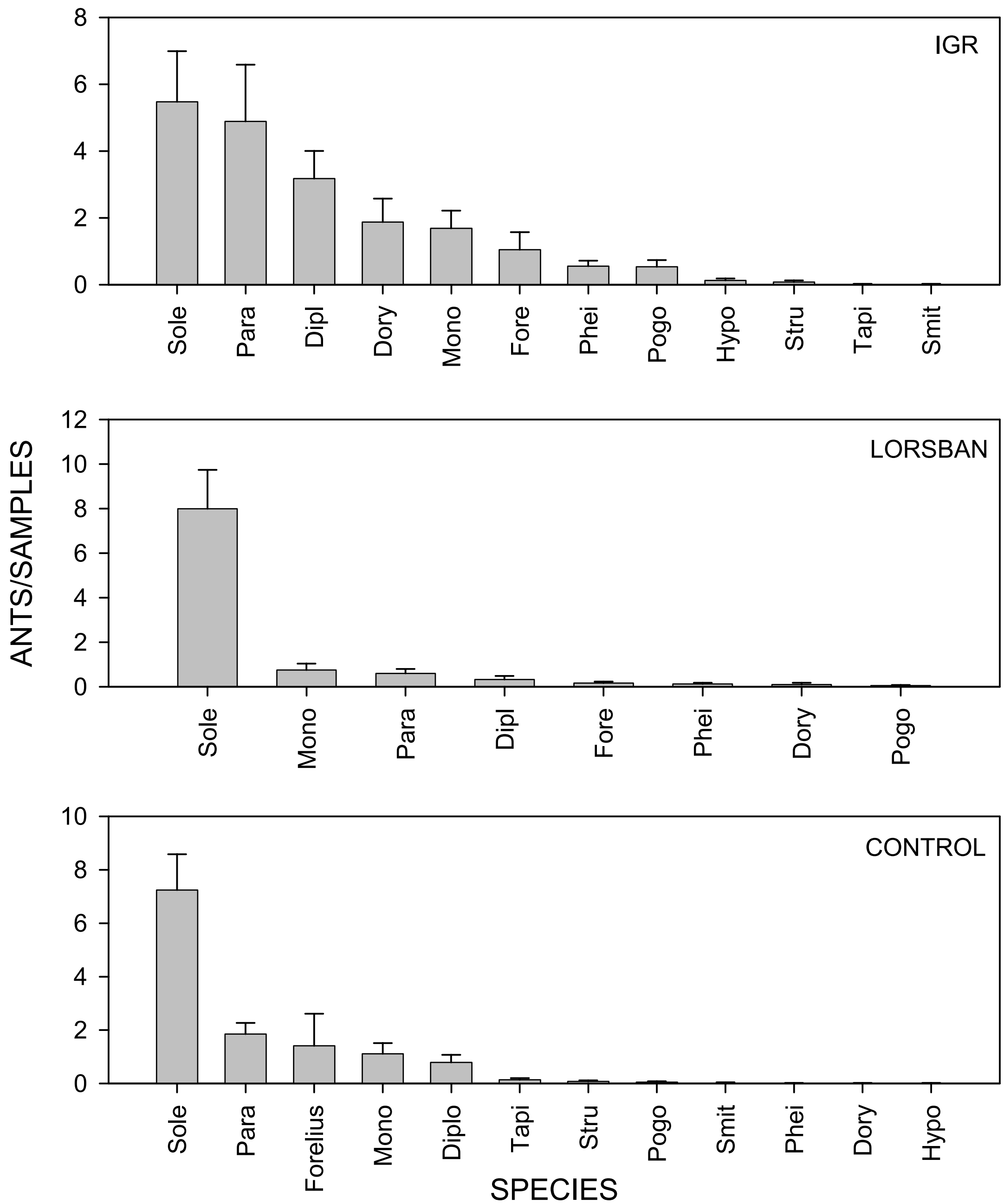

Figure 5. Ant species collected in pitfall traps in all treatments in 2002. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

Table 3. Shannon (H') index estimated for each treatment among years and among different methods (NA= data not collected for that year in that method).

	Treatment								
	2000			2001			2002		
Sampling Technique	IGR	LOR	CON	IGR	LOR	CON	IGR	LOR	CON
Pitfall traps	1.72	1.37	1.21	1.89	1.15	1.32	1.85	0.84	1.35
Baited vials	1.44	0.66	0.45	1.48	0.29	0.25	NA	NA	NA
Direct Sampling	1.45	0.70	0.45	NA	NA	NA	NA	NA	NA
Nest survey	0.90	0.11	0.33	1.28	0.81	0.43	0.99	0.75	0

Table 4. Evenness diversity (E_H) index estimated for each treatment among years and among different methods (NA= data not collected for that year in that method).

	Treatment								
	2000			2001			2002		
Sampling Technique	IGR	LOR	CON	IGR	LOR	CON	IGR	LOR	CON
Pitfall traps	0.63	0.52	0.46	0.68	0.43	0.47	0.74	0.40	0.54
Baited vials	0.69	0.34	0.25	0.71	0.18	0.18	NA	NA	NA
Direct Sampling	0.74	0.39	0.23	NA	NA	NA	NA	NA	NA
Nest survey	0.56	0.10	0.23	0.71	0.58	0.39	0.55	0.54	0

Table 5. Simpson (*D*) diversity index estimated for each treatment among years and among different methods (NA= data not collected for that year in that method).

	Treatment								
	2000			2001			2002		
Sampling Technique	IGR	LOR	CON	IGR	LOR	CON	IGR	LOR	CON
Pitfall traps	3.67	2.64	2.51	5.22	2.07	2.57	5.27	1.56	2.70
Baited vials	3.78	1.61	1.25	3.97	1.16	1.14	NA	NA	NA
Direct Sampling	3.49	1.53	1.29	NA	NA	NA	NA	NA	NA
Nest survey	2.04	1.04	1.16	2.99	2.06	1.28	2.99	1.62	0

Table 6. Chao 1 diversity index estimated for each treatment among years and among different methods (NA= data not collected for that year in that method).

	Treatment								
	2000			2001			2002		
Sampling Technique	IGR	LOR	CON	IGR	LOR	CON	IGR	LOR	CON
Pitfall traps	15.75	14.05	14	18	15.44	15	14	8	13.16
Baited vials	10	5.12	4	12.5	1.16	1.14	NA	NA	NA
Direct Sampling	7	6.5	7	NA	NA	NA	NA	NA	NA
Nest survey	5.12	3.5	4	6	5.12	3	3	4	1.5

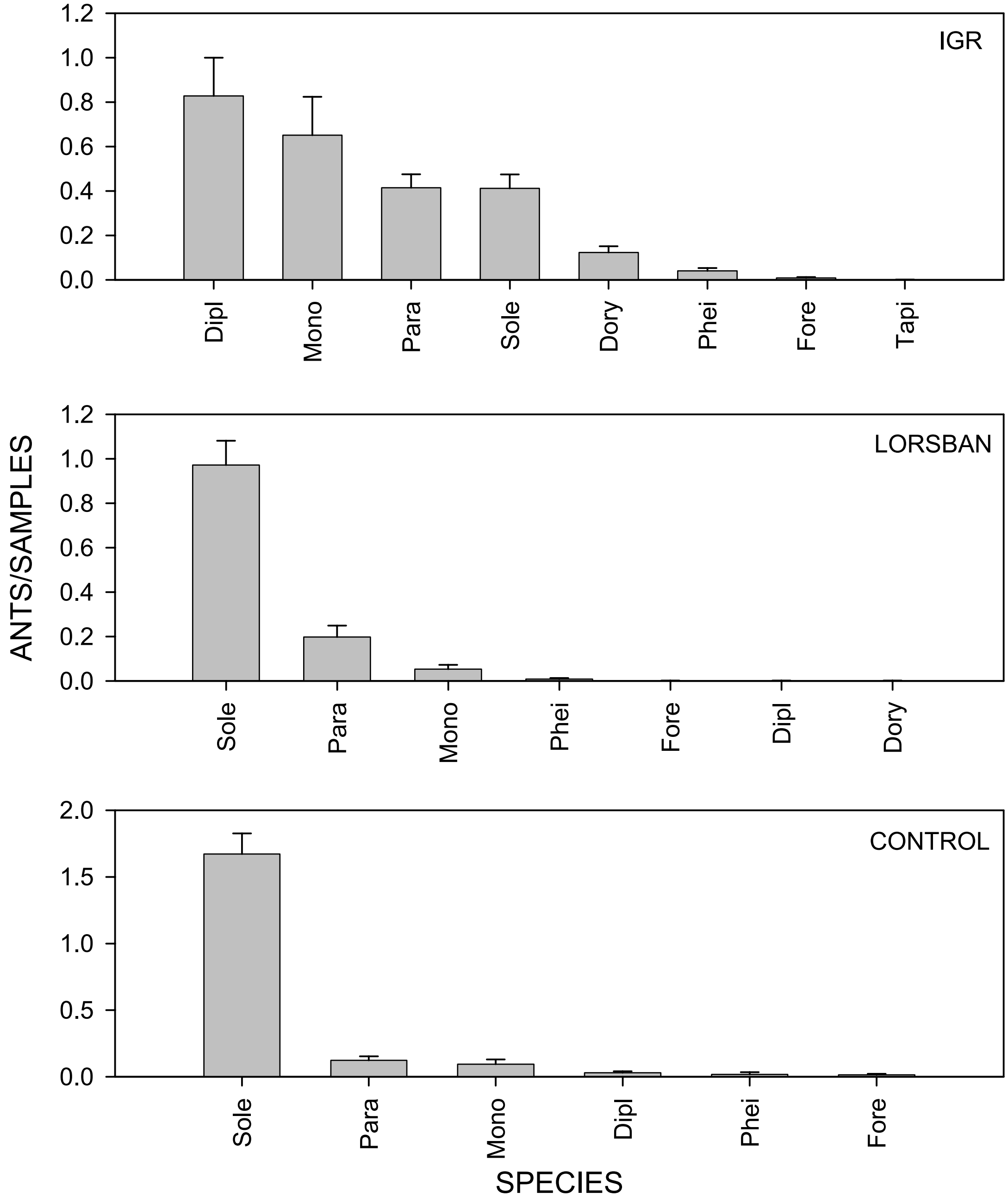

Figure 6. Ant species collected in baited vials in all treatments in 2000. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Phei= *Pheidole*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

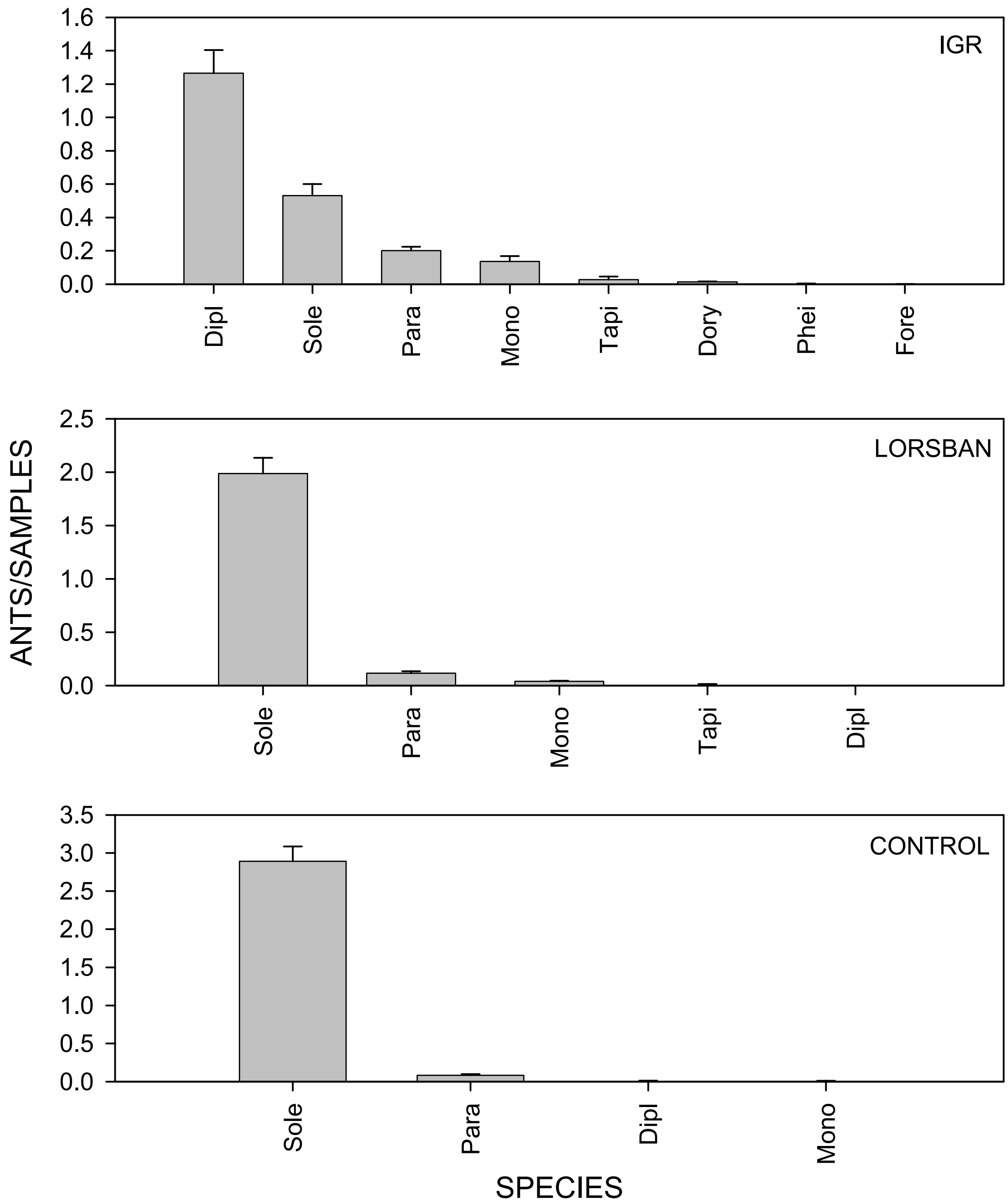

Figure 7. Ant species collected in baited vials in all treatments in 2001. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Phei= *Pheidole*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

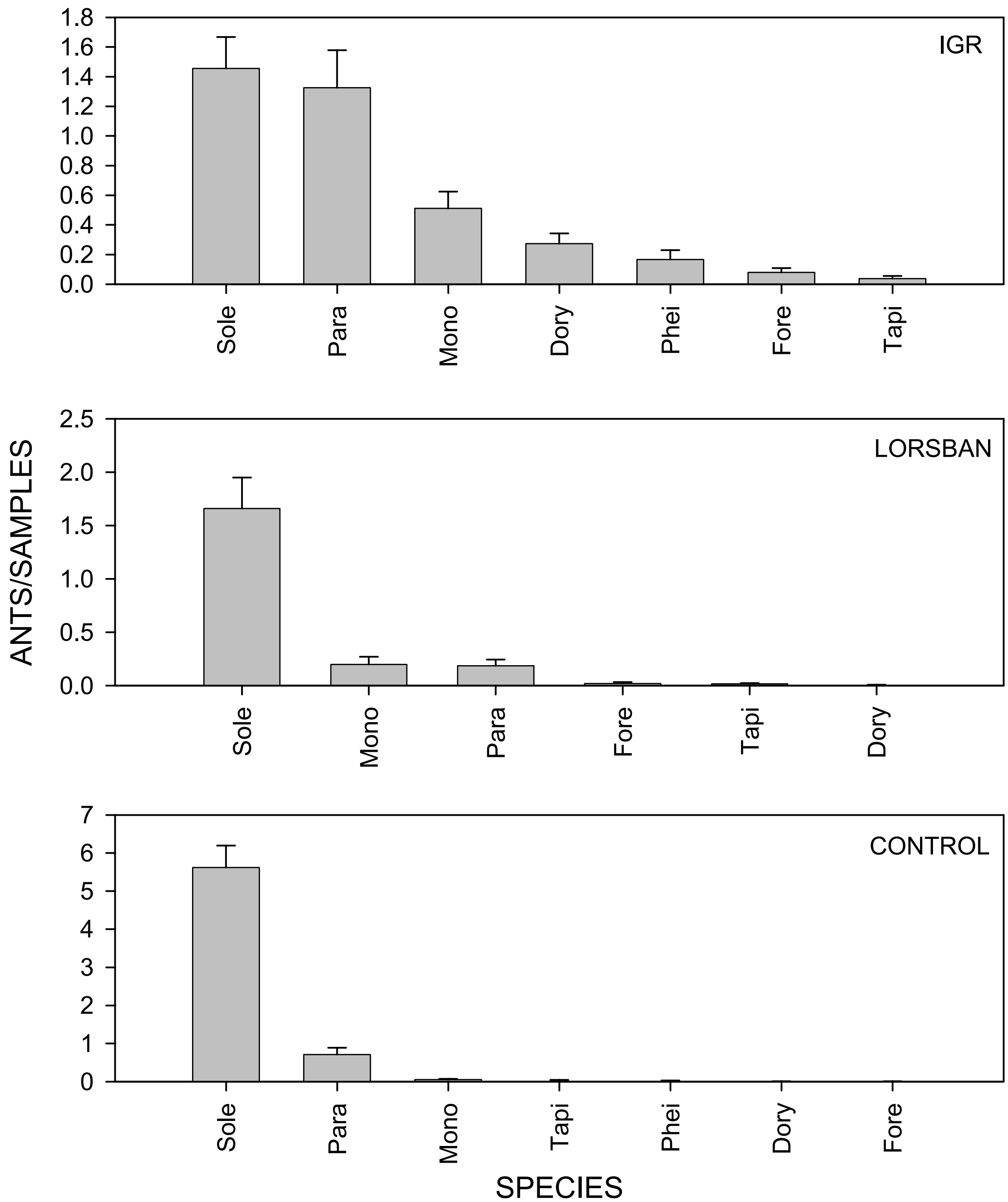

Figure 8. Ant species collected with direct sampling in all treatments in 2000. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Phei= *Pheidole*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

indices comparing chlorpyrifos and control should be similar.

Baited Vials. There were eight species found using vials in IGR plots, seven in chlorpyrifos and six in the controls in 2000 (Fig. 6). Eight species were found in IGR plots, five in chlorpyrifos and four in the controls in 2001 (Fig. 7). Diversity of ants recruiting to vials was greater in IGR plots and were significantly different when compared to chlorpyrifos and controls in 2000 (ANOVA, 2 df; $F= 25.73$; $P<0.05$). Chlorpyrifos did not differ from the control in 2000 (ANOVA, 2 df; $F= 25.73$; $P=0.872$). IGR treatment was significantly higher from the control (ANOVA, 2 df; $F= 14.006$; $P<0.05$) but not from chlorpyrifos in 2001 (ANOVA, 2 df; $F= 14.006$; $P=0.07$). Diversity of ants recruiting to baited vials remained high in IGR plots in 2001 (Tables 3, 4, 5, 6).

Direct Sampling. Seven species of ants were collected on pecan trees located in IGR plots, six in chlorpyrifos and seven in the controls in 2000 (Fig. 8). *S. invicta* was the most abundant ant species collected among the treatments. Diversity the of ant assemblage was significantly higher in IGR compared to the other treatments, (ANOVA, 2 df; $F= 26.430$; $P<0.05$) indicating the reduction of fire ants in IGR positively affected the diversity, abundance and composition of ant species that forage in the trees (Tables 3, 4, 5, 6). In addition, RIFA was also reduced in chlorpyrifos treatment indicating some effect of the treatment on this species. However ants were observed and collected in approximately seven days after the chlorpyrifos trunk applications.

Survey of Ant Colonies. Five ant species were recorded in transects inside IGR plots, three in chlorpyrifos, and four in the control plots in 2000 (Fig. 9). *S. invicta* was most frequently encountered in all treatments and *D. flavus* exhibited a slight increase following IGR treatment (Fig. 9). Seven ant species recorded in IGR, five in chlorpyrifos and three in Control plots in 2001 (Fig. 10). *S. invicta* consistently dominated in chlorpyrifos and Control, while *D. flavus* dominated in IGR. Six species found in IGR, four in chlorpyrifos and one in the control in 2002 (Fig. 11). Numbers of fire ant mounds remained stable in chlorpyrifos plots while in the controls an inexplicable reduction of mounds was observed (Fig. 11). *S. invicta* increased slightly in IGR plots from 2001 to 2002 and other ant species remained active and their nests were observed coexisting near fire ant mounds. *D. flavus* remained the second most abundant species in these IGR plots and seemed to do adapt after the first stages of fire ant reinfestation. Pitfall trap data indicated RIFA remained present in Control plots and RIFA mounds were also observed outside the transects the day data were collected. A single survey was made in 2002.

Diversity of ant species was significantly higher in IGR in 2000 when compared to the controls (ANOVA, 2 df; F= 2.866; P=0.033) and chlorpyrifos significantly lower (ANOVA, 2 df; F= 2.866; P=0.062). IGR ant diversity greatly differed in 2001 and was significantly higher in IGR, and lower in chlorpyrifos compared to the control (ANOVA, 2 df; F= 13.135; P<0.05). In 2002 the diversity remained higher in IGR plots compared to the Control and to chlorpyrifos (ANOVA, 2 df; P<0.05). Diversity of ant nests remained high in IGR plots throughout the study period but it was only significantly higher in 2001 (ANOVA, 2 df; P<0.05) (Tables 3, 4, 5, 6).

Effect of Treatments on the Ant Assemblage and Individual Species. Ants were active throughout the year. Seasonal activity of ground dwelling ant assemblages

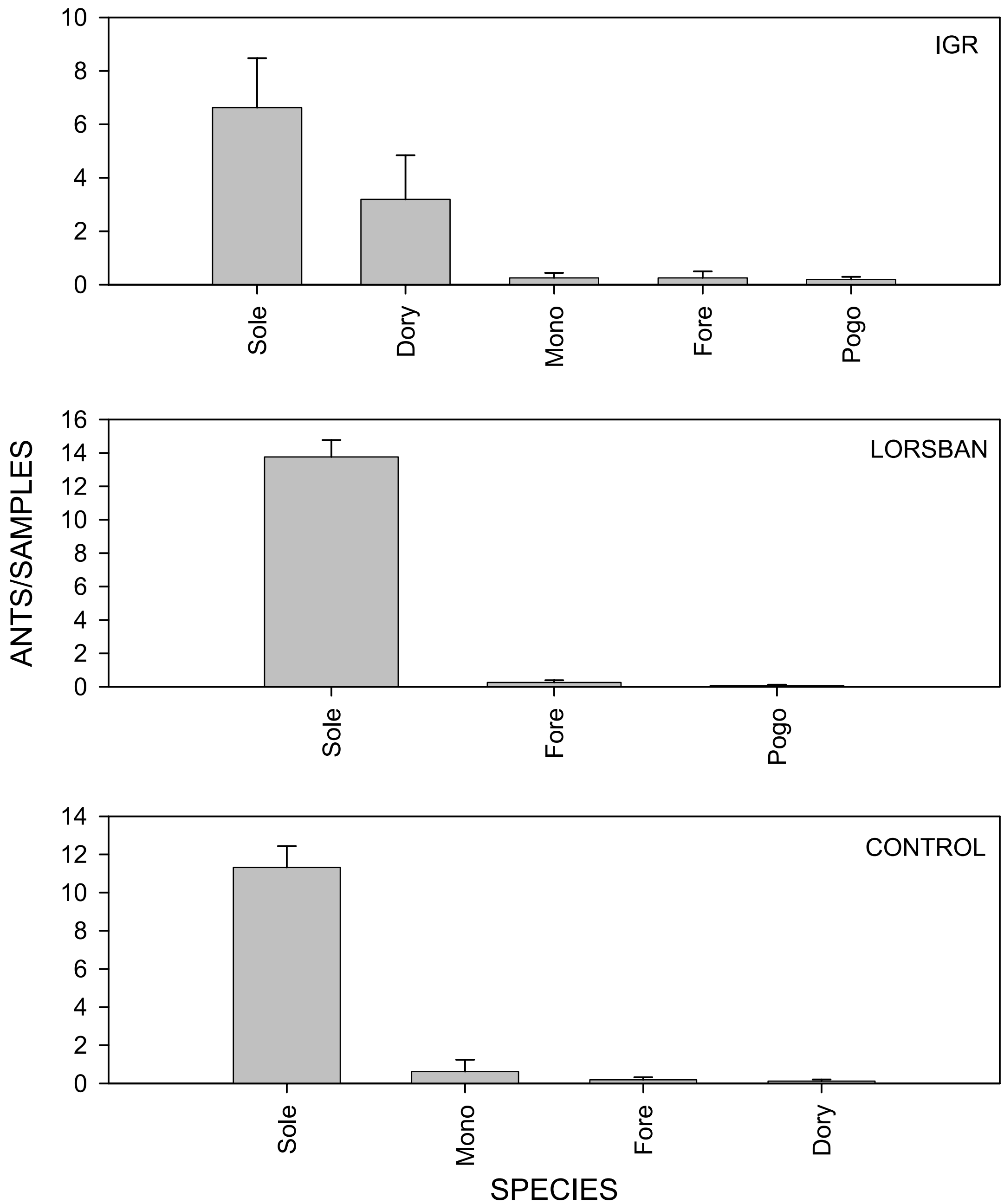

Figure 9. Ant species detected in colony surveys in all treatments in 2000. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Pogo= *Pogonomyrmex*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

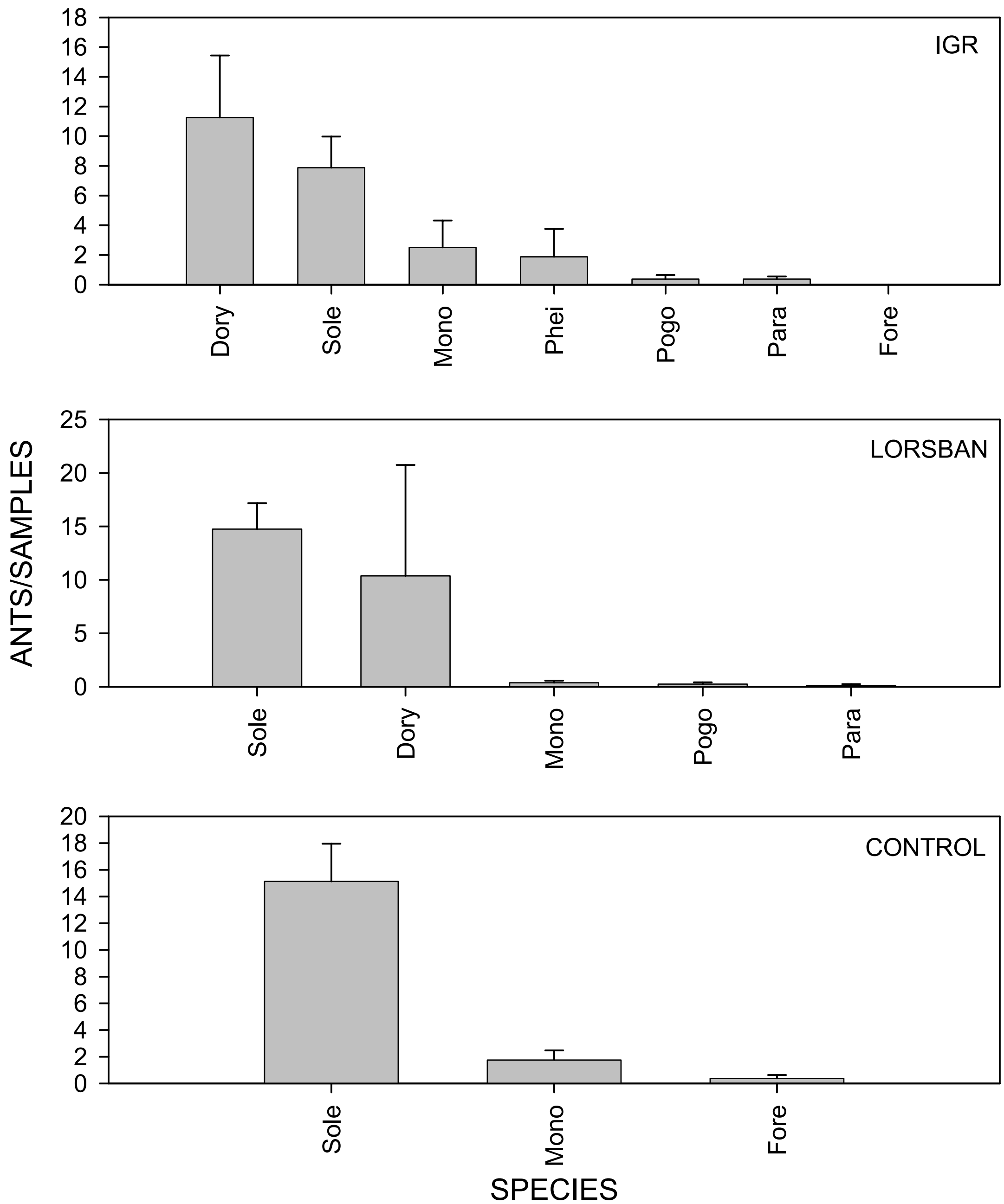

Figure 10. Ant species detected in colony surveys in all treatments in 2001. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Pogo= *Pogonomyrmex*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

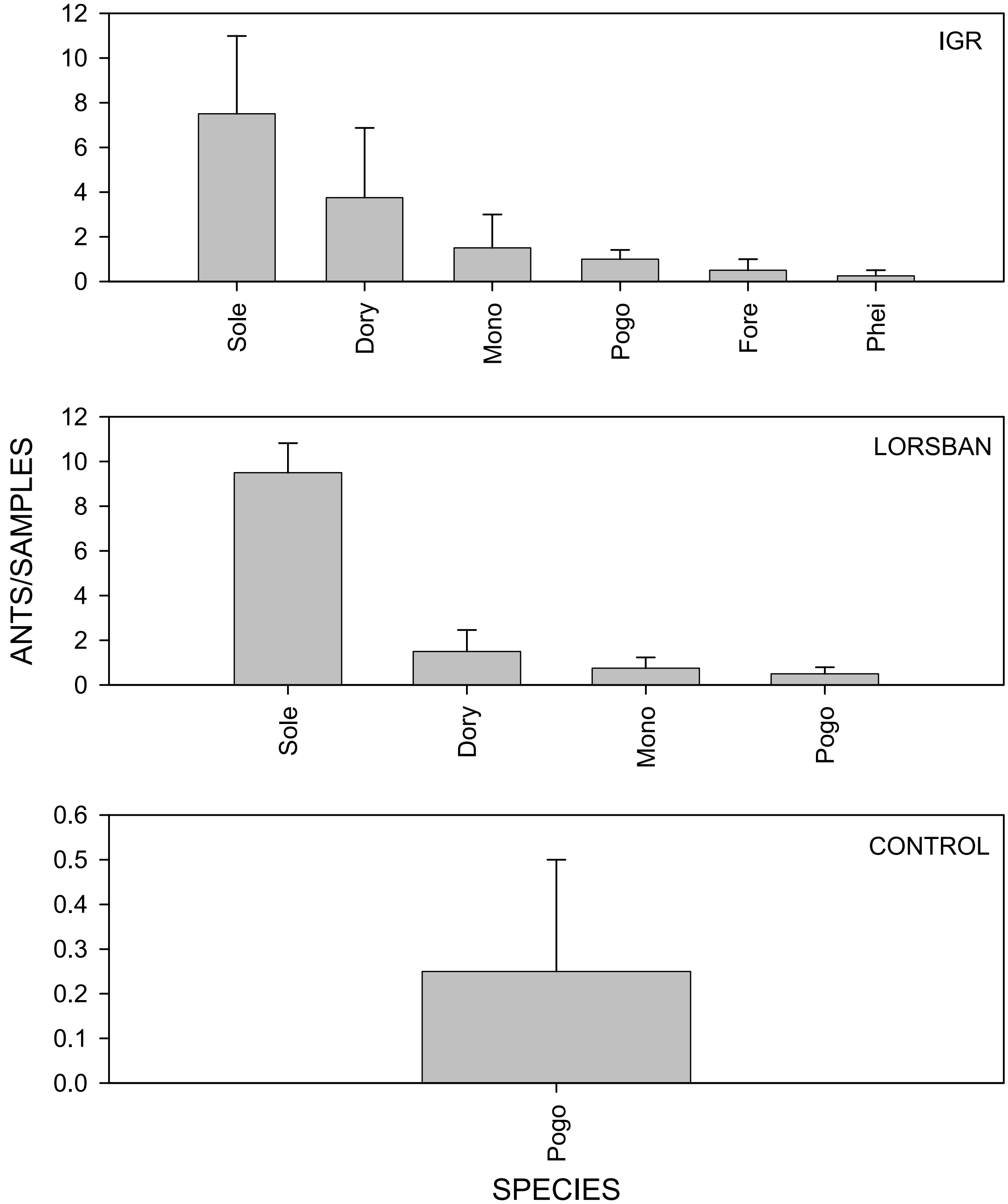

Figure 11. Ant species detected in colony surveys in treatments in 2002. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Pogo= *Pogonomyrmex*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

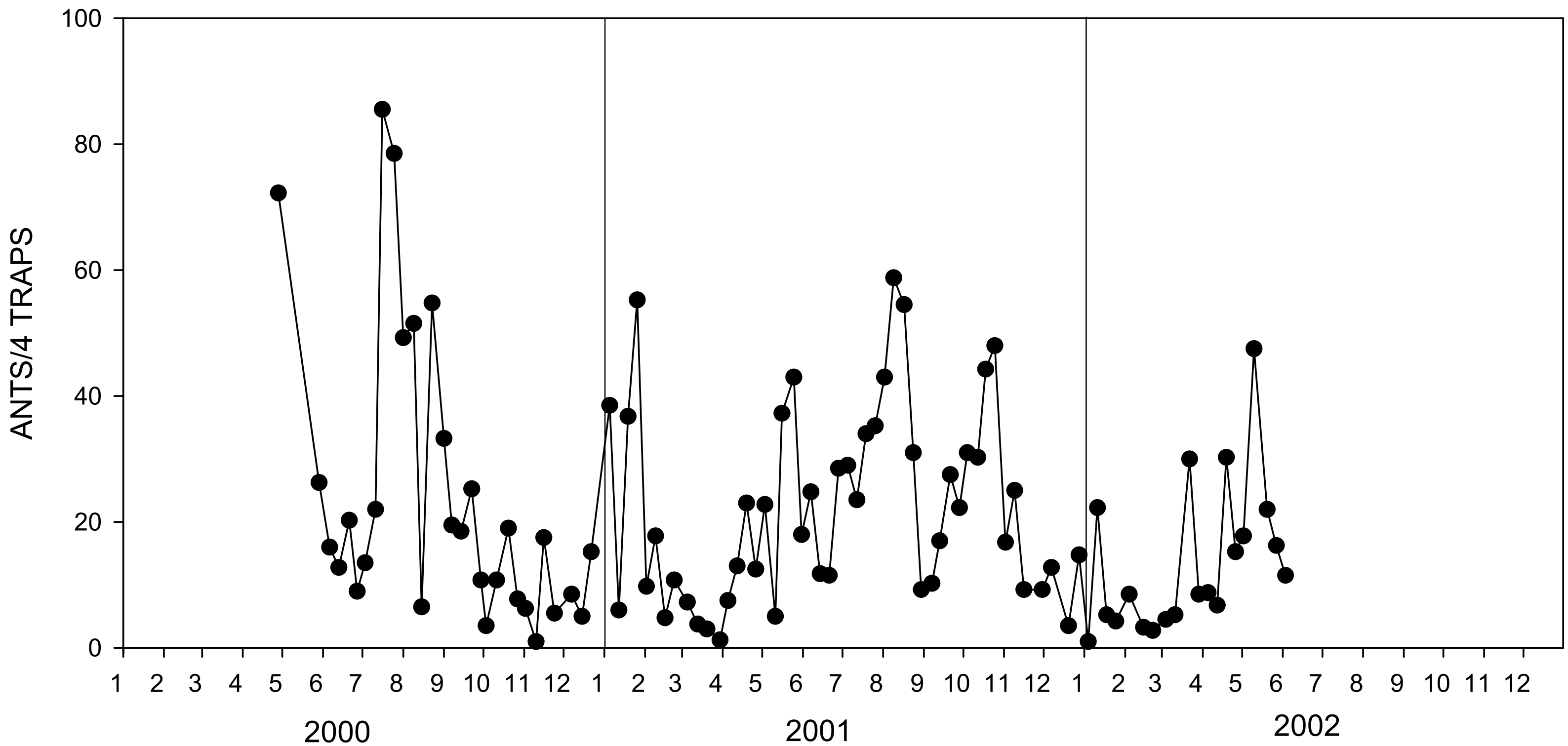

Figure 12. Activity and seasonality of the ant assemblages detected using pitfall traps in years 2000, 2001 and 2002 for this orchard (all the species).

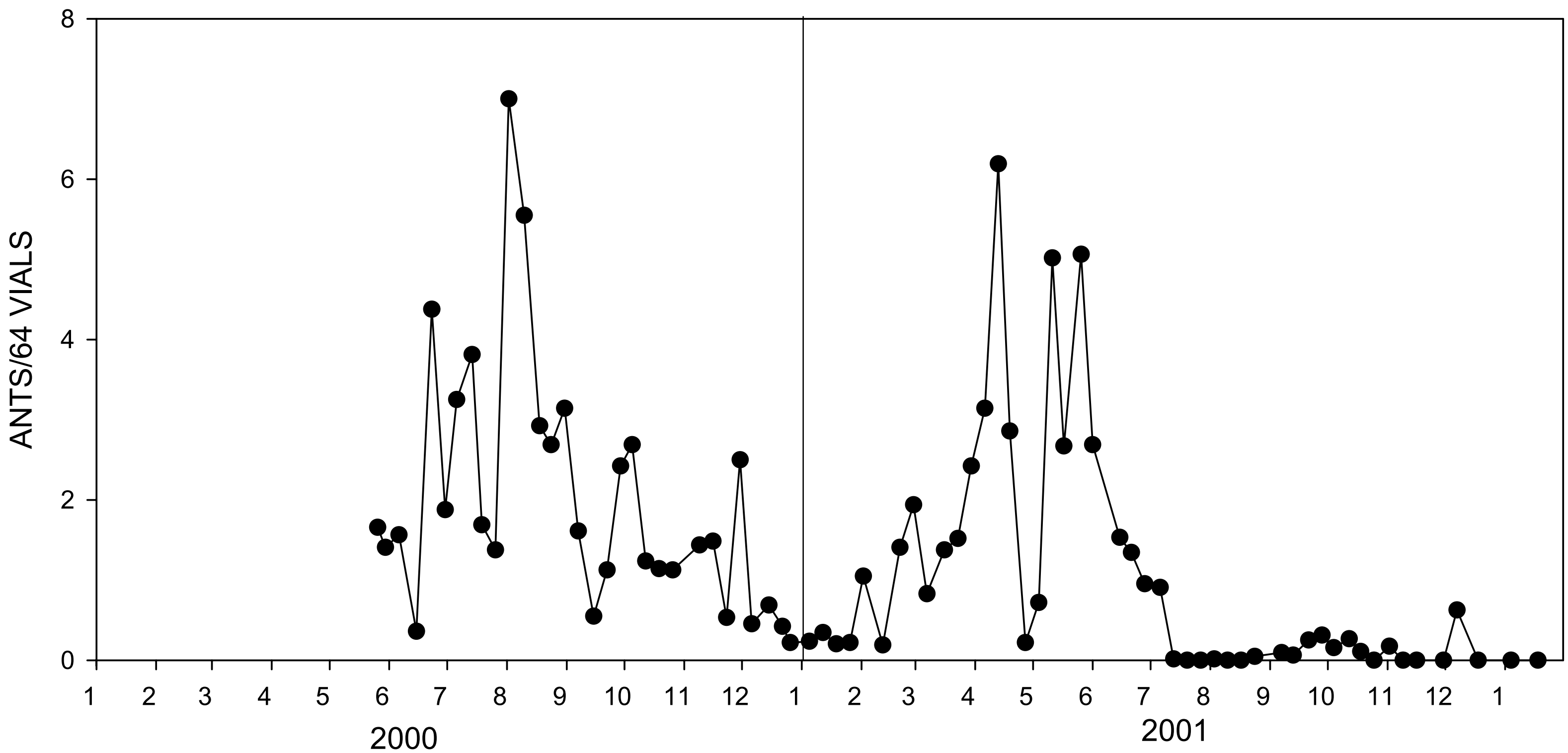

Figure 13. Foraging behavior and seasonality recorded for ant assemblages in pecan orchards for years 2000, and 2001 (all the species). Baits consisted of cat food as source of protein and candy as source of carbohydrate.

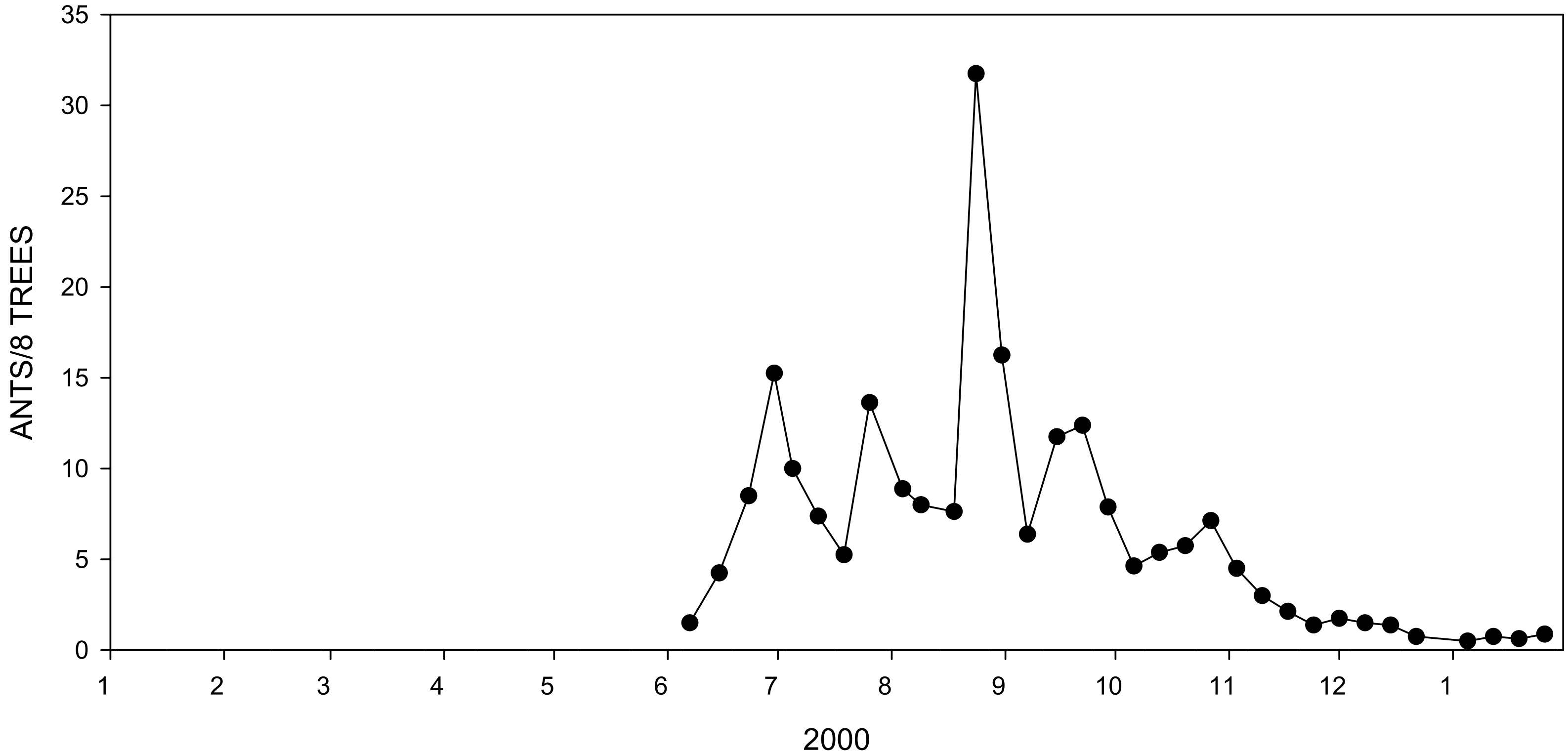

Figure 14. Ants collected from the trees using the direct sampling method in pecan orchards in 2000 ((all the species).

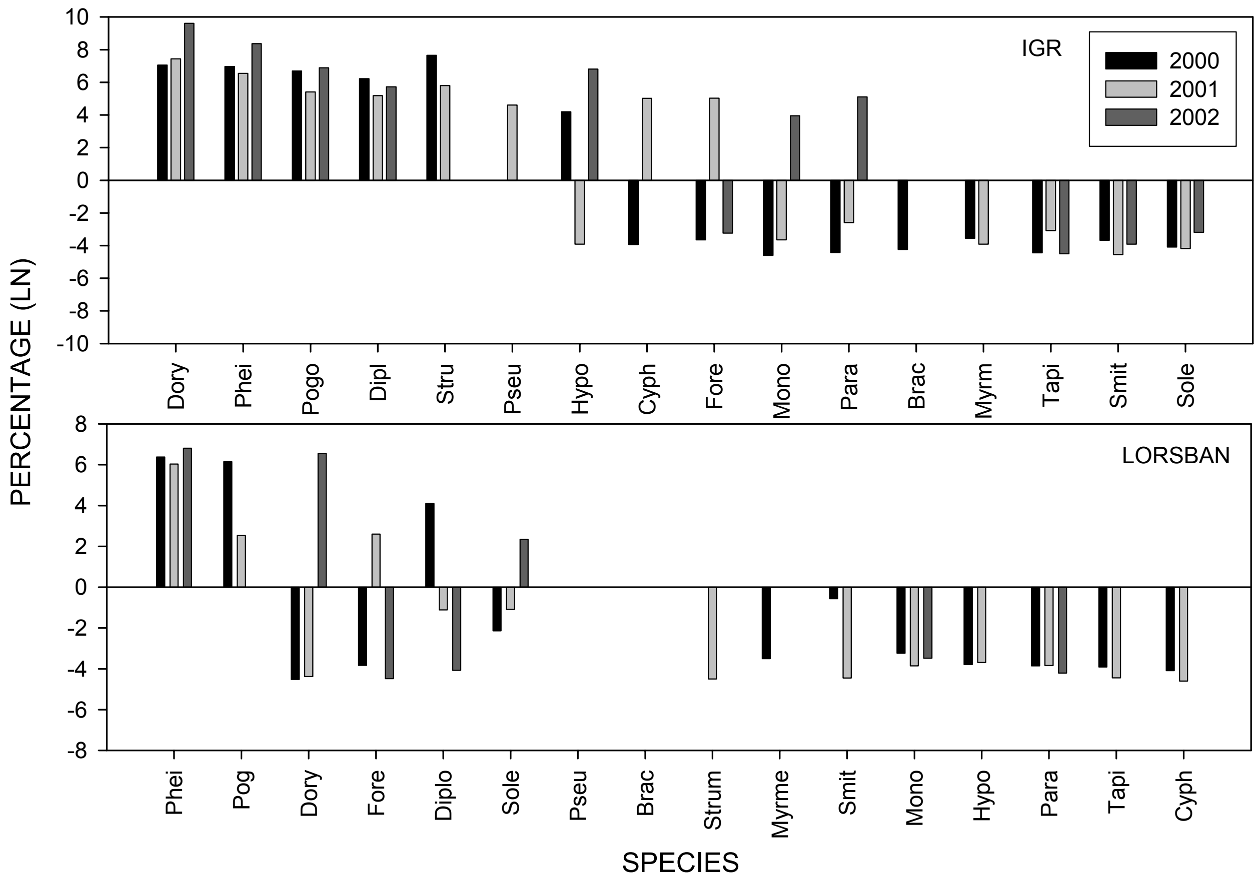

Figure 15. Ant species percentage (ln) increase or reduction collected in pitfall traps in all treatments in 2000, 2001 and 2002 in relation to the Control. (Dory= *Dorymyrmex*; Phei= *Pheidole*; Pogo= *Pogonomyrmex*; Diplo= *Diplorhoptrum*; Strum= *Strumigenys*; Pseu= *Pseudomyrmex*; Hypo= *Hypoponera*; Cyph= *Cyphomyrmex*; Fore= *Forelius*; Mono= *Monomorium*; Para= *Paratrechina*; ; Brac= *Brachymyrmex*; Myrm= *Myrmecina*; Tapi= *Tapinoma*; Smit= *Smithistruma*; Sole= *Solenopsis*;).

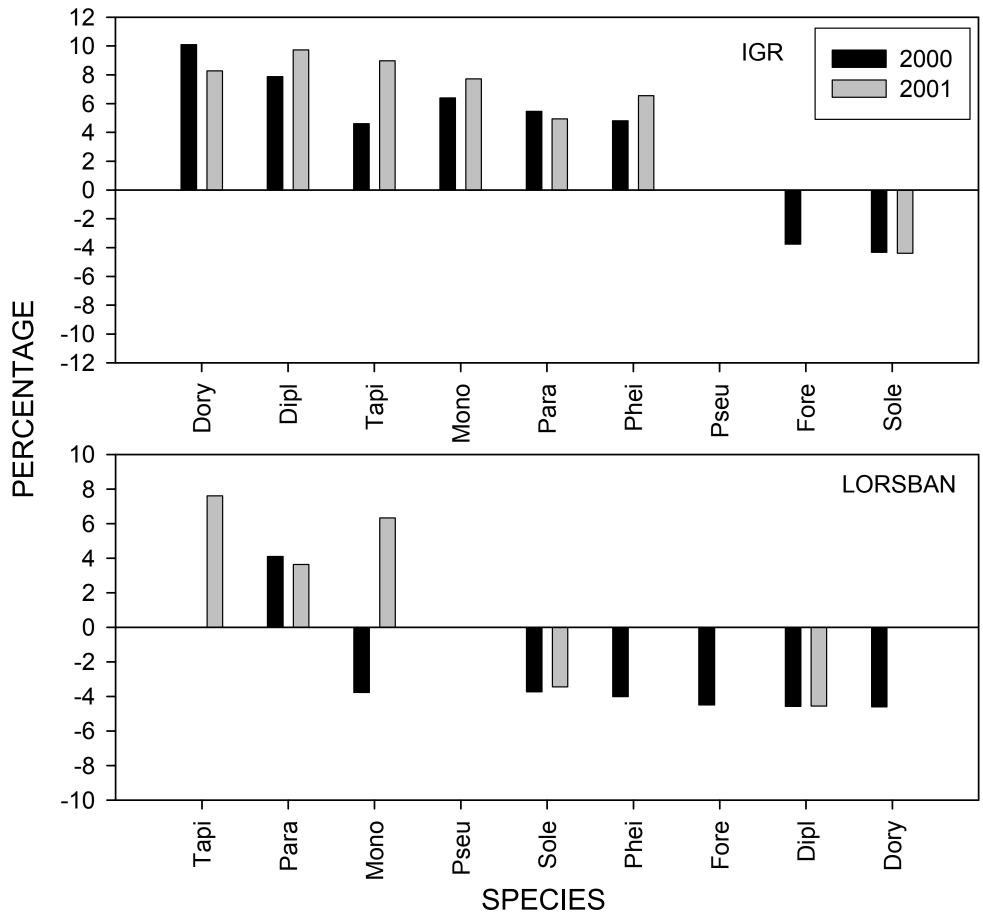

Figure 16. Ant species percentage (ln) increase or reduction collected in baited vials in all treatments in 2000 and 2001 in relation to the Control. (Dory= *Dorymyrmex*; Diplo= *Diplorhoptrum*; Tapi= *Tapinoma*; Mono= *Monomorium*; Para= *Paratrechina*; Phei= *Pheidole*; Pseu= *Pseudomyrmex*; Fore= *Forelius*; Sole= *Solenopsis*).

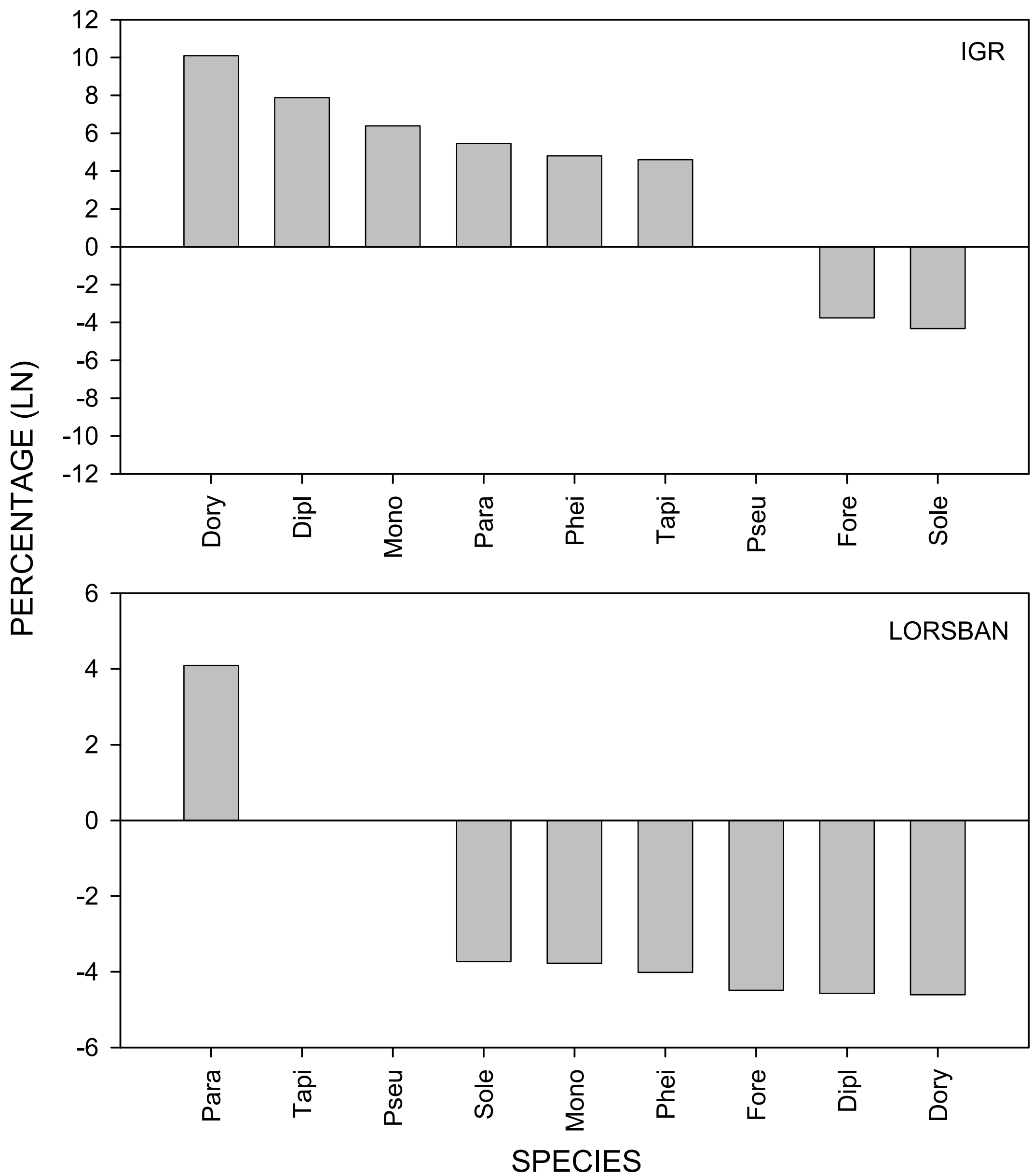

Figure 17. Ant species percentage (ln) increase or reduction collected in direct sampling in all treatments in 2000 in relation to the Control. (Dory= *Dorymyrmex*; Diplo= *Diplorhoptrum*; Mono= *Monomorium*; Para= *Paratrechina*; Phei= *Pheidole*; Tapi= *Tapinoma*; Pseu= *Pseudomyrmex*; Fore= *Forelius*; Sole= *Solenopsis*).

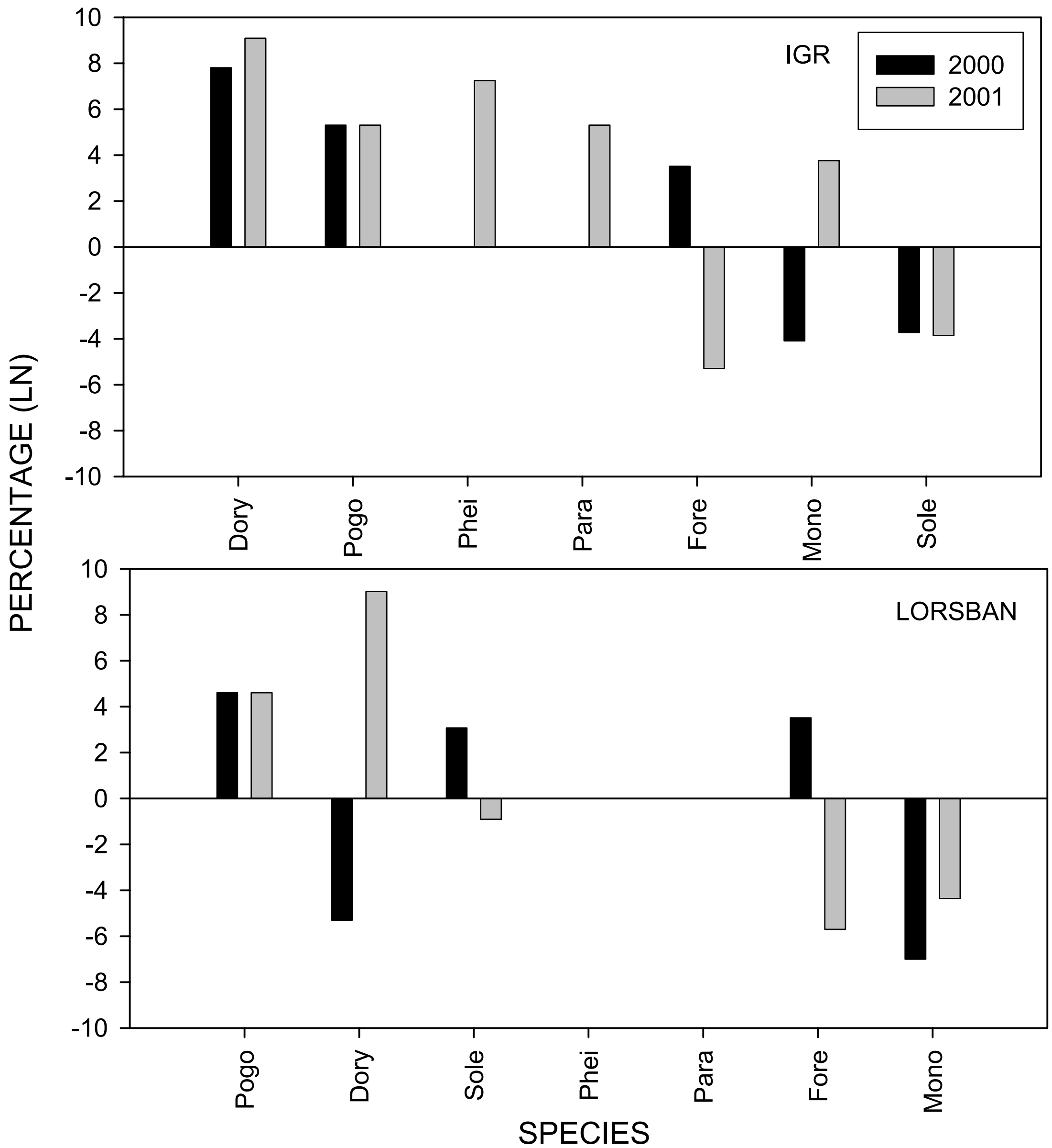

Figure 18. Ant species percentage (ln) increase or reduction in colony surveys observed in all treatments in 2000 and 2001 in relation to the Control. (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Pogo= *Pogonomyrmex*; Dory= *Dorymyrmex*; Tapi= *Tapinoma*).

monitored using pitfall traps show fluctuating densities throughout the two year sample period (Fig. 12). Foraging ants collected in baited vials are shown in Fig. 13. Ants detected by visual inspection were observed using the tree as a foraging area (Fig. 14) and some species may be able to establish satellite colonies in them. The phenology and seasonality of these ant species can also be related to their predatory behavior. Their phenology resembles generalist predatory insects that have a functional response to pest increase (Liao et al 1984, Harris and Li 1996, Liao et al. 1984) and later on are able to switch from prey to prey, by successfully exploiting available resources. This is a strong argument for conservation of ants that needs further attention.

Species within the ant assemblage can be favored, disfavored or unaffected by various treatments (Markin et al 1974, Summerlin et al 1977, Phillips et al 1986, Roth et al 1994, Matlock and De La Cruz 2003). This can be observed by increase, decrease or neutral response in density (pitfalls), foraging (baited vials, direct sampling) and colony appearance (colony transect survey). The response of each species to IGR and chlorpyrifos treatments was assessed by comparing the density of each species in their respective treatment to their density in the Control using data from the four sampling methods (Figs. 15, 16, 17, 18). IGR pitfall data showed seven species consistently were equal or greater in density compared to control, four fluctuate in density, and six were consistently equal to or less than their respective density in the control (Fig. 15). *D. flavus* showed the greatest increase in response to IGR treatments and *S. invicta* the greatest decrease. Bait vial data in IGR plots showed six species had an insignificant decrease compare to Control, one species was unaffected and two were consistently reduced compared to Control (Fig. 16). *D. flavus* most consistently increased and *S. invicta* showed the greatest decrease. Direct sampling in IGR plots showed six species higher in density and only *S. invicta* reduced in density compared to the control (Fig. 17). Colony surveys in IGR plots showed that four species increased, one fluctuated and two decreased compared to the control. *D. flavus* was the species that increased the most and *S. invicta* decreased the most (Fig. 18).

IGR treatments had the greatest adverse impact on *S. invicta* among species comprising the ant assemblage; *S. invicta* was significantly reduced in IGR plots compared to those in the Control. Given that *S. invicta* dominated the ant assemblage in this orchard (killing competing ants outright, raiding nests, locating and removing food resources, thoroughly disrupting previous relationships in the preexisting ant assemblage occurring before RIFA invasion, to name a few), the responses of the remaining species in the ant assemblage to a reduction in RIFA dominance reflects an ecological succession process involving the capacity of each species in the assemblage to respond to the absence or reduction of RIFA. Additionally, RIFA continues to occur as the first or second (except bait vials in 2000) most abundant species in IGR plots throughout the study. An understanding of the effect of the treatments themselves on the phenology and abundance of each native ant species is needed to understand behavior associated to the reduction of *S. invicta*. Fifteen native ant species were found in infested and RIFA reduced areas and coexisting with this invasive species. Several species of native ants appear to pose some resistance to the invasion. A brief review is presented of the interactions of each ant species with RIFA during and after the treatments.

Subfamily Dolichoderinae. *Dorymyrmex flavus* McCook, an epigeic and occasional arboreal species is known as the "pyramid ant" or the "lion ant," presumably because of their aggressive habits. Nests are found on the orchard floor, usually in open and sunny places, and mainly in sandy soil. They have a typical and conspicuous nest with a single opening in a circular crater in a volcano-like, 5-10 cm diameter. This species was recorded by all sampling methods (Figs. 19, 20, 21, 22) indicating a widespread presence at low density in the ant species hierarchy under natural conditions. This species seemed to be one of the most affected species by the presence of fire ants. Its abundance increased following the IGR treatment, which seemed to improve the fitness of this species. In Chapter III the interactions between *D. flavus* and *S. invicta* are described in more detail. This species also appeared to be somewhat resistant to the reinvasion of fire ants. Abundance for 2000, 2001 and 2002 recorded in pitfall traps (Fig. 19) in IGR plots was significantly higher and statistically different for each year compared to the Control (ANOVA, 2 df; $P<0.05$). Foraging behavior recorded using baited vials (Fig. 20) was also significantly higher for 2000 and 2001 among treatments (ANOVA, 2 df; $P<0.05$). Direct sampling showed increased abundance in IGR (Fig. 21). *D. flavus* was more frequently collected by direct sampling in IGR plots compared to other treatments and significantly higher (ANOVA, 2 df; $P<0.05$). Nests of *D. flavus* were more frequently found in IGR plots for 2000, 2001 and 2002 (Fig. 22), although it was significantly higher in 2000 (ANOVA, 2 df; $P=0.023$). No differences were found in nest density in 2001 and 2002 (ANOVA, 2 df; $P>0.05$).

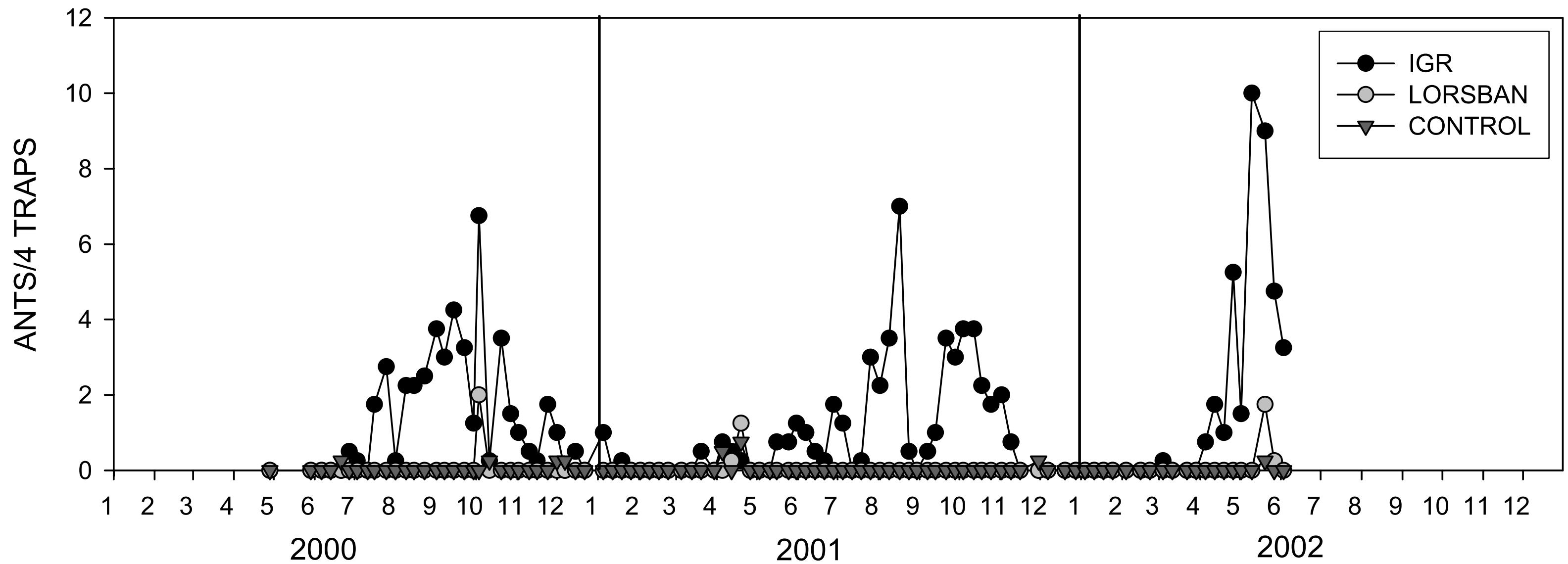

Figure 19. *Dorymyrmex flavus* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

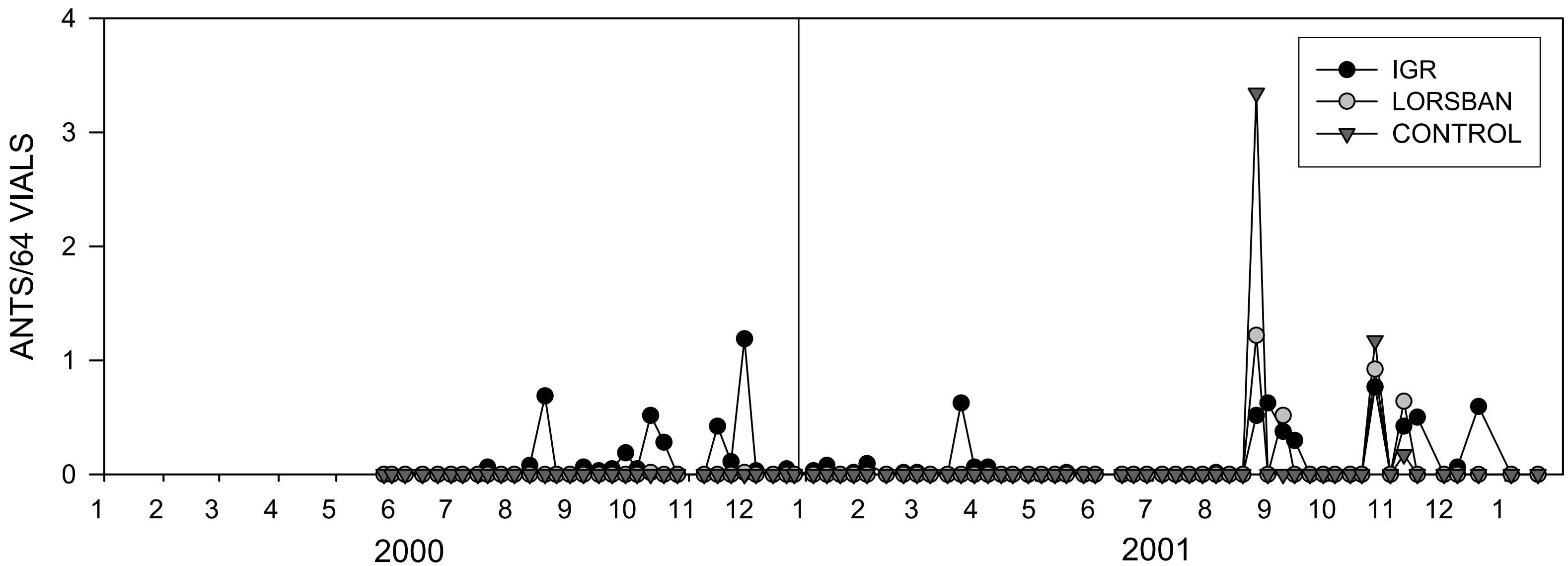

Figure 20. *Dorymyrmex flavus* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

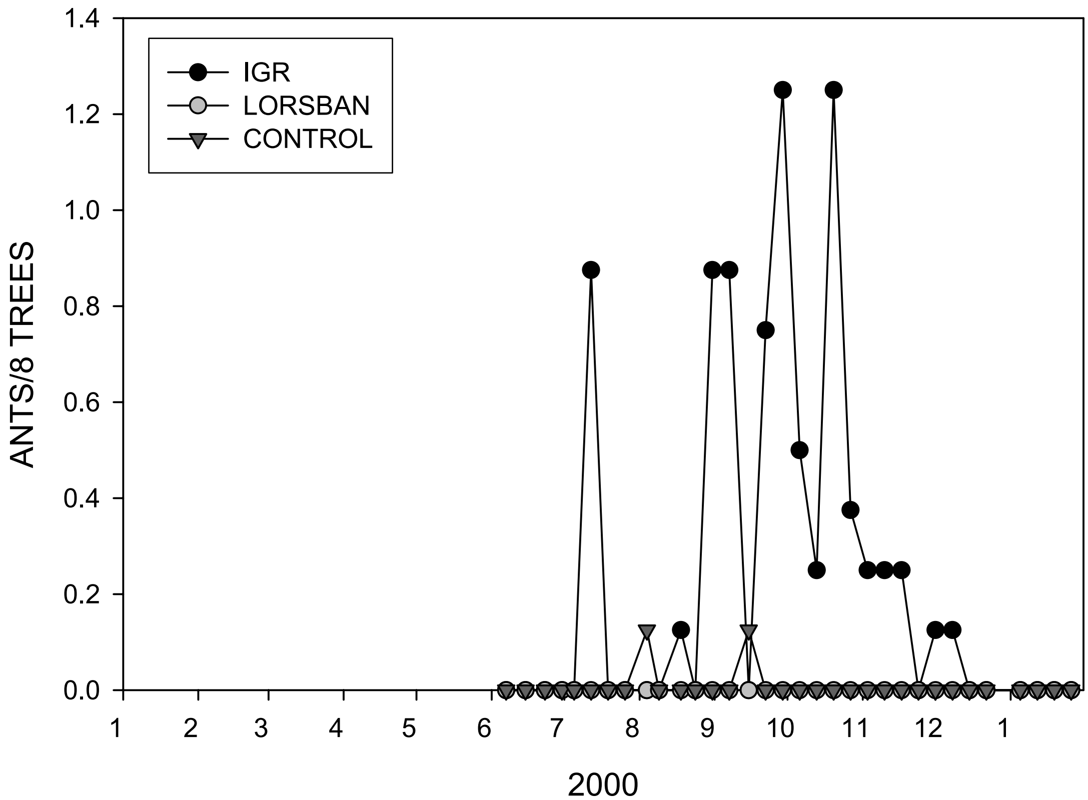

Figure 21. *Dorymyrmex flavus* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

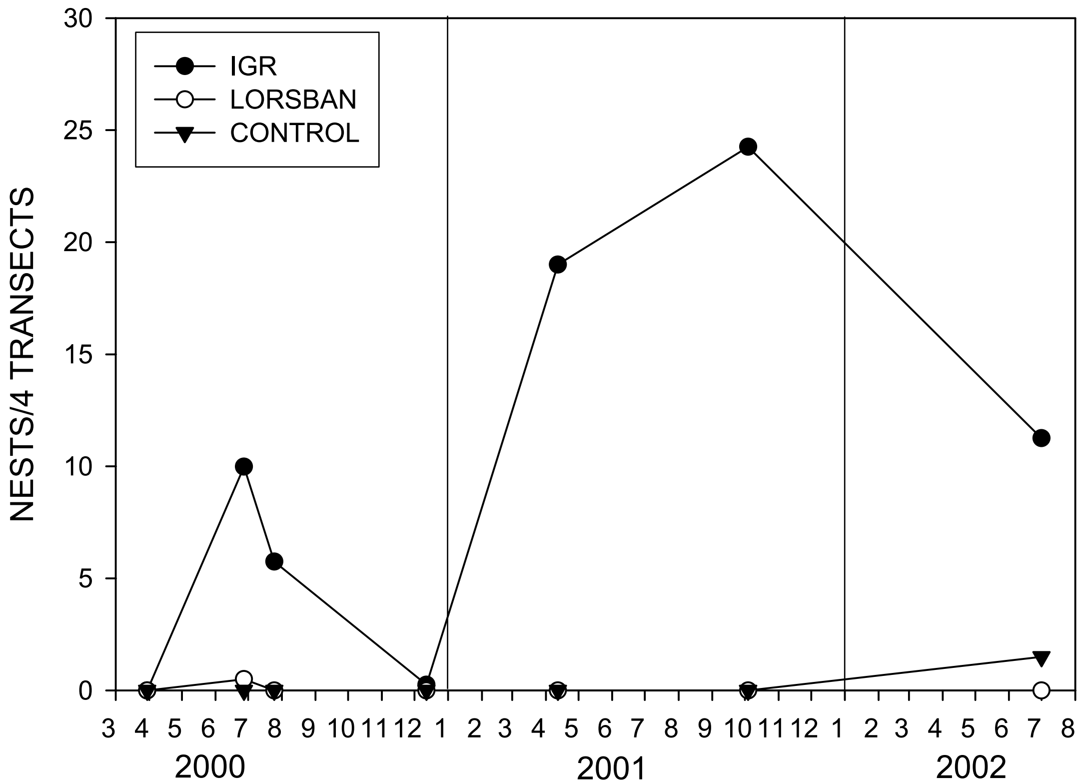

Figure 22. *Dorymyrmex flavus* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

Forelius pruinosus (Roger), an epigeic species known as "cheese ant," constructs their nests in the ground in sandy and open areas similar to *D. flavus*. Nests also resemble *D. flavus* and may be confused with them, but the crater is smaller with a single hole. *F. pruinosus* was regularly observed using all sampling methods and showed up best in pitfall traps (Fig. 23), relative to other techniques (Figs. 24, 25, 26). This species was somewhat affected when RIFA was treated with IGR bait (Figs. 23, 24, 25, 26). Pitfall trap data however indicated no significant differences were found among treatments or years (Fig. 23). The same was found for baited vials (Fig. 24) and direct sampling (Fig 25), and no effect was found on activity between treatments or years. Nest colonies also remained stable (Fig. 26) throughout the seasons and the treatments, with no significant differences found (ANOVA, 2 df; $P>0.05$). This species has been reported killing and feeding on fire ants, which may indicate they survive in infested areas and are able to coexist with the red imported fire ant (Wheeler and Wheeler 1973). Also, it has been reported that *F. pruinosus* prevent workers of fire ant from leaving their nest to forage, which may help to reduce the fitness of the RIFA colony (Rao and Vinson 2004).

Tapinoma sessile (Say), an epigeic and occasionally arboreal species is known as the "odorous ant." This species nests in a wide variety of microhabitats, from open fields and pastures to areas with trees. *T. sessile* is known to be attracted to honeydew, and to tend honeydew-excreting insects such as aphids and mealybugs (Smith 1972, O'Keefe et al. 2002). This species was detected with pitfall traps (Fig. 27) and found foraging in pecan trees (Fig. 28) when pecan aphid outbreaks were occurring. No significant differences between treatments were found in 2000 for this species

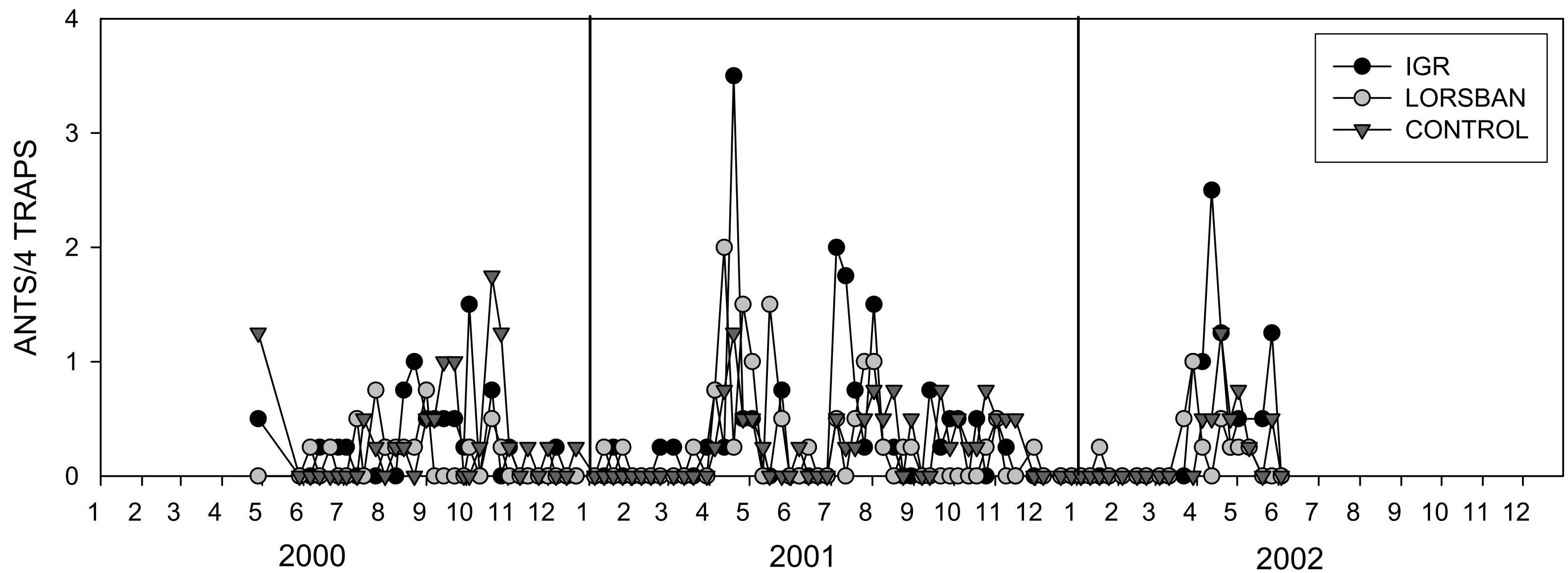

Figure 23. *Forelius pruinosus* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

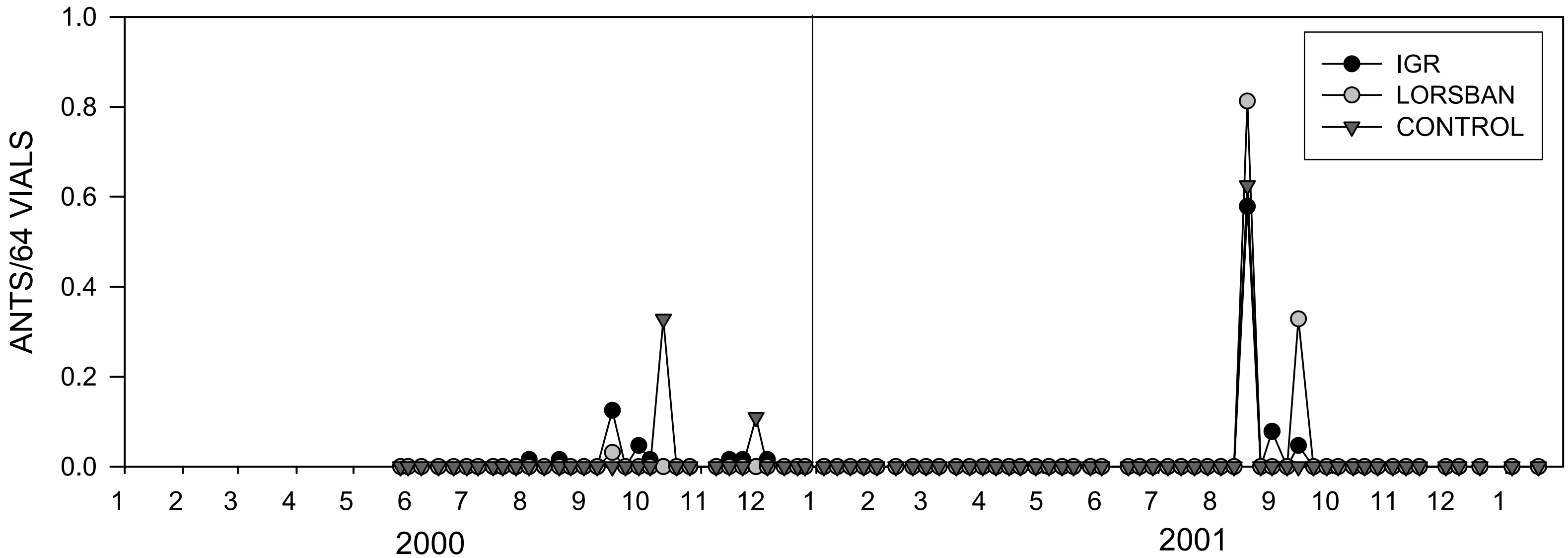

Figure 24. *Forelius pruinosus* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

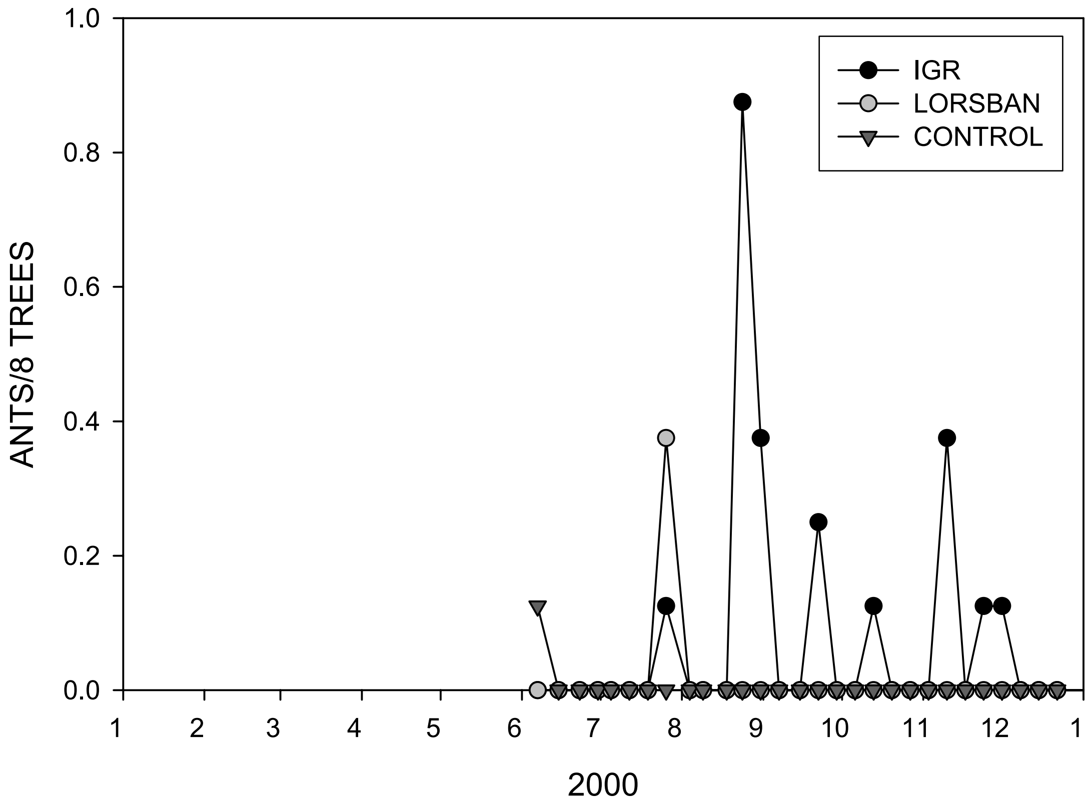

Figure 25. *Forelius pruinosus* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

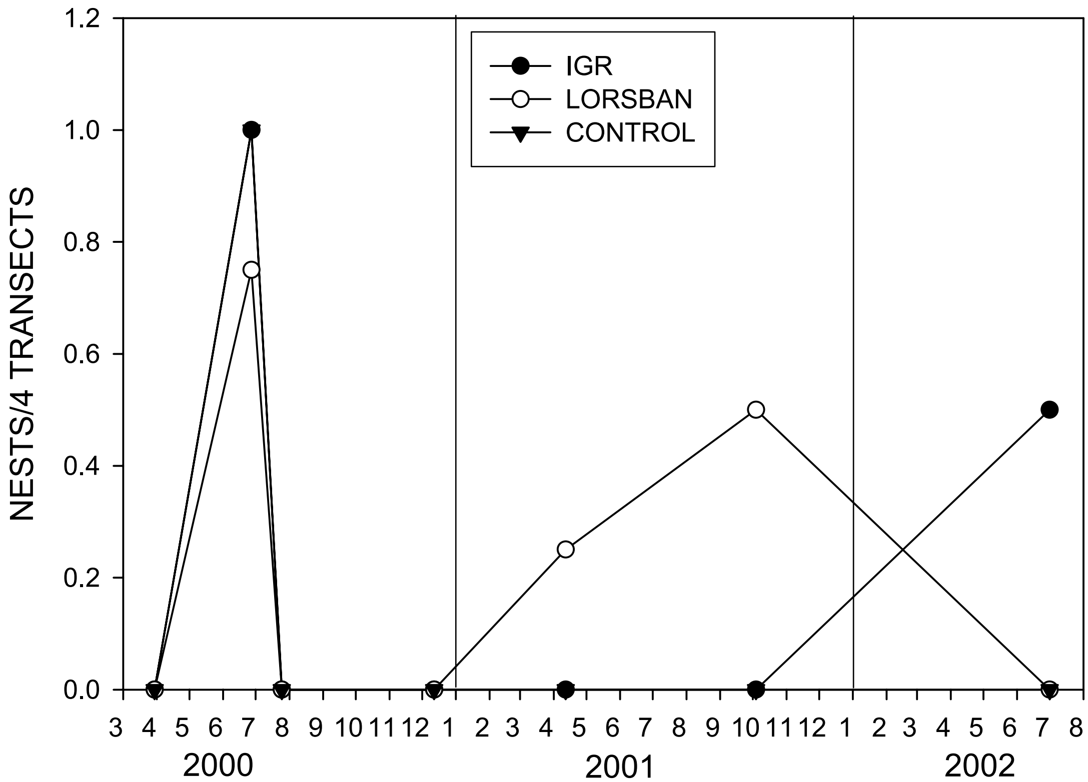

Figure 26. *Forelius pruinosus* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

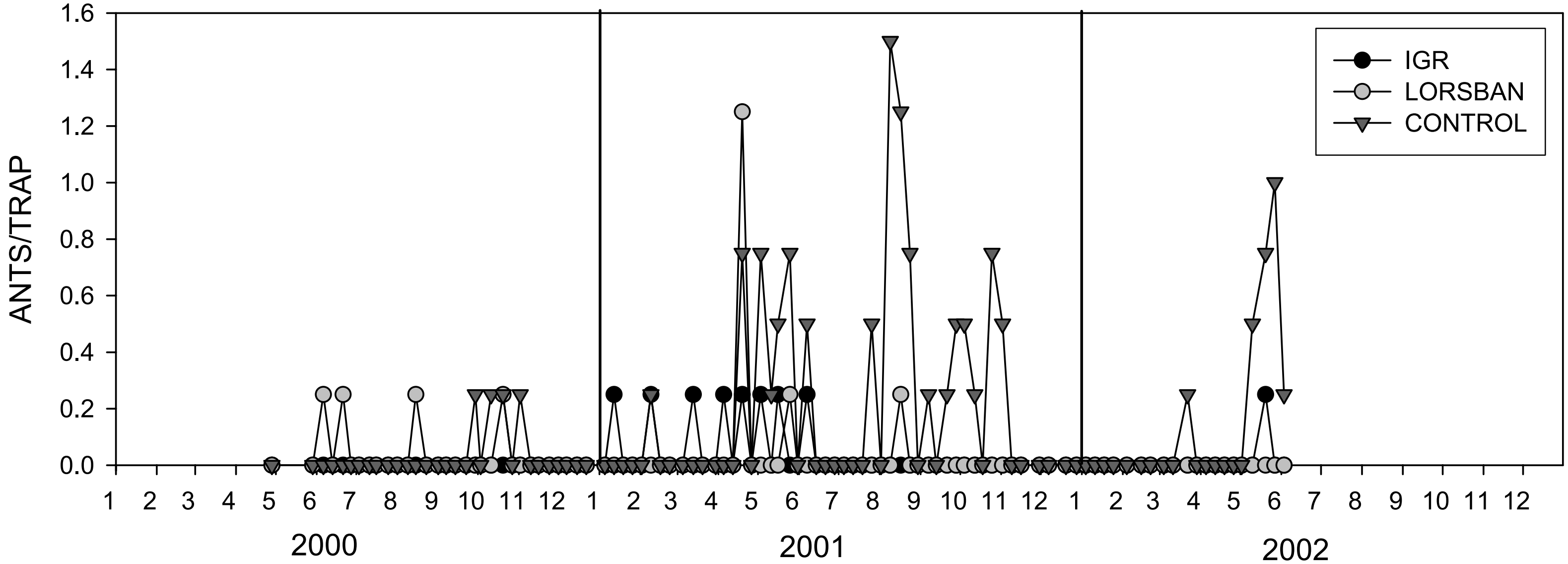

Figure 27. *Tapinoma sessile* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

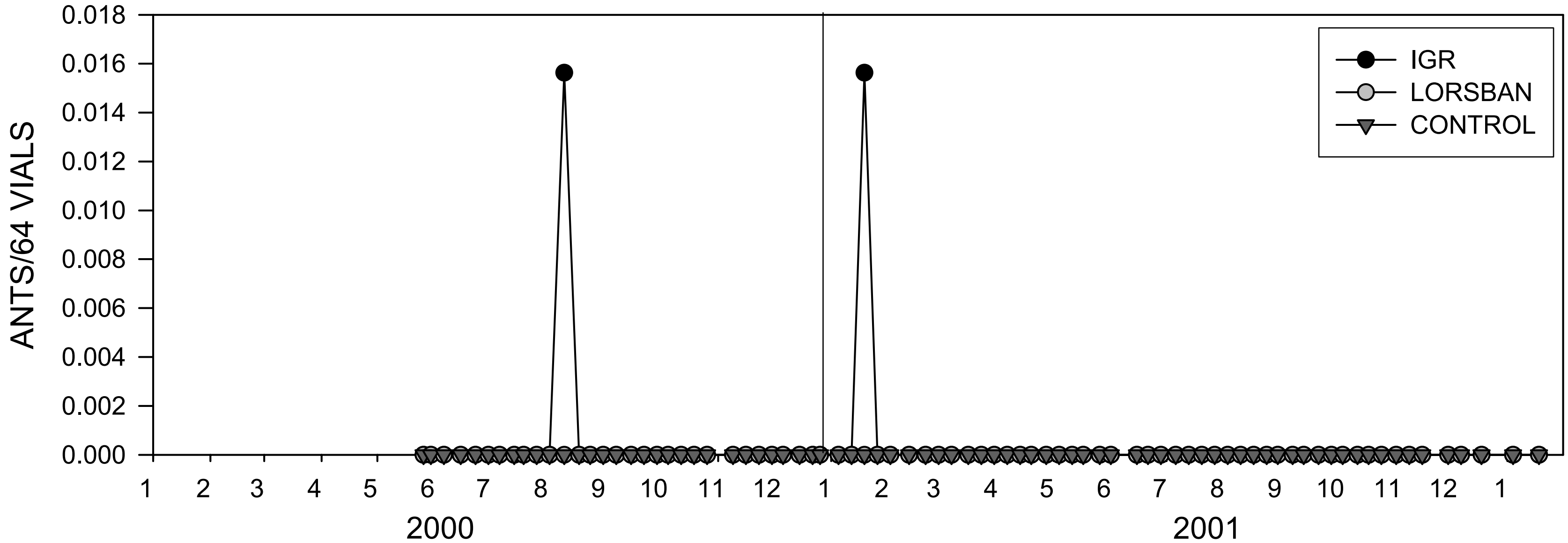

Figure 28. *Tapinoma sessile* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

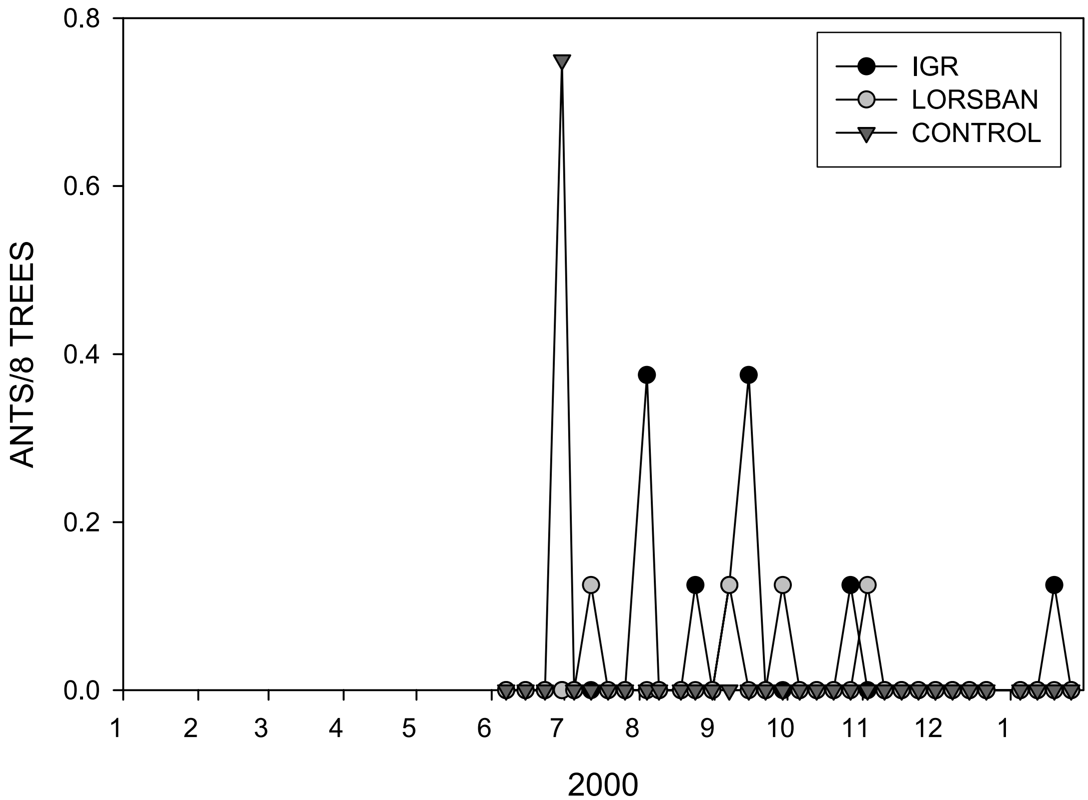

Figure 29. *Tapinoma sessile* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

in pitfall traps, but it was significantly higher in Control plots in 2001 and 2002 in pitfall traps, but it was significantly greater in Control plots in 2001 and 2002 (ANOVA, 2 df; P<0.05) and coexisting with RIFA (Fig 27). *T. sessile* was not frequently collected in baited vials (Fig. 28). Direct sampling also showed *T. sessile* was significantly higher in Control plots in 2000 (ANOVA, 2 df; P=0.024) when ants were observed actively foraging and scouting on trees within these plots (Fig. 29).

Subfamily Formicinae. *Brachymyrmex sp.*, a small epigeic-hypogeic ant was just detected only a few times in pitfall traps (Fig. 30). This species build inconspicuous nests under rocks and pieces of wood and are known to feed on honeydew excreted by aphids and mealybugs (O'Keefe et al. 2002). *Brachymyrmex* was significantly greater in 2000 in the Control plots and significantly higher in IGR and chlorpyrifos plots in 2001 (ANOVA, 2 df; P<0.05), no specimens were collected in 2002.

Paratrechina sp., an epigeic species known as "crazy ant," an apt eponym characterizing peripatetic locomotion behavior including even jumping at times. *Paratrechina* feeds on honeydew produced by aphids and mealybugs, and also is an active predator of some insect species (Smith 1972, O'Keefe et al. 2002). This species forms ground nests under objects and often under wood. *Paratrechina sp.* was detected by all methods (Figs. 31, 32, 33, 34) and occurred throughout the year.

This species was frequently collected in pitfall traps among the treatments and throughout the years (Fig. 31). No significant differences were found among treatments in 2000 and 2001 (ANOVA, 2 df; P<0.05), but it was significantly greater in IGR plots

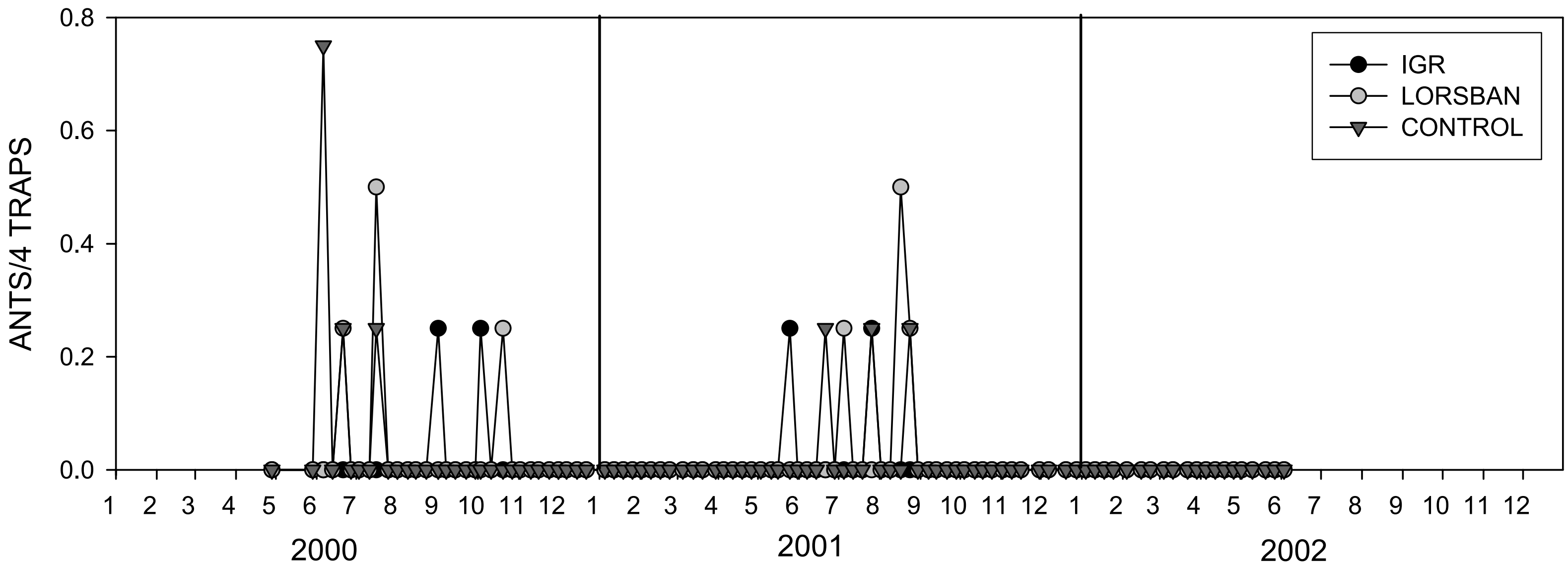

Figure 30. *Brachymyrmex sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

in 2002 (ANOVA, 2 df; P<0.035). It was collected in baited vials (Fig. 32) and it was significantly higher in IGR and chlorpyrifos plots in 2000 (ANOVA, 2 df; P=0.038). No significant differences were found among treatments in 2001. Direct sampling (Fig. 33) results indicate no significant differences among treatments in 2000. In colony surveys (Fig. 34), nests of this species were not observed in 2001, but other techniques consistently showed its presence. *Paratrechina sp.* is found coexisting with RIFA. This is another species which is considered predacious on RIFA, preying upon newly mated queens (Whitcomb et al. 1973, Stimac and Alves 1994) and also coexisting with RIFA in other habitats (Porter and Savignano 1990).

Subfamily Myrmecinae. *Cyphomyrmex wheeleri* Forel, an epigeic species known as "fungus ant" was only detected in pitfall traps (Fig. 35). *C. wheeleri* build small colonies in the ground and often in exposed areas of the orchard, or under rocks. This species usually feed on fungi they grow and sometimes are found scavenging (Smith 1972, O'Keefe et al. 2002). This species was observed in all treatments seeming to coexist with RIFA.

Monomorium minimum (Buckley), an epigeic and arboreal species known as the "little black ant." *M. minimum* nests in exposed areas, mainly of clay and dark soil. Usually scavengers, they may occasionally prey upon other insects. *M. minimum* occurs throughout the year and is easily detected using the different sampling techniques (Figs. 36, 37, 38, 39). This species was one of the most common species collected in all treatments. *M. minimum* did not show significant differences in pitfall traps among

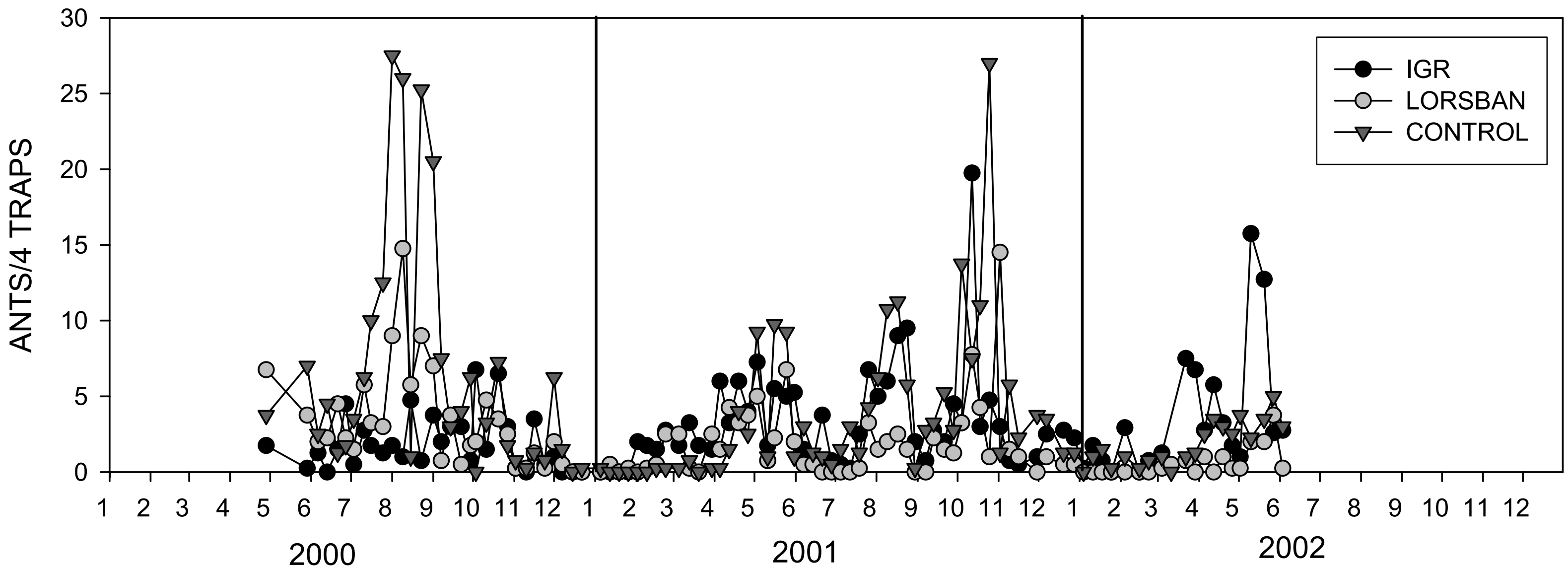

Figure 31. *Paratrechina sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

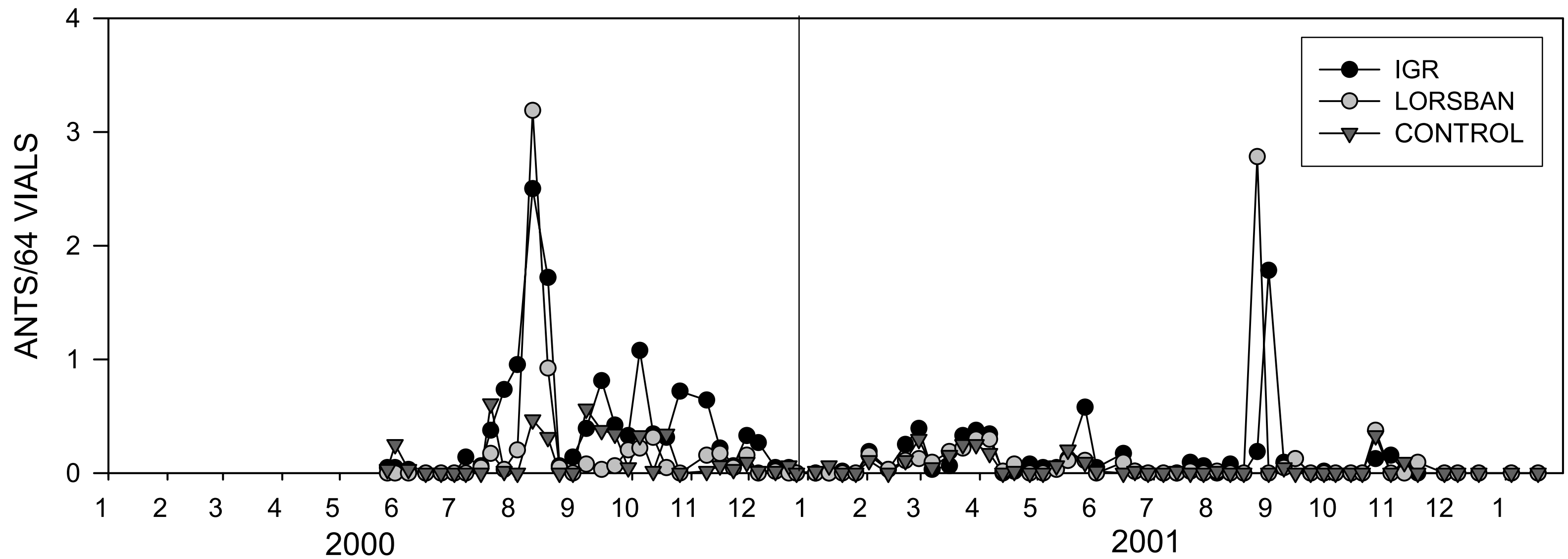

Figure 32. *Paratrechina sp.* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

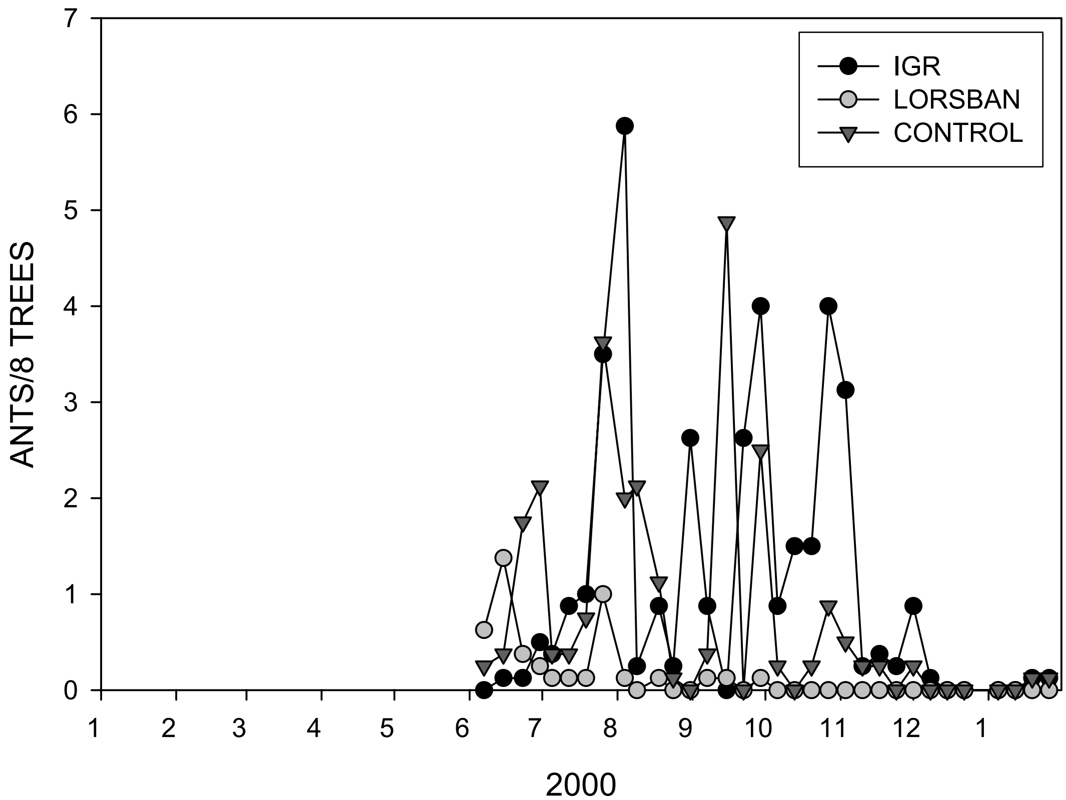

Figure 33. *Paratrechina sp.* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

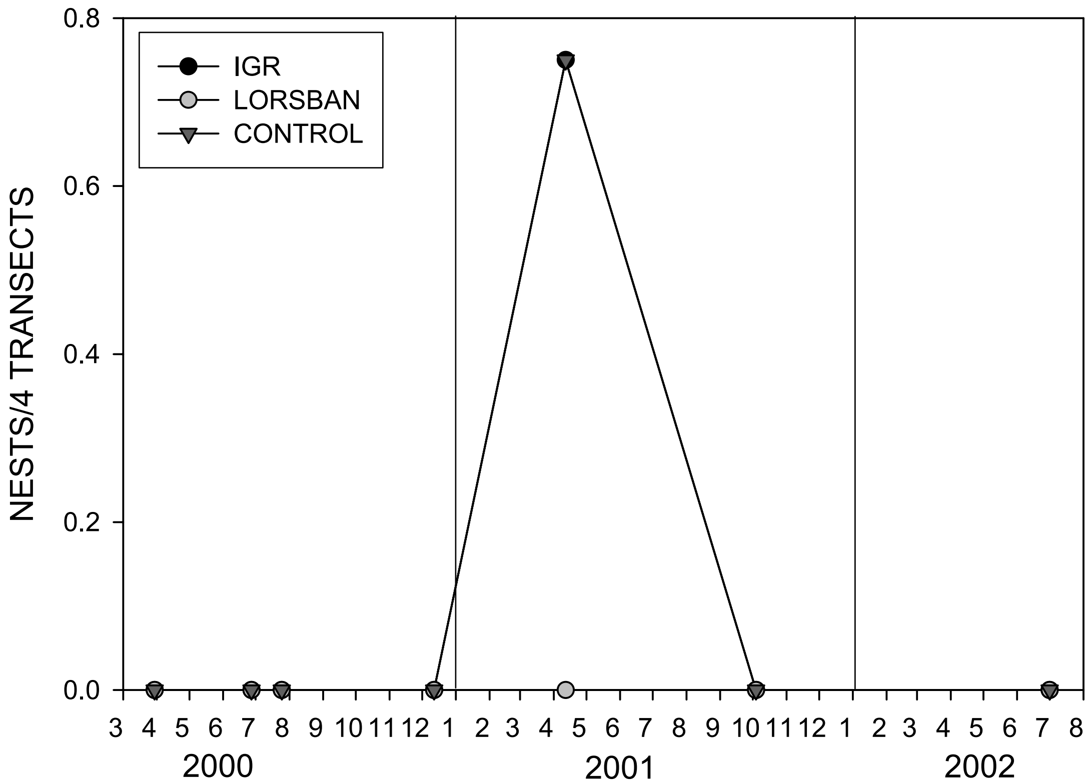

Figure 34. *Paratrechina sp.* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

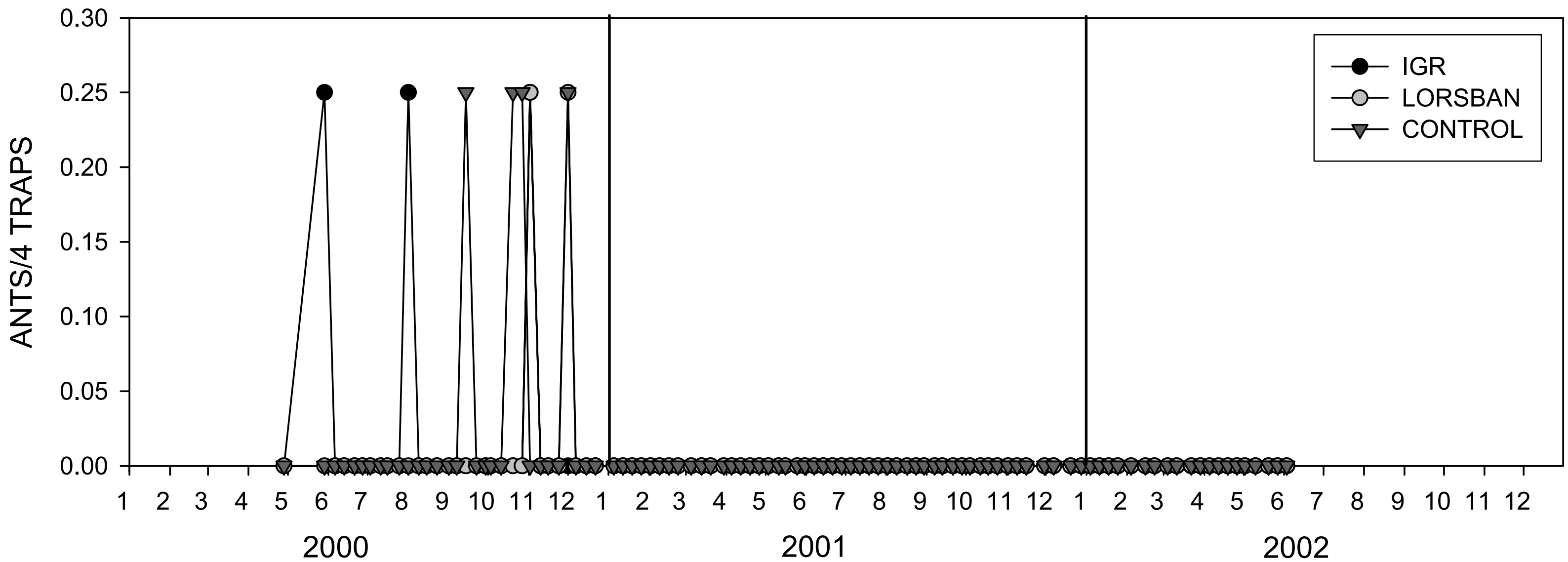

Figure 35. *Cyphomyrmex sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

treatments for any year (Fig. 36). Numbers of ants recruited to baited vials remained stable throughout the year, and did not show major differences among treatments in 2000 (Fig. 37). However, numbers were significantly higher in baited vials located in IGR plots 2001 (ANOVA, 2 df; $P=0.029$). This species was also collected in all treatments using direct sampling (Fig. 38). Nests of this species were consistently found among all treatments and did not show significant differences among treatments (ANOVA, 2 df; $P>0.05$) (Fig. 39). This species has also been reported coexisting with RIFA (Porter and Savignano 1990), as an important predator of newly mated queens and being capable of attacking and destroying new and small RIFA colonies (Nichols and Sites 1991). Rao and Vinson (2004) suggested this species interacts with fire ants by attacking and eliminating their workers.

Myrmecina americana Emery, an epigeic species is a specialist predator of mites. *M. americana* usually nest under rocks, in moist and shady areas. It was found only once in pitfall traps during the entire study. This very rare and cryptic species is assumed to prefer woody habitat surrounding this agroecosystem. This species was collected only on a few occasions (Fig. 40) and interactions with RIFA were not well established.

Pheidole sp., an epigeic species commonly found in this orchard in sandy soil and open areas. *Pheidole* is often seen coexisting with *D. flavus*. Trails are dense near the nest that they provision with seeds and plant materials. Colonies are easily spotted and do not represent any economic impact in pecan production.

This species was collected in all treatments in pitfall traps (Fig. 41). *Pheidole* did not show significant differences in 2000 and 2001 but was significantly higher in IGR

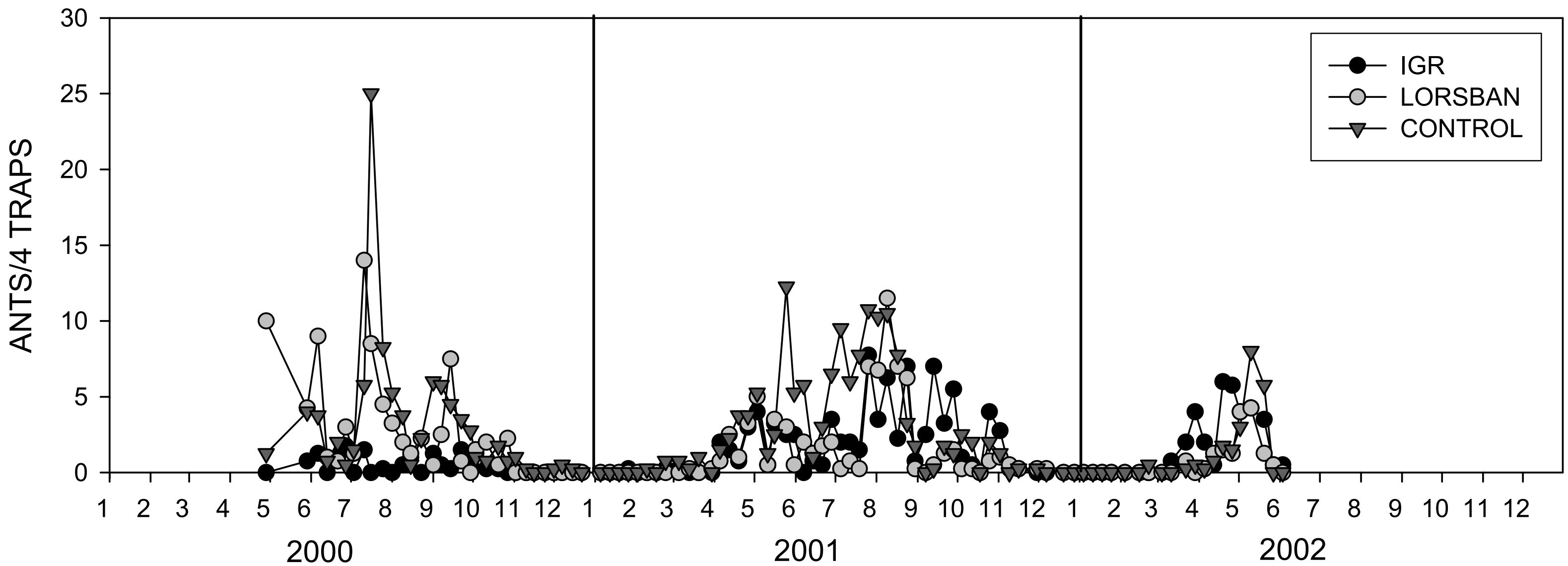

Figure 36. *Monomorium minimum* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

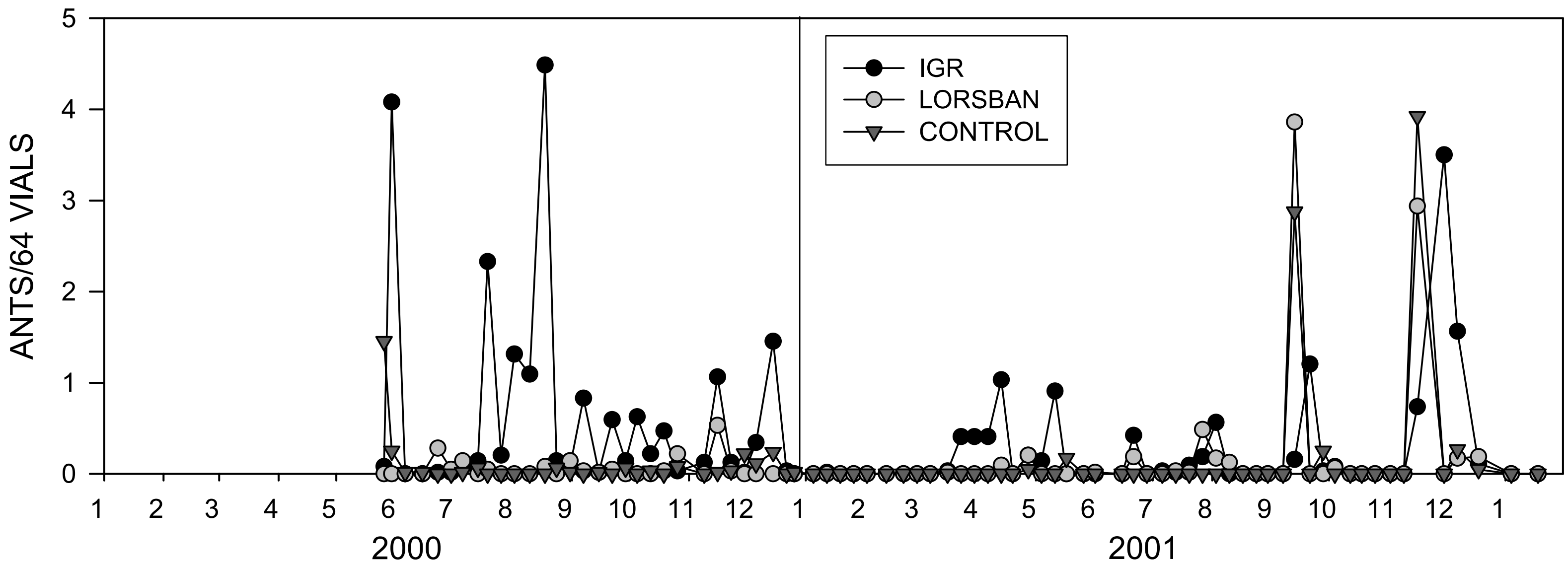

Figure 37. *Monomorium minimum* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

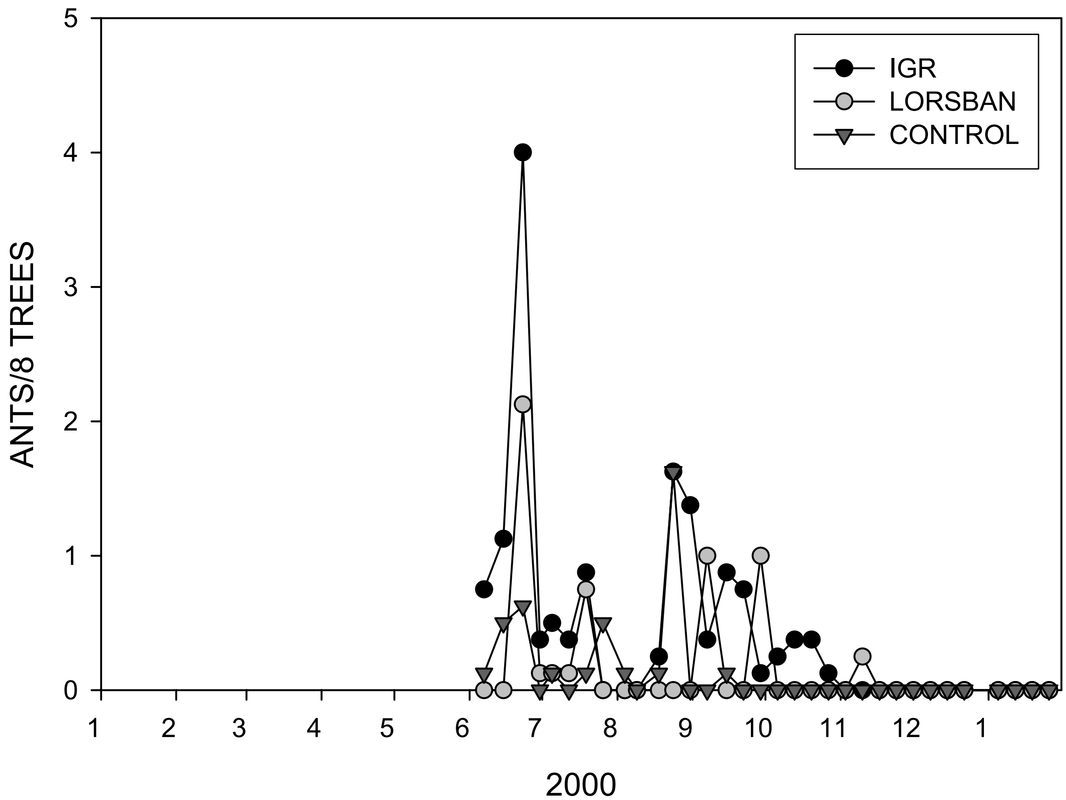

Figure 38. *Monomorium minimum* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

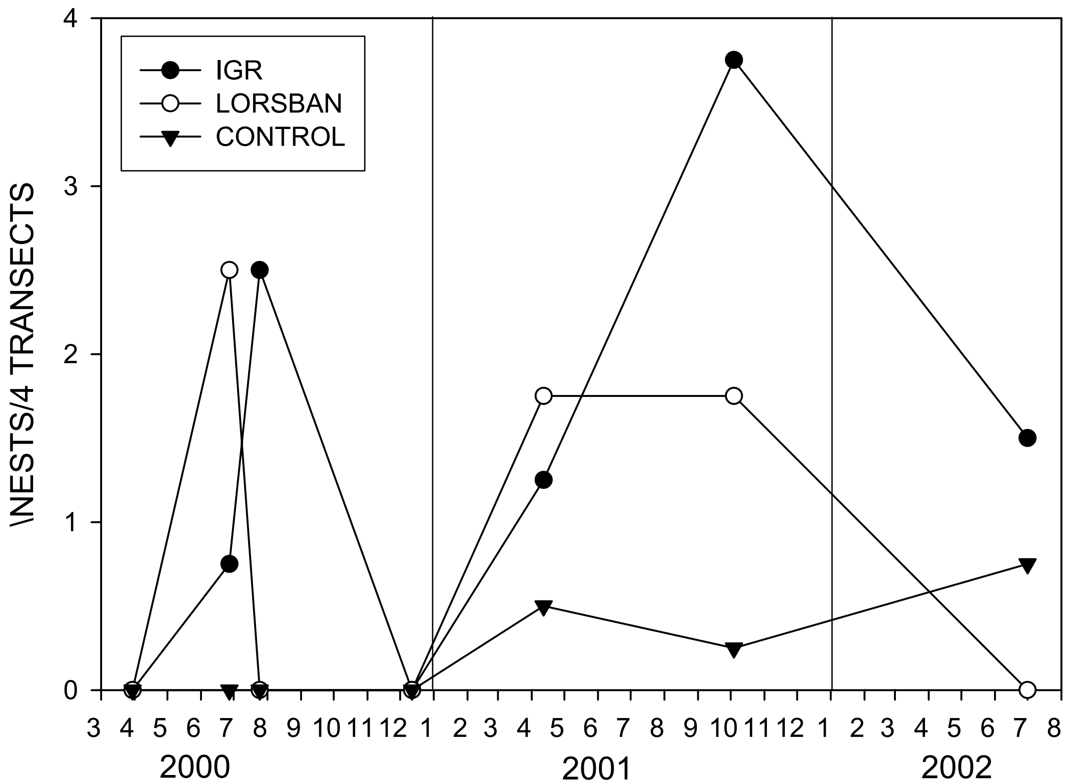

Figure 39. *Monomorium minimum* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

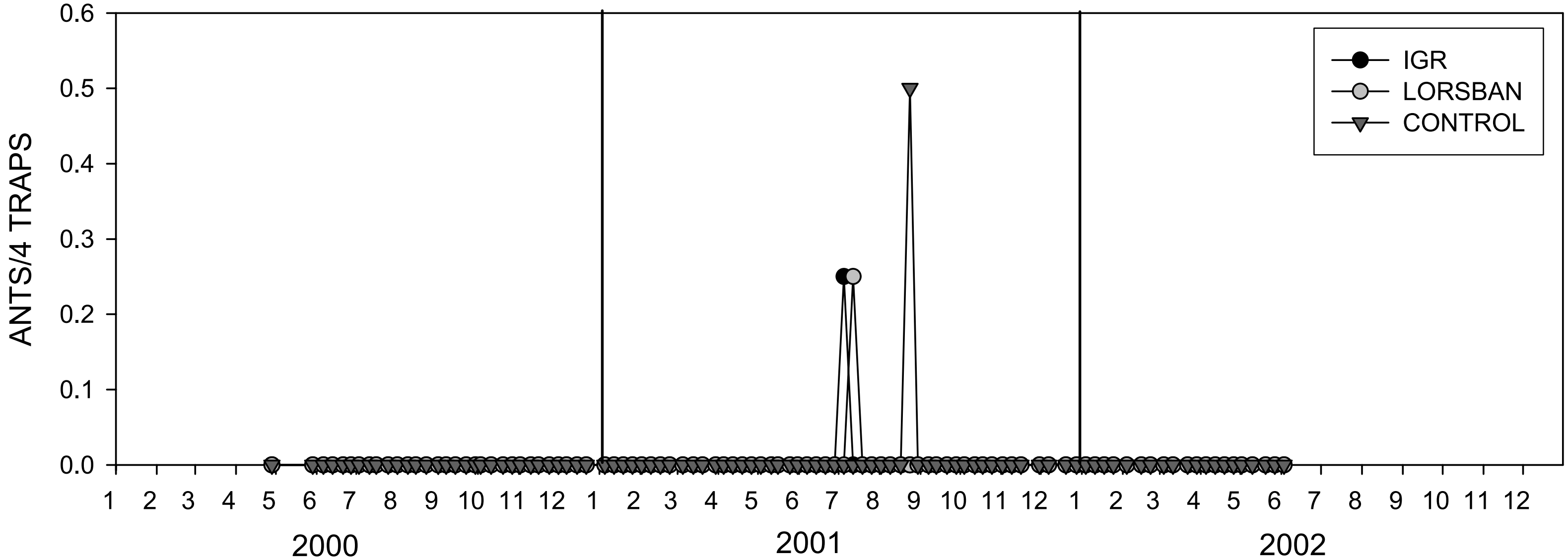

Figure 40. *Myrmecina sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

plots in 2002 (ANOVA, 2 df; P=0.004). It was not collected often in baited vials in 2000 and 2001 (Fig. 42) but numbers were significantly higher in IGR plots (ANOVA, 2 df; P=0.004). This species was occasionally collected in the trees using direct sampling (Fig. 43), but did not differ from Control. *Pheidole* was observed on many occasions in trees foraging nearby RIFA trails. Nest density did not differ among treatments (Fig. 44). This species has been reported attacking RIFA workers and destroying small colonies, and they can defeat RIFA in defensive combat (Bhatkar 1973, Jones and Phillips 1987). Also, this species has been found preying upon brood of fire ants (Rao and Vinson 2004). *Pheidole* was positively affected by the IGR treatment (Figs. 15, 16, 17, 18).

Pogonomyrmex barbatus (Smith), an epigeic species known as "harvester ant" is commonly found in this orchard in sandy soil and open areas. *P. barbatus* is the largest ant in size found in this area. This species is often seen coexisting with *D. flavus*. Trails are dense near the nest that they provision with seeds and plant materials. Colonies are easily spotted and do not represent any economic impact in pecan production. *P. barbatus* was found in pitfall traps and detected during colony surveys (Figs. 45, 46). In pitfall traps, this species did not show significant differences among treatments in 2000 and 2001 but it was significantly higher in IGR plots in 2002 (ANOVA, 2 df; P<0.05) (Fig. 45). Colony density remained stable in 2000, 2001 and 2002 (Fig. 46) and they were not significantly different among treatments (ANOVA, 2 df; P>0.05). It was observed coexisting with RIFA. This species has been reported attacking RIFA workers, and killing queens (Taber 2000). Mound and ant density increased in the IGR treatment (Figs. 15, 16, 17, 18).

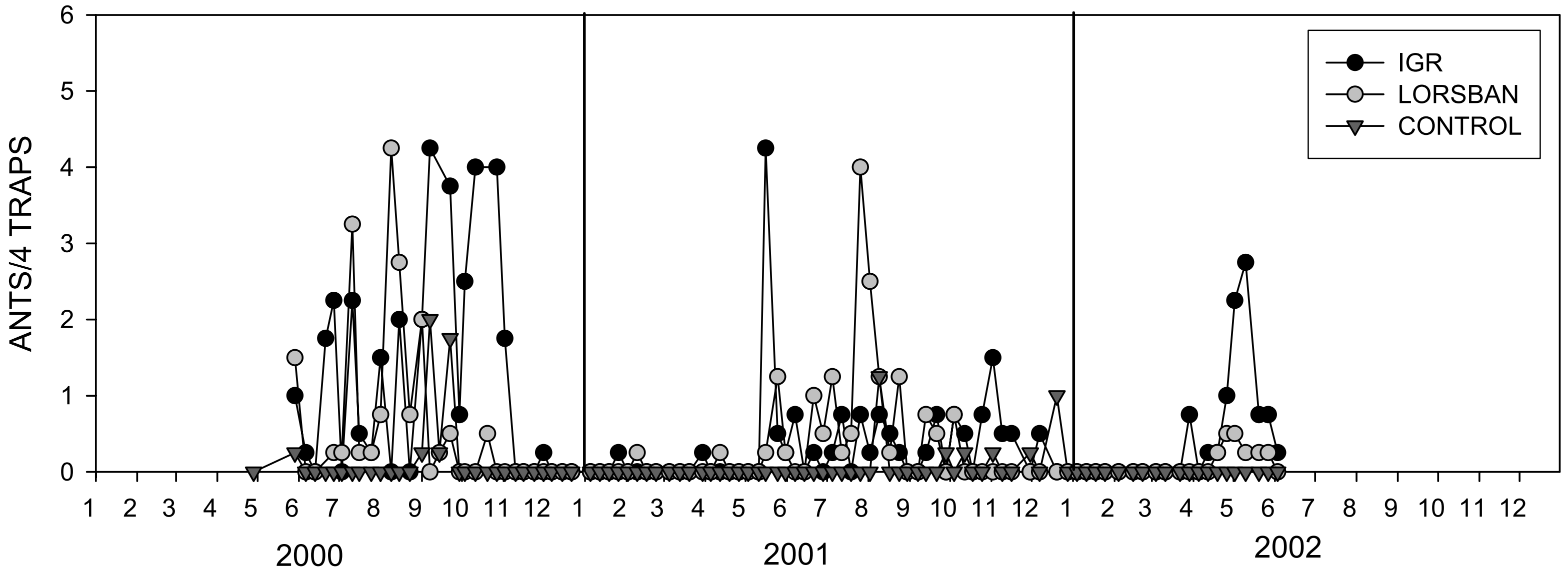

Figure 41. *Pheidole sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

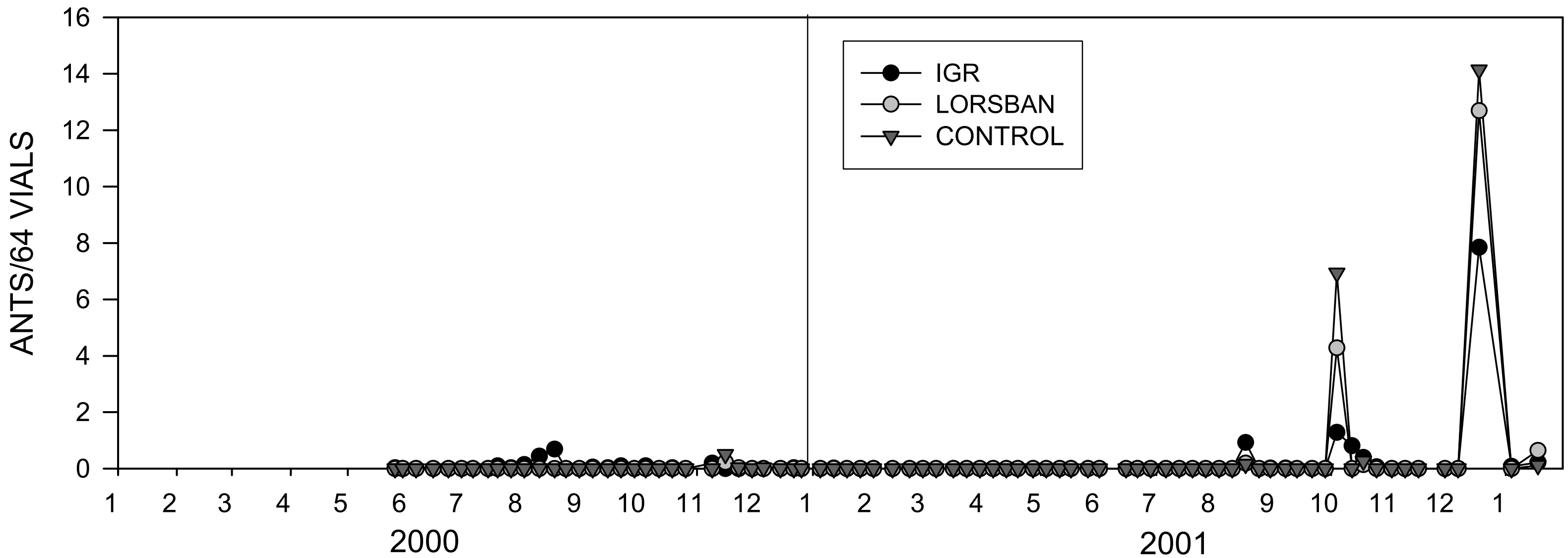

Figure 42. *Pheidole sp.* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

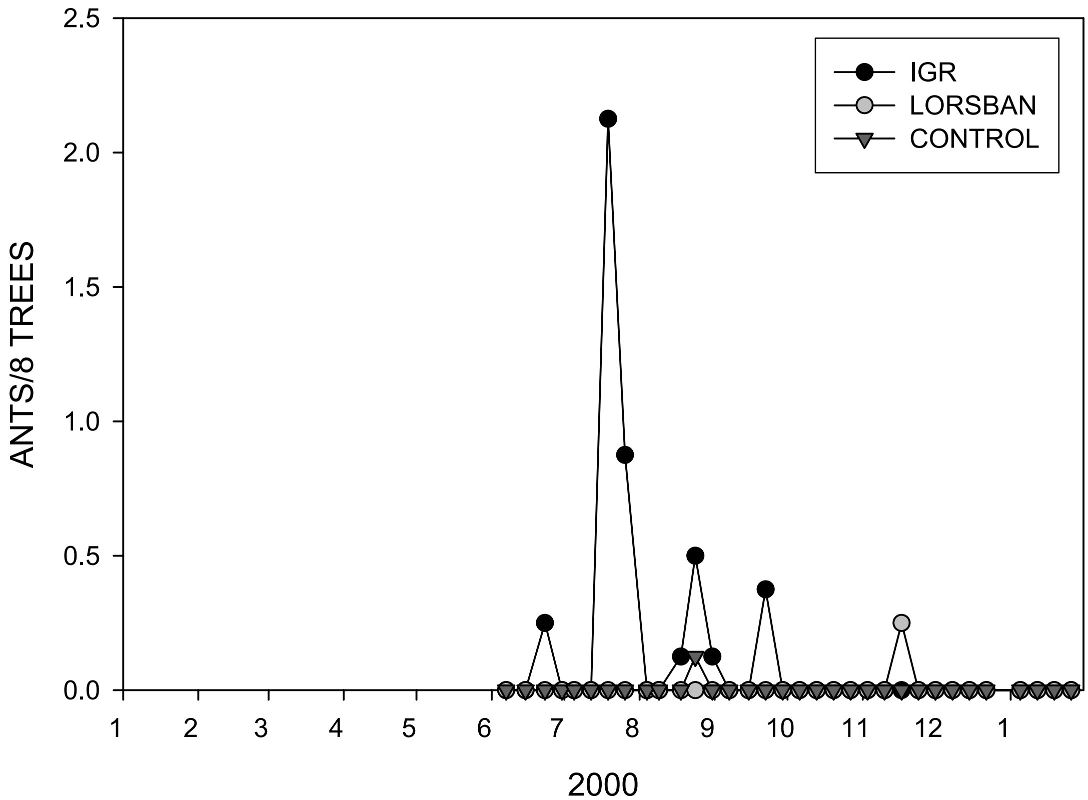

Figure 43. *Pheidole sp.* relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

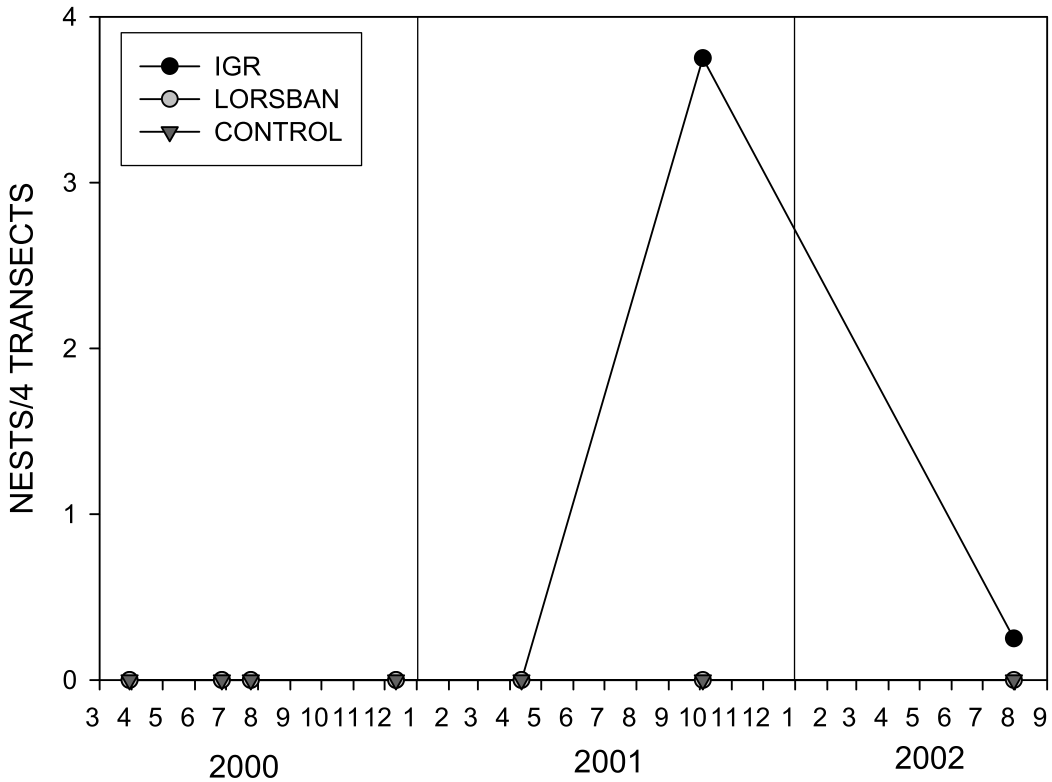

Figure 44. *Pheidole sp.* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

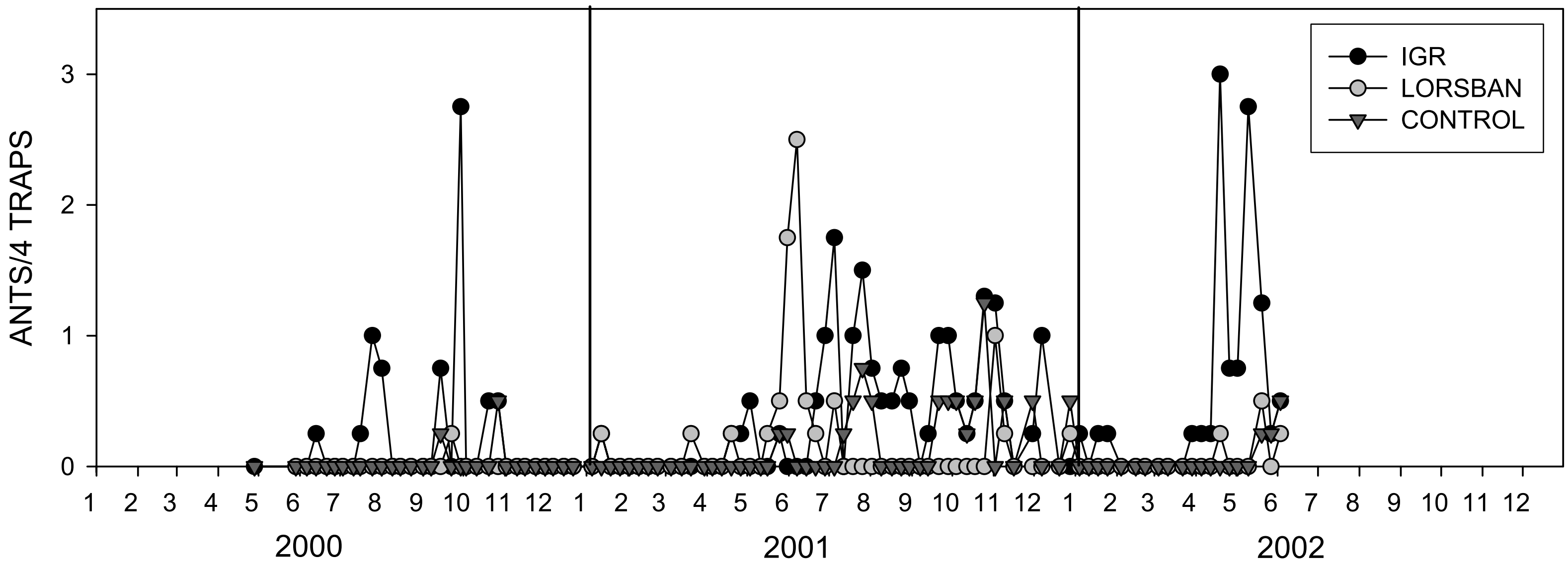

Figure 45. *Pogonomyrmex barbatus* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

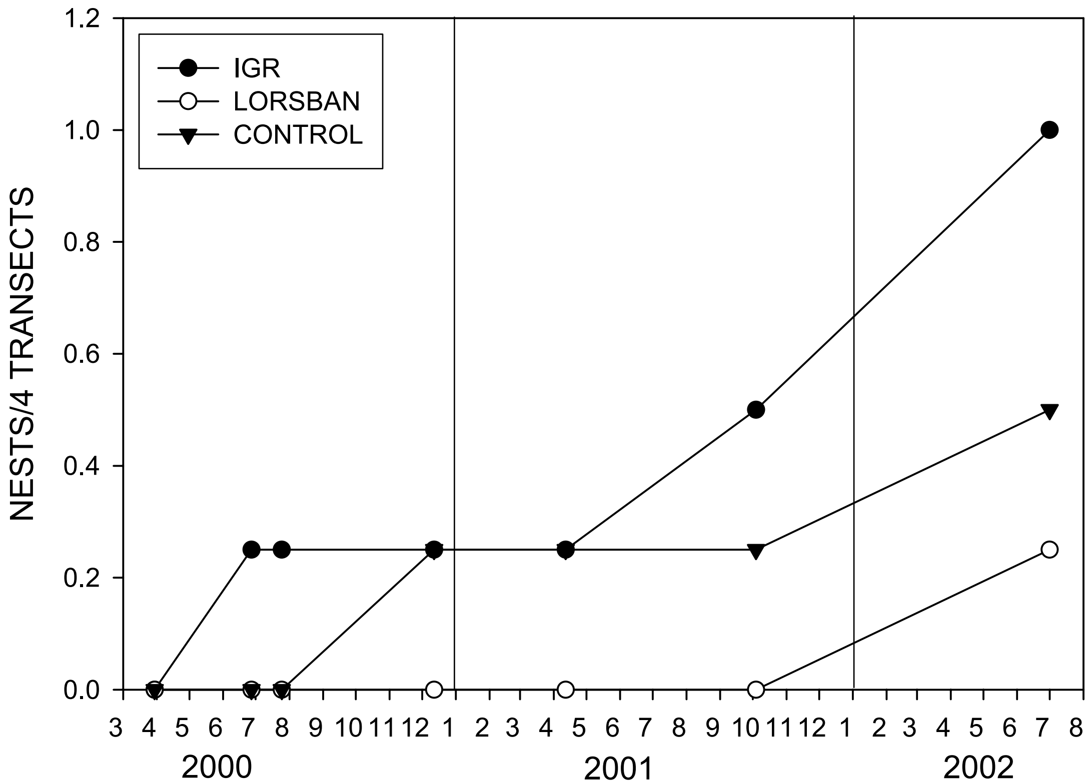

Figure 46. *Pogonomyrmex barbatus* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

Smithistruma sp., an hypogeic to epigeic species that is known to feed on small soft bodied insects such as collembolans and to also exploit nectar and aphid honeydew production (Dejean 1985). This small species was frequently collected in pitfall traps (Fig. 47). No significant differences were found among treatments in 2000, 2001 and 2002 (ANOVA, 2 df; $P>0.05$). The results indicate this species is coexisting with RIFA. *Smithistruma* has been reported somewhat resistant to RIFA (Ward 1987).

Solenopsis invicta, an epigeic species is known as the "red imported fire ant." *S. invicta* formed conspicuous mounds (up to 70 cm diameter and up to 30 cm in height) of loose earth on the ground floor of the orchard. This species is the most abundant species recorded during this study and the only species within the ant assemblage reported to have an economic impact in pecan production (Tedders et al. 1990, Dutcher et al. 1999, Harris et al. 2003). Colonies can be large (more than 100,000 individuals), usually constructed in exposed areas but also regularly found adjacent to pecan tree trunks. *S. invicta* was also observed in arboreal small colonies in the pecan trees preying on other insects and tending mealybugs. This species was found using all methods (Figs. 48, 49, 50, 51) and represented the most significant contribution in terms of biomass production within the ant assemblage. *S. invicta* may disrupt natural enemies efficiency (Tedders et al. 1990, Harris et al. 2003) but further studies are needed to understand the fire ant-aphid–natural enemy interaction.

The red imported fire ant was collected during all years in all treatments by all sampling techniques. In IGR plots RIFA showed a significant reduction in density in pitfall traps compared to other treatments (Fig. 48). Numbers of RIFA were significantly

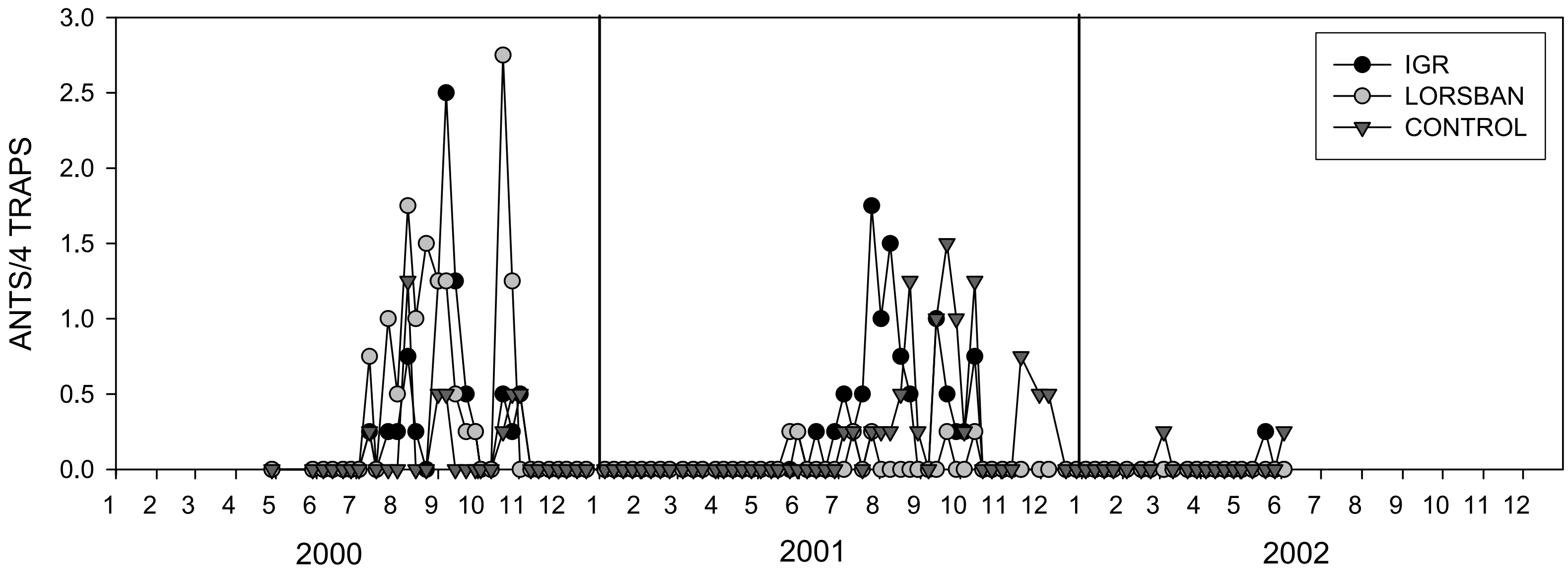

Figure 47. *Smithistruma sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

lower in 2000 (ANOVA, 2 df; $P=0.030$) and in 2001 (ANOVA, 2 df; $P<0.05$). No differences were found in 2002 when treatment was not applied, and RIFA was observed reinfesting IGR plots (ANOVA, 2 df; $P=0.421$). Foraging behavior was also affected (Fig. 49) for 2000 and 2001. Numbers of RIFA were significantly lower in IGR plots compared to the other treatments (ANOVA, 2 df; $P<0.05$). IGR plots had fewer RIFA workers foraging in the trees (Fig. 50), differences were found in the direct sampling method among treatments (ANOVA, 2 df; $P<0.05$), indicating the prolonged effectiveness of IGR and the shorter residual effectiveness of chlorpyrifos trunk treatment. RIFA recovered and was frequently observed foraging in trees three to seven days after Lorsban® trunk application. Mound density remained low in IGR plots during 2000 and 2001 (Fig. 51) and recovered in year 2002. Numbers were significantly lower in 2000, 2001 (ANOVA, 2 df; $P<0.05$) but not in 2002. These results showed the efficacy of IGR treatments, (Extinguish™) to decimate fire ant populations, reduce foraging behavior efficiency and enhance the native ant assemblage. Chlorpyrifos showed some differences but not consistently throughout the time and may also potentially alter the behavior of other ant species that are active in the trees.

Solenopsis (=Diplorhoptrum) sp., a hypogean-epigeic-arboreal species known as "thief ant," is a cryptic species generally not found on the soil surface. *Diplorhoptrum* is an opportunistic species that constructs underground nests near other colonies, from which they steal food and other resources. This species was abundant in pitfall traps (Fig. 52), sporadically found in baited vials (Fig. 53) and seems to be active throughout the year. This minuscule ant was consistently found throughout the study.

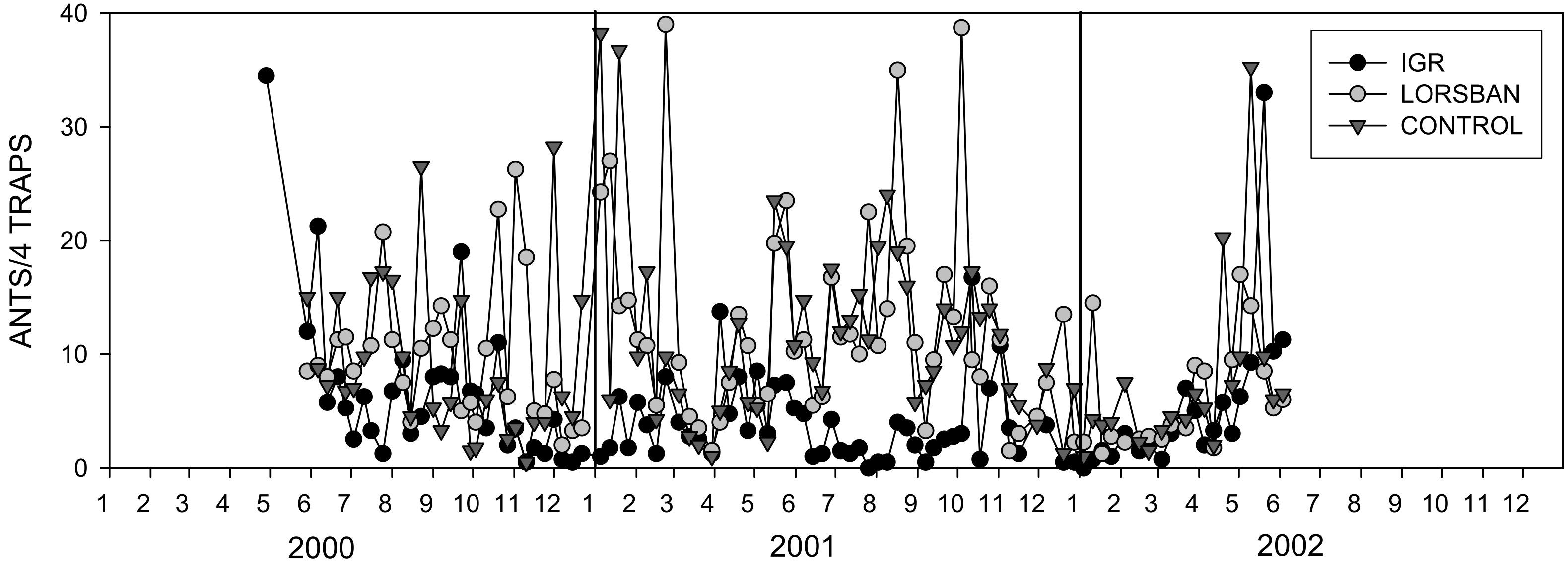

Figure 48. *Solenopsis invicta* Buren relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

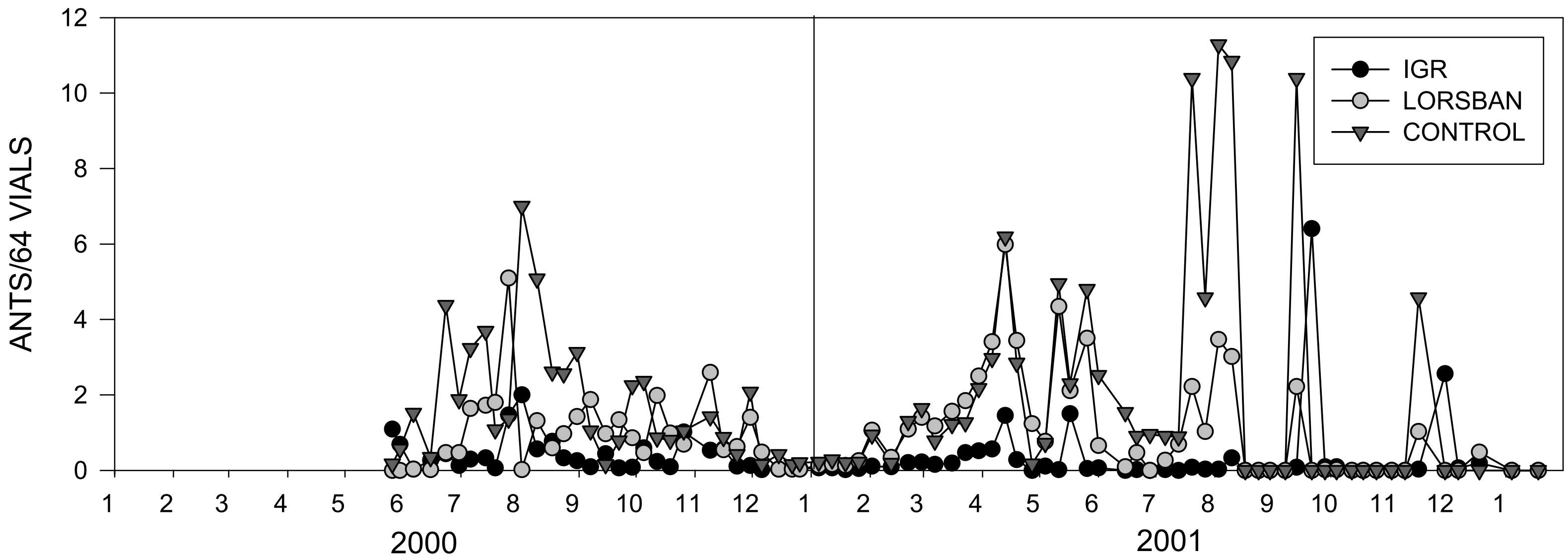

Figure 49. *Solenopsis invicta* Buren relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

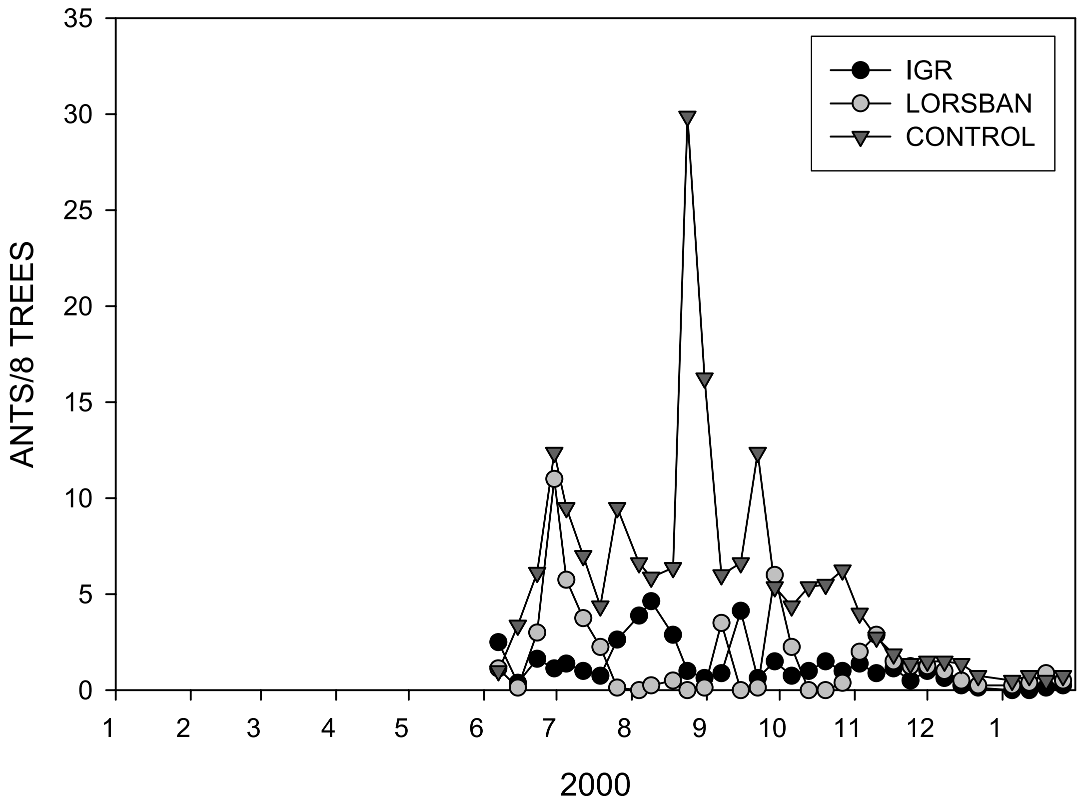

Figure 50. *Solenopsis invicta* Buren relative abundance (number of ants/8 trees) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

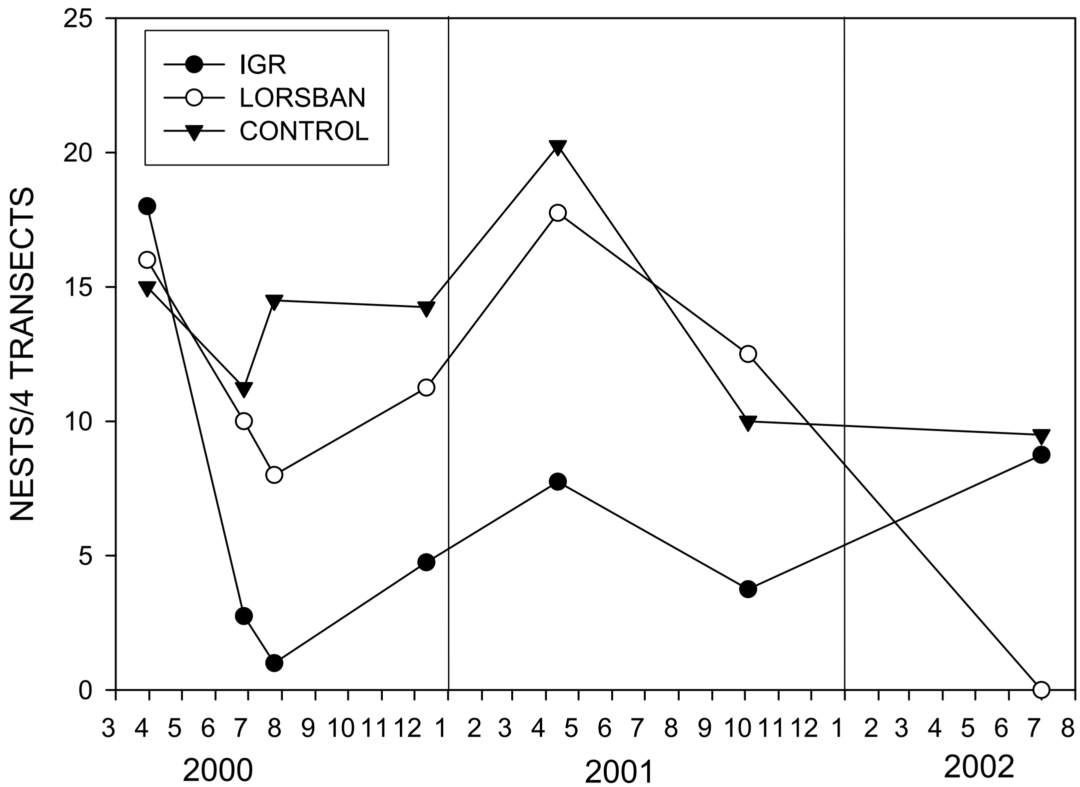

Figure 51. *Solenopsis invicta* relative nest abundance (number of nests/4 transects) and seasonality in Mumford, TX, in three different treatments (IGR, Lorsban and Control).

Diplorhoptrum was collected in pitfall traps in all the treatments (Fig. 52) and did not show significant differences in 2000 (ANOVA, 2 df; P>0.05). It was significantly greater in IGR plots in 2001 and 2002 compared to the Control and chlorpyrifos plots (ANOVA, 2 df; P<0.05) indicating this species is somewhat affected by RIFA. However, it has been observed that *Diplorhoptrum* may coexist with RIFA (Porter and Savignano 1990). This species was abundant in baited vials in IGR plots in 2000 and 2001 (Fig. 52) and it was significantly different compared to other treatments (ANOVA, 2 df; P<0.05). This species was uniformly benefited by the IGR treatments (Figs. 15, 16, 17, 18).

Strumigenys sp., a small hypogeic species was found several times during the study in pitfall traps (Fig. 54). *Strumigenys* biology is believed to be similar to *Smithistruma* feeding on small soft bodied insects and honeydew and nesting mostly in soil near decaying wood; a few species can live in arboreal plant cavities (Brown and Wilson 1959). This species was only collected in pitfall traps and significantly higher in IGR (Fig. 54) in 2000, 2001 and 2002 (ANOVA, 2 df; P<0.05). This is another cryptic species believed to resist RIFA invasion (Ward 1987) that was found at low density in RIFA infested plots. This species increased in density in the IGR treatment (Figs. 15, 16, 17, 18).

Subfamily Ponerinae. *Hypoponera opacior* (Forel), an epigeic species is known as the "legionnaire ant." It is a generalist predator and scavenger. Colonies are small and inconspicuous. *H. opacior* nest in moist soil in shady areas. This species was collected in pitfall traps (Fig. 55) and found throughout the year in just few occasions (Fig. 55). Data

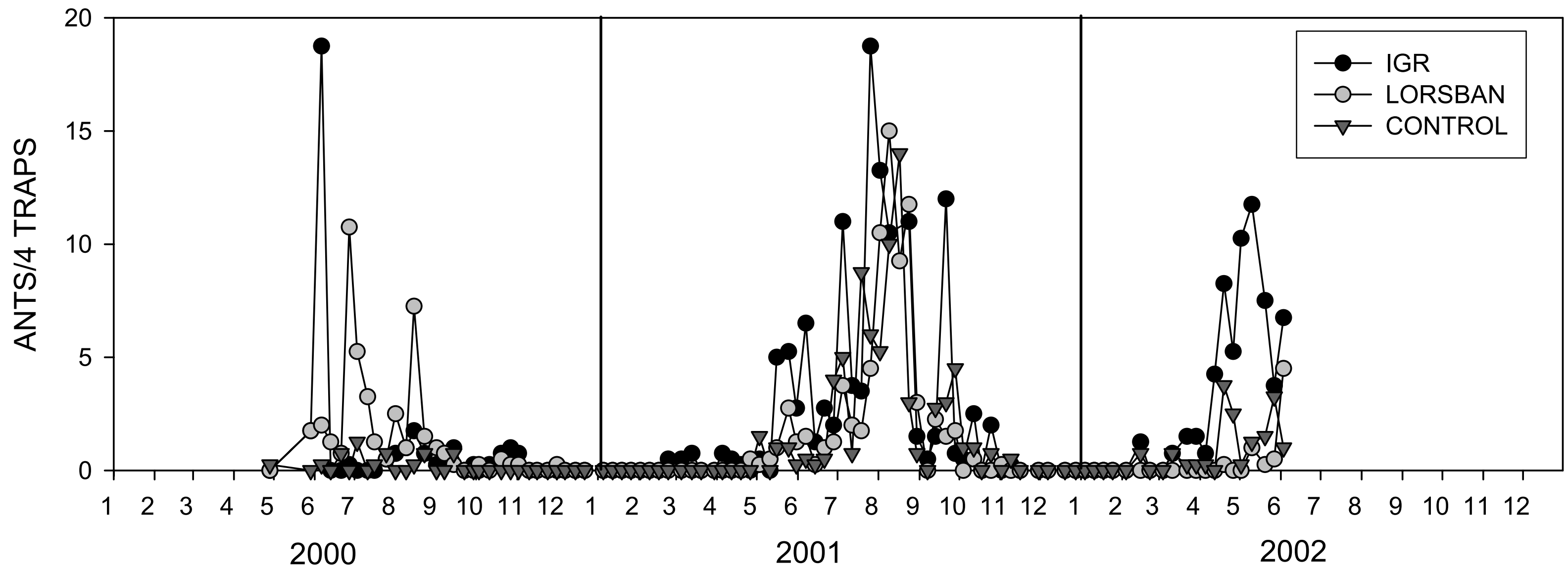

Figure 52. *Diplorhoptrum sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

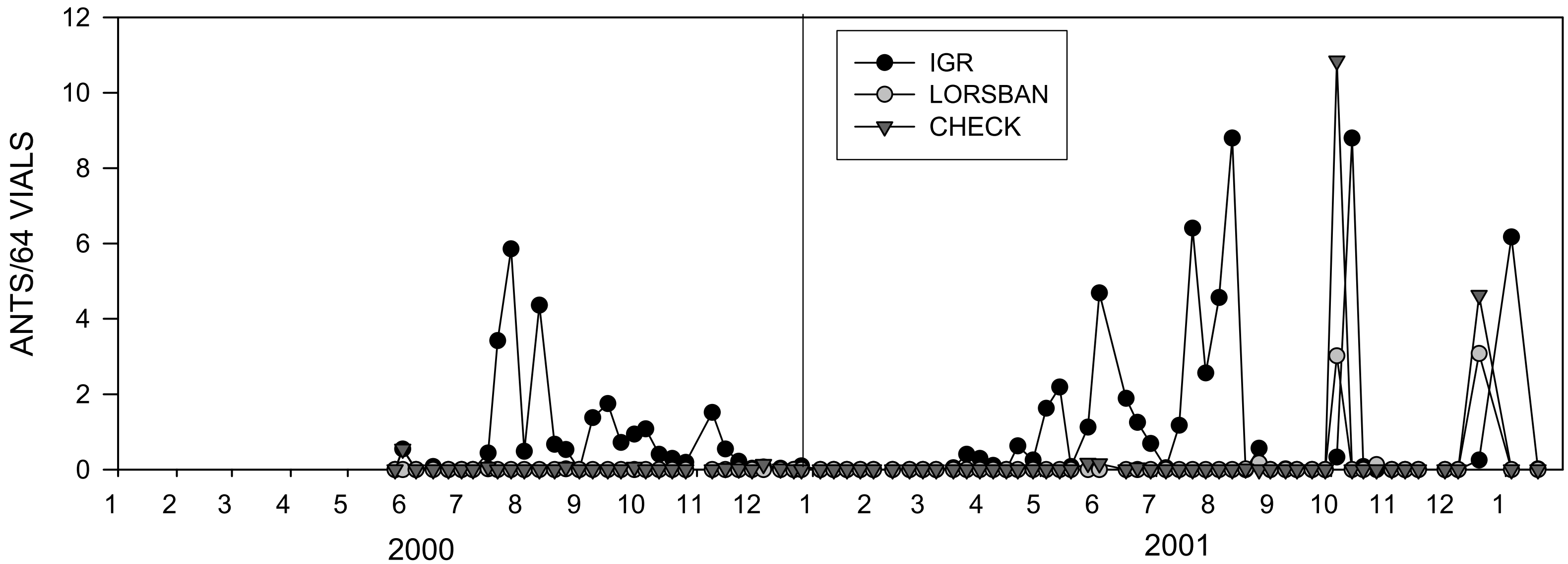

Figure 53. *Diplorhoptrum sp.* relative abundance (number of ants/64 vials) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

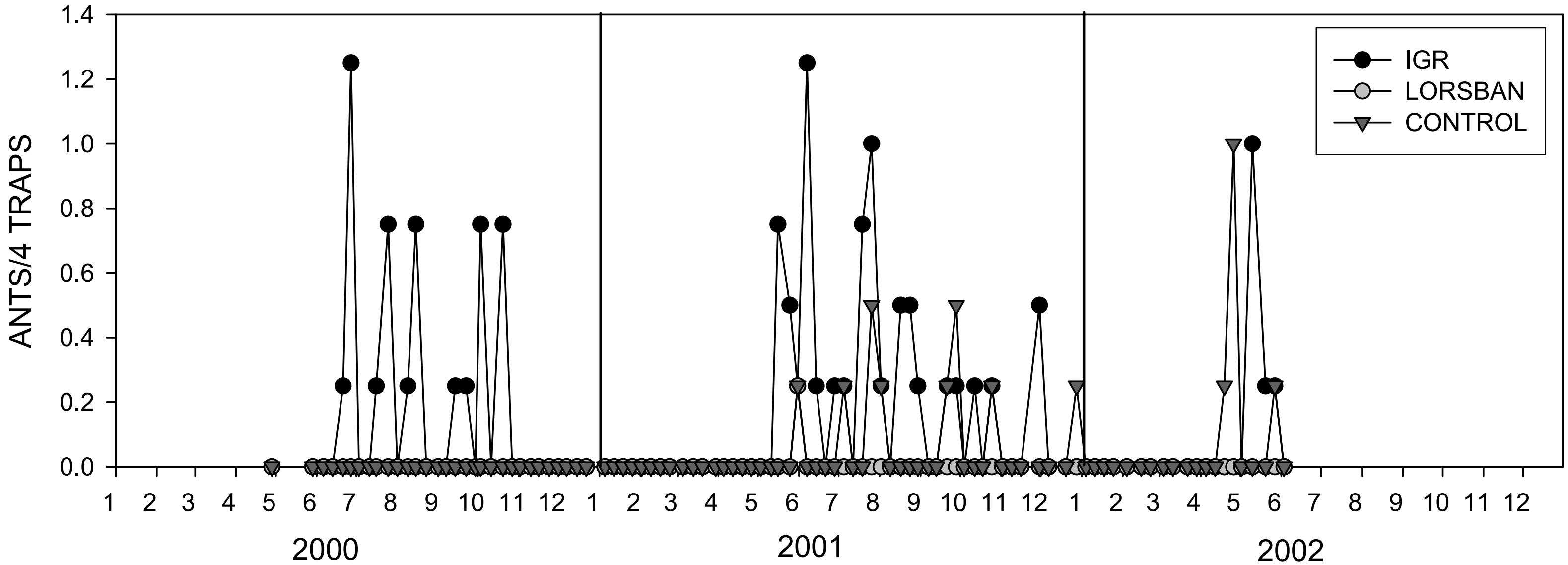

Figure 54. *Strumigenys sp.* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

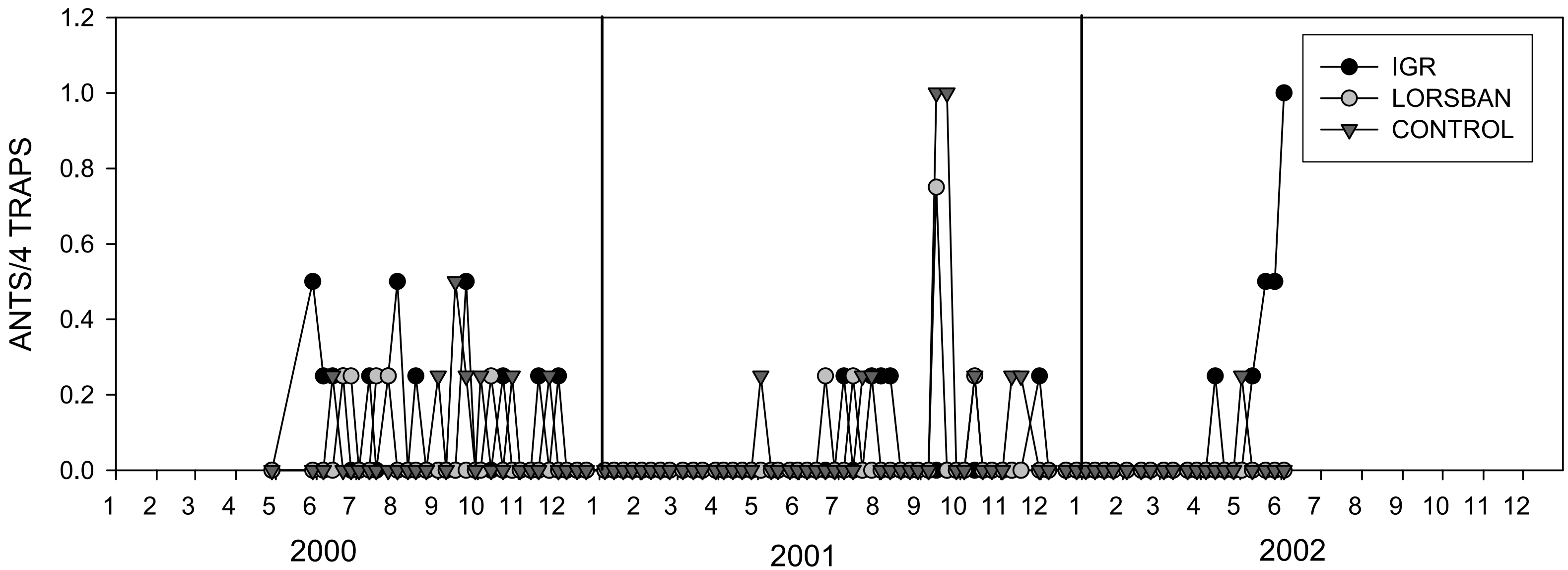

Figure 55. *Hypoponera opacior* relative abundance (number of ants/4 traps) and seasonality in Mumford, TX, in three different treatments (IGR= Insect Growth Regulator; Lorsban= Chlorpyrifos; and Control).

showed this species was significantly higher in IGR plots in 2000 and in 2002 (ANOVA, 2 df; $P<0.05$) but not in 2001 (ANOVA, 2 df; $P=0.843$). *H. opacior* was found to coexist and resist RIFA presence as has been previously reported (Porter and Savignano 1990). This species did not increase in density in 2000 and 2001 and decreased in 2002 in the IGR treatments (Figs. 15, 16, 17, 18).

Subfamily Pseudomyrmecinae. *Pseudomyrmex sp.*, an arboreal species known as "acacia ant" is a rare species, and was only detected once in pitfall traps and once in baited vials. This species is known to nest in trees and it is probably the only "true" arboreal species inhabiting the pecan agroecosystem. *Pseudomyrmex* is omnivorous, feeding on honeydew, plant tissue and other insects (O'Keefe et al. 2003). This species was collected only once in pitfall traps in RIFA infested areas and once in baited vials in IGR plots.

Correspondence Analysis (CA). CA is an indirect gradient technique that simultaneously ordinates sample and species scores obtained by reciprocal averaging (Quinn and Keough 2002). A half turnover of species occurs in one standard deviation, and full species turnover occurs in four standard deviations (Hoeinghaus et al. 2003). In this analysis, rare species were down weighted and selected Hill's scaling option (scaling the eigenvectors so that dissimilarities between points are chi-squared distances (Quinn and Keough 2002). Pitfall trap data were used for this analysis since it comprised all the species present in this study. The effect of treatments on ant assemblages were examined using CA based on species abundance by treatment matrices for 2000, 2001 and 2002.

The species loadings for each matrix for each year are presented in tables 7, 8 and 9. The analysis revealed similar response patterns of the ant assemblages among years due to the IGR treatment (Figs. 55, 56, 57). In 2000 with two IGR treatments, the first two canonical axes accounted for 30% of the variation, the axis obtained with CA analysis explained 16% of the variation, this axis models the ant assemblage gradient in which large positive scores are associated with greater abundances of *D. flavus, P. barbatus*, *Diplorhoptrum* and *Brachymyrmex*, and low abundances of *M. minimum* as a response of the IGR treatment, and, as well the second canonical axes, that in this year, explained 14% of the variation with large positive scores associated with the increase in abundance of *Pheidole*, *Strumigenys* and *M. minimum* and low abundances of *Diplorhoptrum*, *C. wheeleri, H. opacior* and *Brachymyrmex* (Tables 7, 8, 9), this last species was collected once significantly influencing the score in this analysis. In 2001 with a single IGR treatment, the first two axes accounted for 35% of the variation, the first axis explained 20% of the variation, large positives scores are observed with the increase of *D. flavus*, *P. barbatus*, *Smithistruma*, *Strumigenys* and *Pheidole*, and a decrease in abundance of *T. sessile*, *M. minimum* and *S. invicta*, and, the second axes explained 15% of the variation with large positive scores of *C. wheeleri*, *Diplorhoptrum*, *Pseudomyrmex*, *Strumigenys* and *Myrmecina* and negative scores for *Pheidole* and *M. minimum* (Tables 7, 8, 9). In 2002 without IGR treatment, 42% of the variation was explained with the first two axes, the first one explained 22% of the variation with large positive scores for *Diplorhoptrum*, *Paratrechina* and *H. opacior*, and negative scores for *T. sessile* and *M. minimum*, and, the second axis explained 20% of the variation with

Table 7. Eigenvalues, proportion of variance explained, and species variable loadings (eigenvectors) for the first two axes from CA performed on species (16) collected in pitfall traps in 2000.

	AX 1	AX2
EIGENVALUE	0.3098	0.2499
Proportion (%)	0.16	0.14
Species		
Brachymyrmex sp.	1.5843	-5.5316
Paratrechina sp.	-0.0115	0.4288
Dorymyrmex flavus	5.2891	-0.3235
Forelius pruinosus	1.2528	-1.0994
Tapinoma sessile	-0.4365	-0.9602
Pseudomyrmex sp.	0.0426	-1.73
Hypoponera opacior	0.5898	-1.9756
Cyphomyrmex wheeleri	-0.3156	-2.208
Monomorium minimum	-1.1049	1.1123
Myrmecina sp.	-0.4553	-0.5686
Pheidole sp.	1.4669	3.0158
Pogonomyrmex barbatus	4.7145	0.4705
Smithistruma sp.	1.4047	1.0303
Solenopsis (=Diplorhoptrum)	2.0536	-2.2258
Solenopsis invicta	-0.6087	-0.619
Strumigenys sp.	1.5094	1.4139

Table 8. Eigenvalues, proportion of variance explained, and species variable loadings (eigenvectors) for the first two axes from CA performed on species (15) collected in pitfall traps in 2001.

	AX 1	AX2
EIGENVALUE	0.3045	0.2171
Proportion (%)	0.20	0.15
Species		
Paratrechina sp.	0.4695	-0.8384
Dorymyrmex flavus	4.0226	-0.9041
Forelius pruinosus	0.165	-0.257
Tapinoma sessile	-2.0628	-0.5139
Pseudomyrmex sp.	-0.0862	1.7778
Hypoponera opacior	0.7409	0.6088
Cyphomyrmex wheeleri	0.4842	1.9666
Monomorium minimum	-1.0253	-1.6232
Myrmecina sp.	0.6161	1.3154
Pheidole sp.	1.1318	-1.7083
Pogonomyrmex barbatus	2.9309	-1.434
Smithistruma sp.	1.9516	0.4872
Solenopsis (=Diplorhoptrum)	0.9818	1.8287
Solenopsis invicta	-0.9565	0.572
Strumigenys sp.	1.8268	1.1858

Table 9. Eigenvalues, proportion of variance explained, and species variable loadings (eigenvectors) for the first two axes from CA performed on species (13) collected in pitfall traps in 2002.

	AX 1	AX2
EIGENVALUE	0.3599	0.3224
Proportion (%)	0.22	0.20
Species		
Paratrechina sp.	1.7592	-0.3798
Dorymyrmex flavus	0.7012	0.8709
Forelius pruinosus	-0.3642	0.698
Tapinoma sessile	-1.2828	0.3313
Hypoponera opacior	1.0461	0.1853
Monomorium minimum	-1	3.0428
Pheidole sp.	0.1167	2.18
Pogonomyrmex barbatus	0.6281	0.6609
Smithistruma sp.	0.575	-0.8624
Solenopsis (=Diplorhoptrum)	1.9176	0.3028
Solenopsis invicta	-0.9383	-0.8134
Strumigenys sp.	0.8442	0.3192

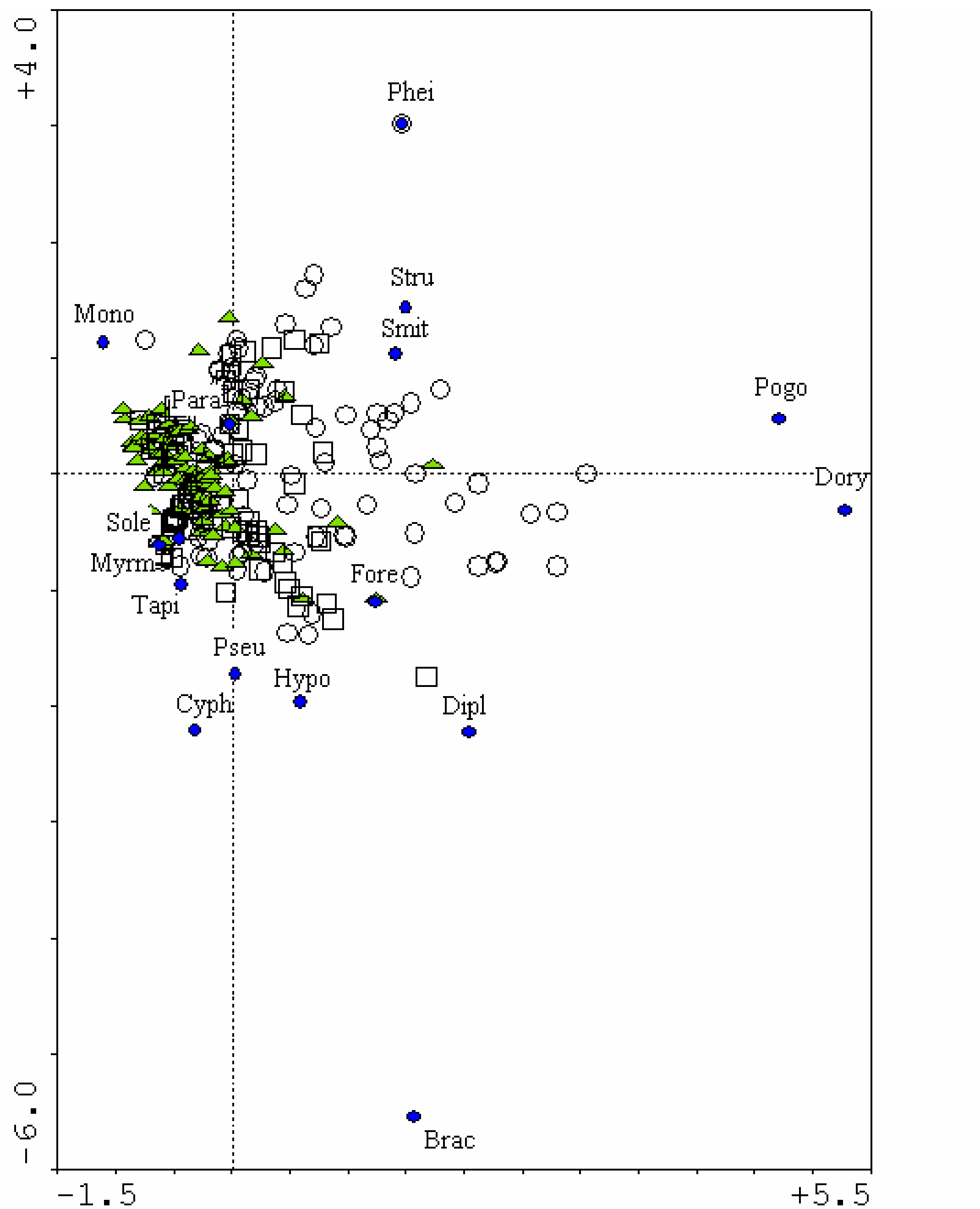

Figure 56. Correspondence analysis (CA) for pitfall traps for 2000. Circles (○) indicate IGR plots, squares (□) indicate Chlorpyrifos plots and triangles (▲) indicate control plots (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

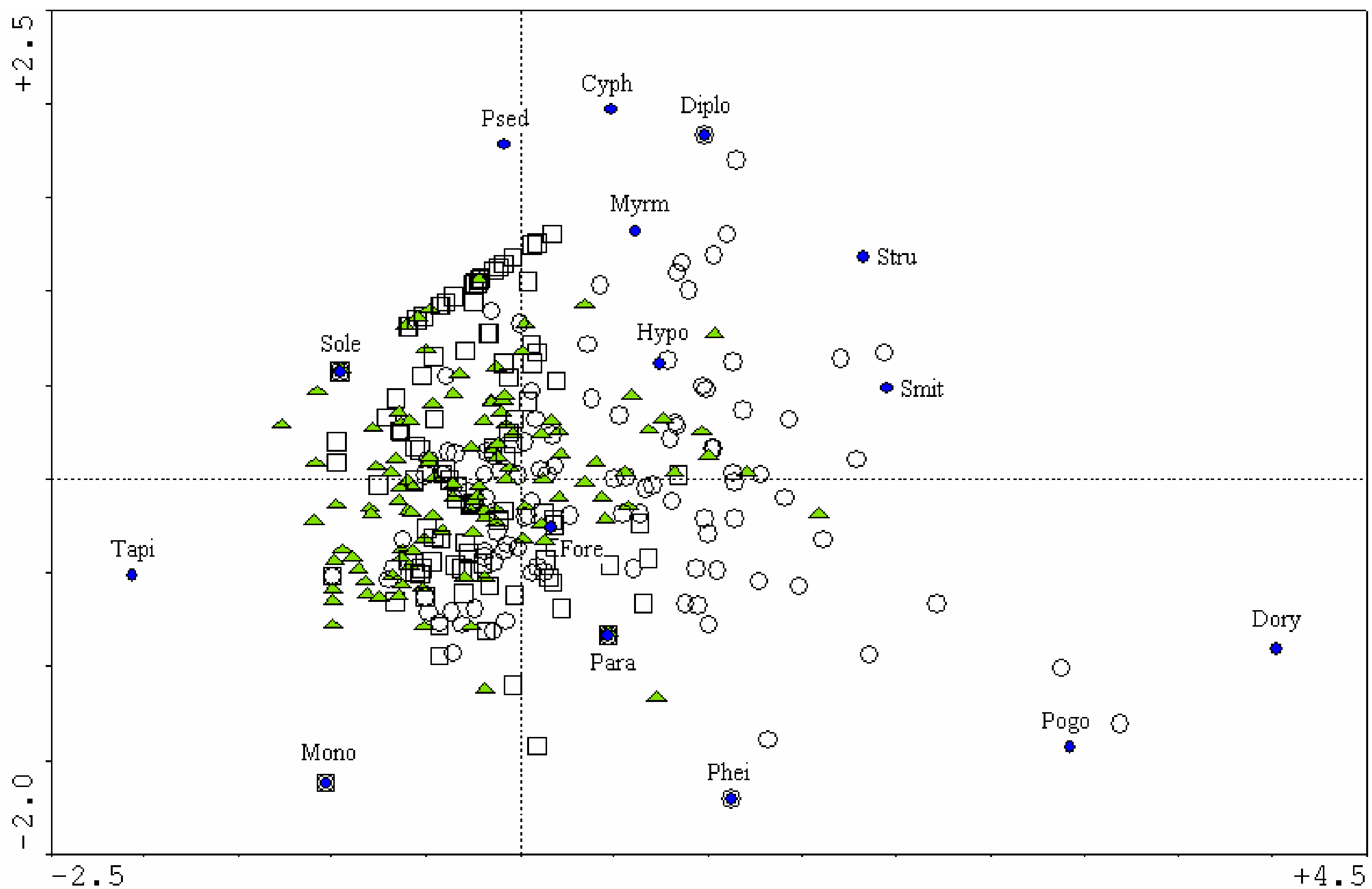

Figure 57. Correspondence analysis (CA) for pitfall traps for 2001. Circles (○) indicate IGR plots, squares (□) indicate Chlorpyrifos plots and triangles (▲) indicate control plots (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

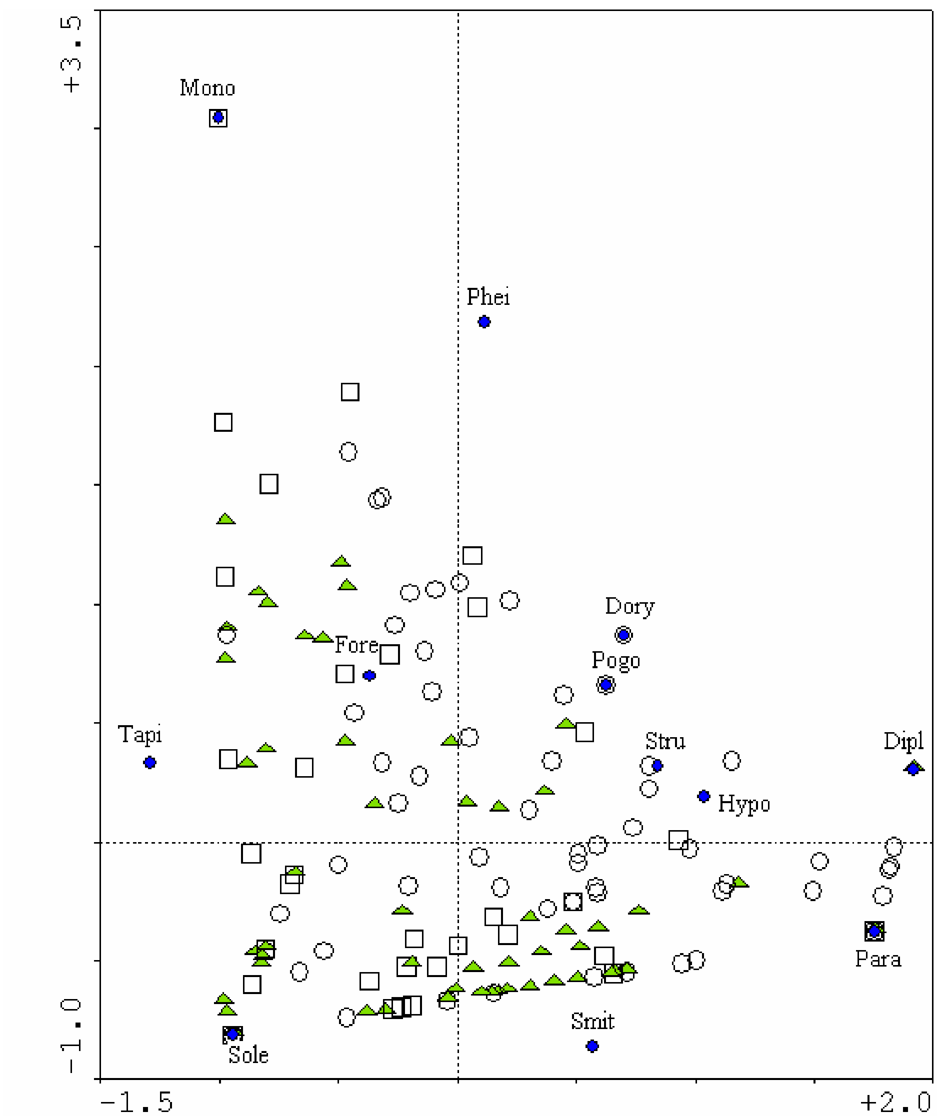

Figure 58. Correspondence analysis (CA) for pitfall traps for 2002. Circles (○) indicate IGR plots, squares (□) indicate Chlorpyrifos plots and triangles (▲) indicate control plots (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

large positive scores of *M. minimum* and *Pheidole* and negative scores for *Smithistruma* and *S. invicta* (Tables 7, 8, 9).

No differences between ant assemblage species composition (Shannon index) among treatments in 2000 were found (ANOVA, 2 df; F=0.562; P=0.291) indicating the IGR treatment did not have a major impact on the assemblage for that year. In 2001 ant diversity in IGR plots was greater compared to chlorpyrifos and Control (ANOVA, 2 df; F=8.553; P<0.05) indicating the bait treatment had some positive effect on the ant assemblage. In 2002 with no treatment applied, IGR plots had the greater diversity values compared to the Control (ANOVA, 2 df; F=60.240; P<0.05) indicating some extended effect on some species of the IGR treatment applied in previous years. CA ordination among years showed ant assemblages were similar in relative species ranking among treatments. Native ant assemblages with some variation as noted above were present despite domination by RIFA in this agroecosystem. However, the densities of several species were affected by treatments, RIFA was reduced by IGR plots in pitfall, bait vials, direct sampling and colony surveys compared to the control and remaining species generally increased (Figs. 3-11, 56, 57, 58).

D. flavus and *P. barbatus* consistently increased following RIFA reduction, it demonstrated the positive effect of IGR treatment. In fact all species in the ant assemblage were affected less by IGR treatment than was RIFA, and all the species were found in Control plots which indicates all these species coexist with RIFA. This last finding disagrees with Porter and Savignano (1990). They argued RIFA has a major effect on native ant assemblages by displacing species and removing them from the system. This is not the case for this particular agroecosystem where species can coexist and survive RIFA infestation. These findings are confirmed by lab experiments conducted by Helms and Vinson (2001) and Rao and Vinson (2004) where they suggested native ant species coexist and can survive while RIFA is present in the environment.

These results are of great value for the conservation of these ant assemblages. The intensity of this sampling revealed the great ability of several species to coexist with fire ants, to survive the RIFA infestation by becoming more specialized and therefore to survive IGR treatment. The results of the CA analysis and the amount of variation explained by the analysis (not higher than 40%) indicated there are many other factors besides the IGR treatment influencing the ant assemblage response such as soil type, vegetation, temperature, humidity, etc and others that were not included in the analysis. Morrison and Porter (2003) reported a positive association between fire ants and other arthropods, and supported some of the findings in this study. The pecan agroecosystem seems to have a suitable microhabitat allowing the coexistence of a diverse ant assemblage with RIFA.

Summary of the Ant Assemblages Inhabiting Pecans. Several species appear well adapted to this agroecosystem. The availability of food resources in this habitat appears to match food use of several ant species present. For example, the high densities of ants during the summer may be a numerical response associated with seasonal population dynamics of pecan aphids and the production of honeydew (Harris et al. 2003). Many of these species are known to seek and consume honeydew, many of them are known to tend aphids, and a few of them are capable of preying on them (Bristow 1991). Aphids diversify the environment they inhabit in many ways: 1) they serve as prey for many predatory species; 2) they produce copious amounts of carbohydrate (honeydew) that attract many species of sugar feeders that could not otherwise access this resource, including ants; 3) the opportunistic sugar feeders attracted to the honeydew exhibit a functional response that concentrates in turn their natural enemies into the affected area within and beneath the pecan canopy; and 4) the honeydew serves as a substrate for microbes like fungi and bacteria, also attracts yet another complex of microbial feeders (Harris and Li 1996).

The aphid and arthropod complex that includes their natural enemies makes this agroecosystem particularly interesting for the conservation of the ant assemblage. It provides resources that guarantee assemblage survival, therefore enhancing the system with more generalist insects important for maintaining the biological control of secondary pests, an important aspect that needs to be addressed in the future. *S. invicta*, *Paratrechina*, *M. minimum* and *Diplorhoptrum* seems to best describe the dominant ant fauna inhabiting pecans in Mumford, Robertson Co. Texas in natural conditions.

Comparison of Sampling Methods. Four sampling techniques were compared.

Pitfall Traps. The data produced by pitfall traps in Control plots included richness and composition, relative abundance of ant foragers in traps and plots, and the frequency of occurrence of species inside the traps. A total of 3,403 ants were collected during 2000, 4,377 during 2001 and 1,082 during 2002. Sixteen species (Species Richness (S)) were recorded using pitfalls; 14 species in 2000, 16 during 2001, and 12 during 2002 (Table 10). *S. invicta*, *Paratrechina sp.* and *M. minimum* were the most abundant species for years 2000 and 2001 and *S. invicta*, *F. pruinosus*, *Paratrechina sp.* and *M. minimum* were the most abundant species for year 2002 (Fig. 59). Shannon diversity index was significantly greater in this method compared to all the other sampling techniques (ANOVA, 3 df; F= 52.46; $P<0.05$) (Fig. 60). Diversity indices of Simpson (D), and Chao 1 and Richness (S) were also higher compared to other sampling techniques excepting the Evenness (E_H) which remain similar among sampling techniques (Table 11). This technique provided the most complete information of species abundance and diversity for this particular environment.

Baited Vials. Ant foragers attracted to vials also provided data on species richness, composition and relative abundance. Baited vials collected seven species consisting of 3,726 ants in 2000, and 8,947 in 2001. Six species were recorded during 2000 and five during 2001. *S. invicta* was the most abundant species in 2000 followed by *Paratrechina sp.* and *M. minimum*. In 2001, *S. invicta* and *Paratrechina sp.* were the most abundant species (Fig. 61). Compared with the other methods, the Shannon diversity index did not show any significant differences when compared with direct sampling (ANOVA, 3 df; F= 52.46; P=0.478) and mound surveys (ANOVA, 3 df; F= 52.46; P=0.737) but it was different and significantly less effective (ANOVA, 3 df; F= 52.46; $P<0.05$) when compared to pitfall traps as mentioned above (Fig. 60). Diversity indices of Simpson (D), and Chao 1 and Richness (S) were similar compared to other sampling techniques except to pitfall traps which were lower. Evenness (E_H) remained similar among all sampling techniques (Table 11).

Table 10. List of ant species detected with all sampling techniques at Mumford, TX during 2000-2002 season.

Family Formicidae	Sampling Technique			
	Pitfall	Vials	Direct	Nest Survey
Subfamily Formicinae				
Brachymyrmex sp.	X			
Paratrechina sp.	X	X	X	X
Subfamily Dolichoderinae				
Dorymyrmex flavus	X		X	X
Forelius pruinosus	X	X	X	X
Tapinoma sessile	X		X	
Subfamily Pseudomymecinae				
Pseudomyrmex sp.	X	X		
Subfamily Ponerinae				
Hypoponera opacior	X			
Subfamily Myrmecinae				
Cyphomyrmex wheeleri	X			
Monomorium minimum	X	X	X	X
Myrmecina sp.	X			
Pheidole sp.	X	X	X	
Pogonomyrmex barbatus	X			X
Smithistruma sp.	X			
Solenopsis (=Diplorhoptrum)	X	X		
Solenopsis invicta	X	X	X	X
Strumigenys sp.	X			
Species Richness (S)	16	7	7	6

Table 11. Diversity indices estimated for each sampling method. (S= Species richness; H'= Shannon; E_H= Evenness; *D*= Simpson; Chao 1= Chao).

Sampling Technique	Diversity index				
	S	*H'*	E_H	*D*	Chao 1
Pitfall traps	16	1.59	0.21	3.53	15
Baited vials	7	0.44	0.22	1.24	4
Direct Sampling	7	0.45	0.23	1.29	7
Nest survey	6	0.40	0.22	1.22	5.12

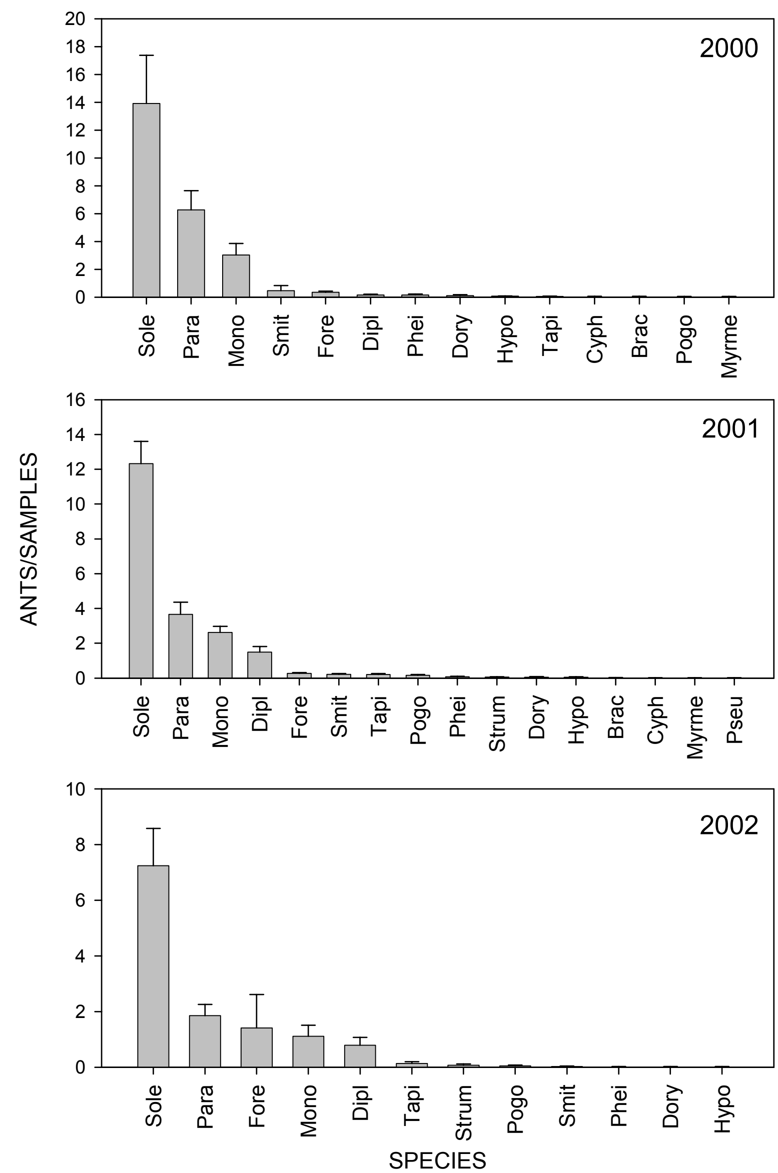

Figure 59. Species recorded in pitfall traps at Mumford, TX. Numbers indicate the average proportion of each species divided the total number of samples and indicated by year (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Smit= *Smithistruma*; Tapi= *Tapinoma*; Pogo= *Pogonomyrmex*; Phei= *Pheidole*; Strum= *Strumigenys*; Dory= *Dorymyrmex*; Hypo= *Hypoponera*; Brac= *Brachymyrmex*; Cyph= *Cyphomyrmex*; Myrme= *Myrmecina*; Pseu= *Pseudomyrmex*).

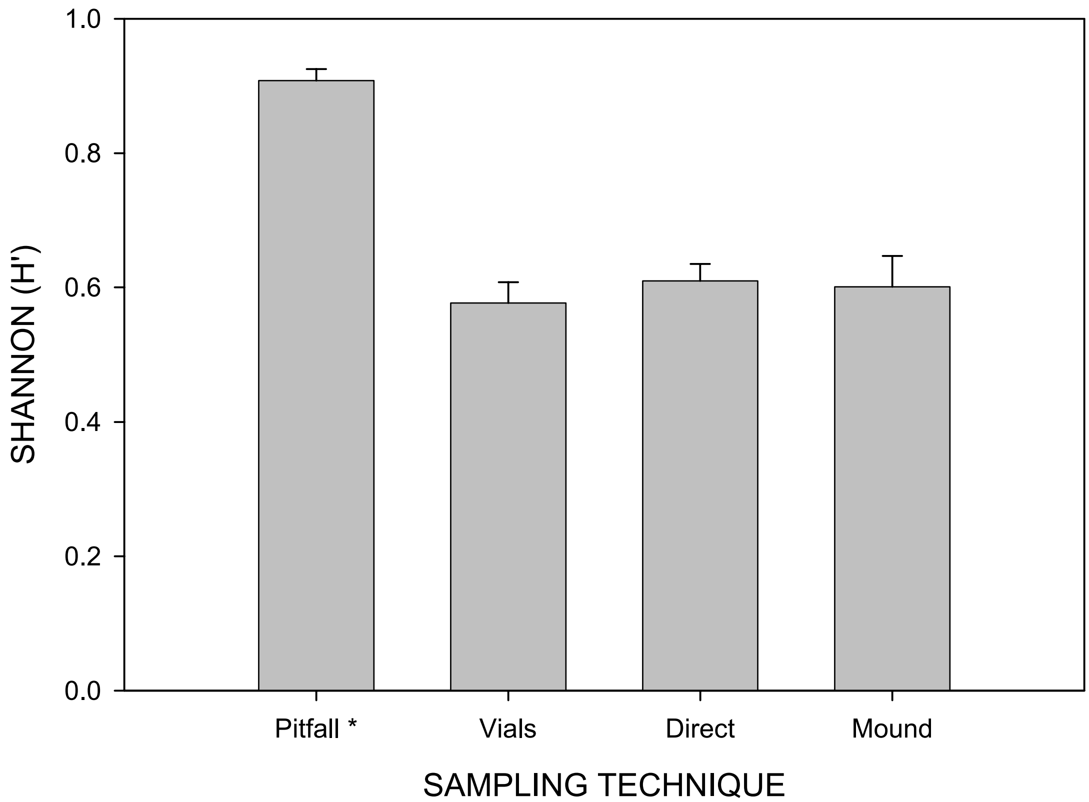

Figure 60. Shannon diversity index among different sampling techniques, Asterisk (*) indicates statistical differences between methods.

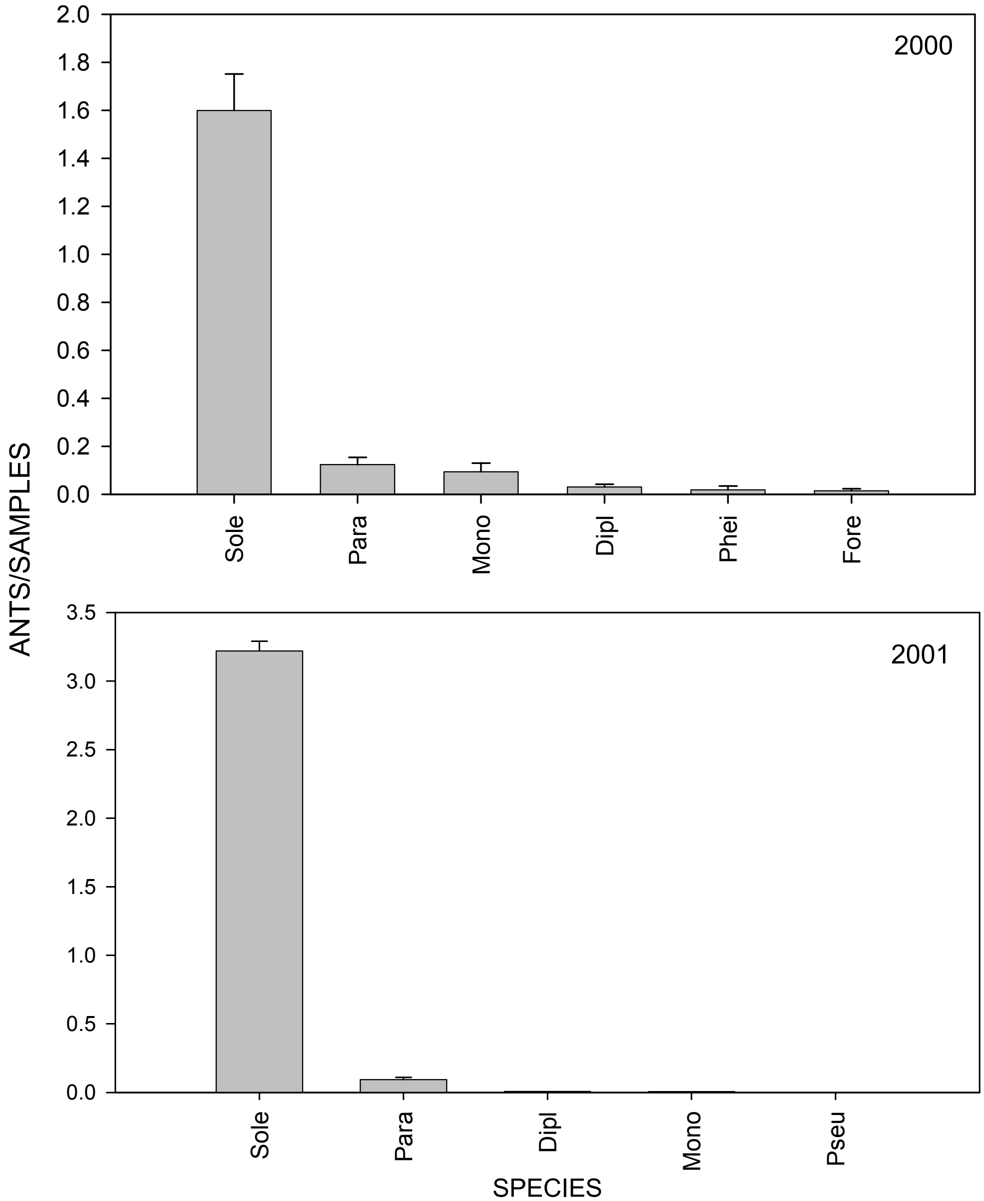

Figure 61. Species recorded in baited vials at Mumford, TX. Numbers indicate the average proportion of each species divided the total number of samples and indicated by year (Sole= *Solenopsis*; Para= *Paratrechina*; Fore= *Forelius*; Mono= *Monomorium*; Diplo= *Diplorhoptrum*; Phei= *Pheidole*; Pseu= *Pseudomyrmex*).

Direct Sampling. Data obtained from direct sampling was used to determine richness, composition, and the frequency of occurrence of ant species in trees. Direct sampling detected seven species from 1,701 ants collected during 2000. *S. invicta* was the most abundant species followed by *Paratrechina sp.* and *M. minimum* (Fig. 62). Shannon diversity index calculated for direct sampling did not show any significant differences when compared to baited vials (ANOVA, 3 df; F= 52.46; P=0.478) and colony surveys (ANOVA, 3 df; F= 52.46; P=0.856) but it was significantly less efficient when compared to pitfall traps (ANOVA, 3 df; F= 52.46; P<0.05) (Fig. 60). Diversity indices of Simpson (D), and Chao 1 and Richness (S) were similar compared to other sampling techniques except to pitfall traps which were lower. Evenness (E_H) remained similar among all sampling techniques (Table 11).

Survey of Ant Colonies. Colony survey data were used to examine richness, composition and colony density. There were six species represented by 481 ant nests (colonies) recorded on transects (225 in 2000, 207 in 2001 and 29 in 2002). *Solenopsis invicta* was the most abundant, while *D. flavus* showed some increase in 2001 and 2002 followed by *M. minimum* (Fig. 63). Shannon diversity index estimated for this method did not show significant differences when compared to baited vials (ANOVA, 3 df; F= 52.46; P=0.737) and to direct sampling (ANOVA, 3 df; F= 52.46; P=0.856) (Fig. 60), but the estimate was significantly lower compared to pitfall data as noted above. Diversity indices of Simpson (D), and Chao 1 and Richness (S) were similar compared to other sampling techniques except to pitfall traps which were lower. Evenness (E_H) remained similar among all sampling techniques (Table 11).

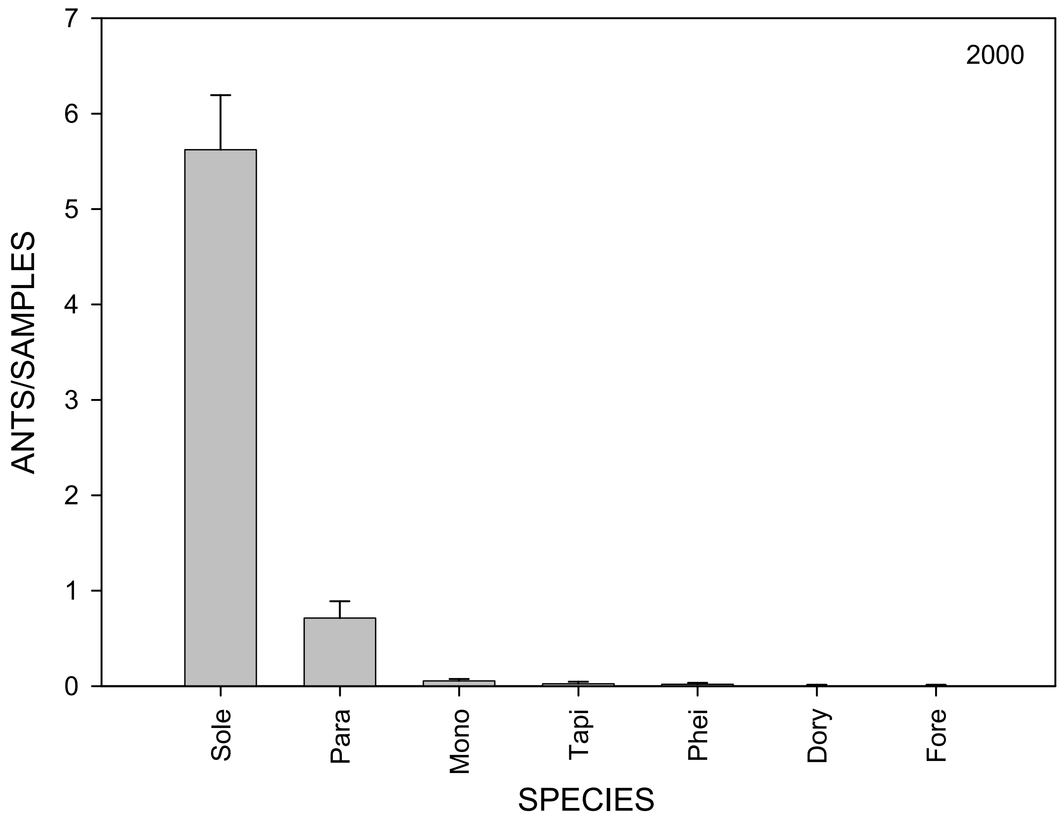

Figure 62. Species recorded using direct sampling method at Mumford, TX. Numbers indicate the average proportion of each species divided the total number of samples and indicated by year (Sole= *Solenopsis*; Para= *Paratrechina*; Tapi= *Tapinoma*; Phei= *Pheidole*; Dory= *Dorymyrmex*; Fore= *Forelius*).

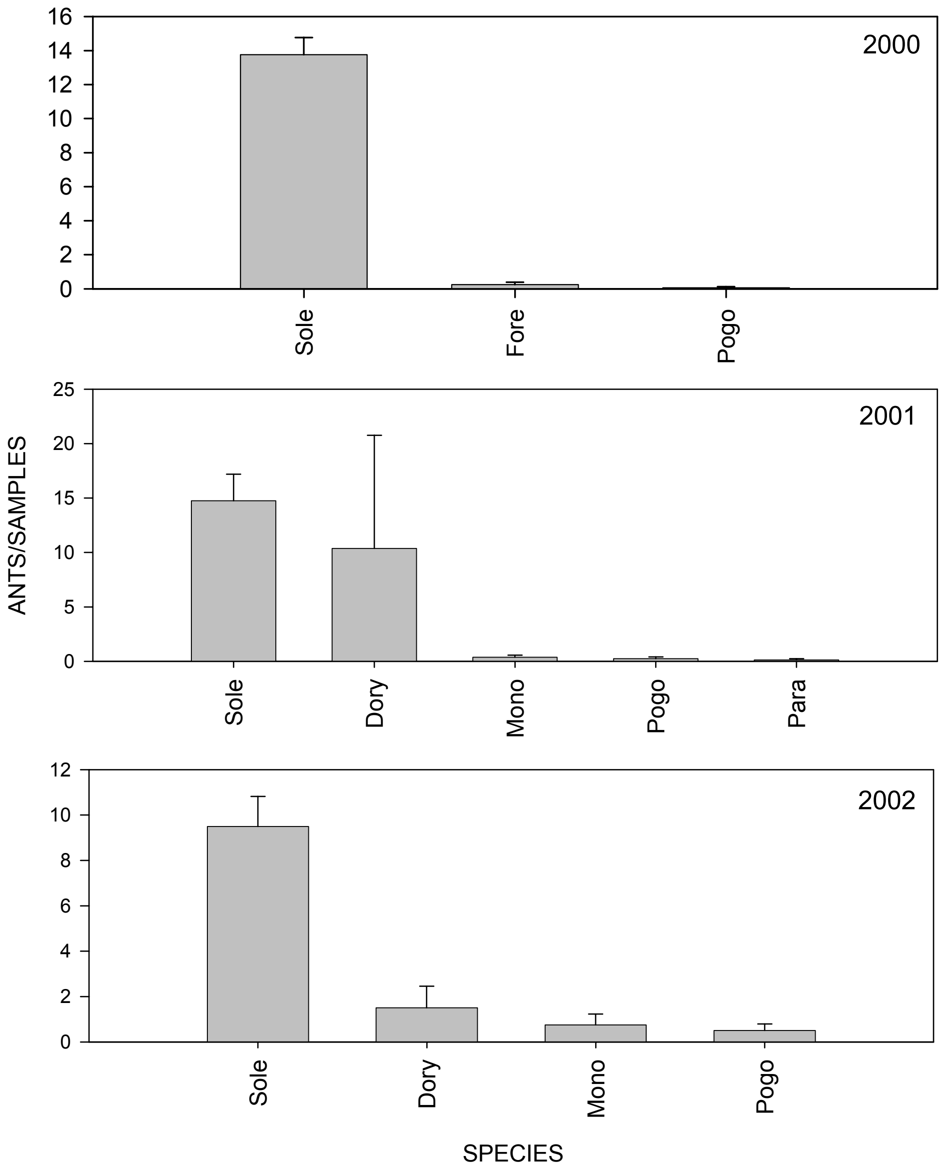

Figure 63. Species recorded in transects using the nest survey method at Mumford, TX. Numbers indicate the average proportion of each species divided the total number of samples and indicated by year (Sole= *Solenopsis*; Fore= *Forelius*; Pogo= *Pogonomyrmex*; Dory= *Dorymyrmex*; Mono= *Monomorium*; Para= *Paratrechina*).

Summary of Sampling Method Efficiency. Pitfall traps collected all ant species (16) found by any method and also captured more individual ants than any other method. However, other considerations are needed before assuming this is the most efficient method and when using it in studies. In spite of the abundance and ease of collection of ants, several features of ant biology complicate their sampling. First, ants are variable and non randomly distributed on several spatial scales. Second, individuals are aggregated into colonies on small scales and those colonies are often dispersed across the landscape owing to competition (Bestelmeyer et al. 2002, Wiernasz and Cole 1995, Crist and Wiens 1996). The relationship between the activity and the abundance of foragers and colony abundance and distribution differ greatly among species. This indicates a combination of several techniques may improve detection with less overall effort. The greater amount of time expended on pitfall traps may account for their higher yield of species compared to other techniques. Studies done over shorter periods may benefit by using a combination of sampling methods. Nevertheless, all ant species that were recorded in pitfall traps in this study were represented after three weeks of sampling using four traps.

Different agroecosystem and the heterogeneity of some agricultural systems may require that more traps be monitored for a longer time to reach a comprehensive plateau (species accumulation curves) to determine if the assemblage is well represented within those samples. The biology of the ant species can influence the choice of sampling methods. Other factors, such as disturbance (mowing, harvesting) may alter the composition of the ant assemblages and alter the diversity and the results among traps.

CHAPTER III

INTERACTIONS BETWEEN THE PYRAMID ANT AND THE RED IMPORTED FIRE ANT

> "Real" wilderness as human beings consider it, viewed over distances of hundreds of kilometers (again, a matter of conceptual scale), is everywhere threatened.
>
> -Holldobler and Wilson, Journey to the Ants

The introduction of the red imported fire ant *Solenopsis invicta* Buren has been reported as the cause of the reduction in density (but not extinction) of many native ant fauna in the US through direct and indirect competition (Camilo and Phillips 1990, Porter and Savignano 1990, Jusino-Atresino and Phillips 1994, Wojcik 1994, Wojcik et al. 2001). Interactions between native ant fauna and RIFA may be affected by the use of baits for the control of fire ants in managed and unmanaged areas. Effects on local ant fauna include direct toxic effect of the bait, how they respond to the reduction/suppression of RIFA, and what impact this may have on these assemblages.

The domination by RIFA of the ant assemblage, as indicated in Chapter II, in the pecan agroecosystem has occurred through a wide range of possible effects. These include 1) direct competition through aggressive interactions by RIFA that reduce or eliminate colonies of other species, 2) superiority in locating, and utilizing food resources to the exclusion of other ant species and, 3) RIFA domination of space and food resources causing severe disruption of the relationship in the pre-existing ant assemblage resulting in an evolving reordering process that is still underway. These effects are currently reflected in how the ant assemblage in pecan responds to broadcast bait (IGR) and trunk treatment (chlorpyrifos) (Figs. 15, 16, 17, 18) as was discussed in

Chapter II. The ant species that responded most favorable to IGR treatment was *Dorymyrmex flavus*. This response was noted early in this investigation as density increase occurred in the various sampling methods. Additionally, dead fire ants were noted in *D. flavus* middens, indicating this species may be aggressively interacting with the reduced densities of RIFA in the IGR plots and that *D. flavus* may be providing significant competition for RIFA. Middens consisted of piles of remnants of ants and other insects deposited around the nest opening, most of them were dismembered.

Dorymyrmex flavus occurs at low densities in pecan in the presence of RIFA, but results (Chapter II) indicated that reduction of RIFA density with poison bait (Extinguish^{TM}) results in rapid increase and persistence of *D. flavus* for extended periods following treatment. There have been observations recounting interactions between these two species in the field. *D. flavus* has been recorded attacking newly mated queens and males (Whitcomb et at 1973, Nickerson et al. 1975). RIFA remains had also been observed in *D. flavus* middens (Hung 1974, Calixto et al. 2003, 2004). These observations indicate an aggressive interaction may be occurring between these species. These findings prompted the following investigation.

Materials and Methods

Study Site. This part of the study was carried out in a commercial pecan orchard located in Mumford, Robertson. Co., TX. More details of the site are described in Chapter II.

Sampling Methods. Pitfall traps, baited vials, direct sampling and colony survey data collected for and included in Chapter II were used to describe the interactions between these two species.

Analysis of Middens. *D. flavus* nests were sampled by randomly collecting 10 middens (refuse piles) on 31 July 2002. These nests were marked and geo referenced by using a GPS (Trimble® Geo Explorer XT with submeter accuracy). Middens were collected without replacement, returned to the lab, and analyzed. Each midden was inspected for ant remains and these were classified and recorded. A week later, on 8 August 2002, middens that had accumulated in the interim were collected again from near the same nests and processed as before.

Analysis of *D. flavus* Behavior. Three video cameras (Sony® Digital Handycam MicroMV DCR-IP5) were located above three *D. flavus* nests to record their behavior; the field of view was approximately 15 cm by 15 cm. Each nest was located 1 meter from an active RIFA nest. Recording sessions began at 1300 h and ended at 1500 h. Sessions were conducted at intervals of 10 minutes every 20 minutes on 4, 14, 18, 19, 22, 25 September and on 15 October 2003. Observations were made during the afternoon after determination that *D. flavus* was more active during those hours. Tapes were reviewed and two behaviors were observed and quantified, 1) the frequency of *D. flavus* bringing fire ants into the nest, and, 2) *D. flavus* removing fire ants from the nest. Other unusual behaviors involving aggression between these two species were also noted.

Analysis of Middens With Marked Fire Ants. Individual RIFA mounds were chosen at random within IGR treated plots in 2003. Colonies were approximately the same size. Each one of the mounds was assigned a color, disturbed and ants were sprayed with paint trying to mark as many ants as possible (Bhatkar et al. 1991, Wojcik et al. 2000). Mounds were left for 48 hours, then all *D. flavus* mounds within 1.5 meters surrounding each RIFA mound were located and *D. flavus* middens collected. Samples were returned to the laboratory and inspected for fire ants, recording marked and unmarked RIFA remains.

***D. flavus* Nest Density After Fire Ant Reinfestation.** A GPS unit (Trimble® Geo Explorer XT with submeter accuracy) was used to survey and map all *S.s invicta* and *D. flavus* mounds in IGR and Control plots on 4, 14 and 18 September 2003. The size of the area sampled was 0.4856 hectares (four by six tree blocks, trees are spaced 13.716 m by 13.411 m each) inside of each IGR and control block. Distribution and absolute numbers of nests of each species were determined as well their location with respect to each other using the software ArcGIS 8.0 (ESRI™).

Analysis of Data. Data obtained from pitfall traps, baited vials, and direct sampling were analyzed using ANOVA-GLM with the LSD multiple comparison test. A Spearman's Rank Comparison test was used to relate midden size and number of remains collected in middens. Ant colony survey data were analyzed and compared by day and treatment for each species by using a Mann-Whitney U test as well the data obtained with the geo referenced method.

Results and Discussion

These results show *D. flavus* densities increased following RIFA reduction using

IGR baits. The change in density was still apparent more than two years after the last IGR treatment as documented in the following results:

Pitfall Traps. Abundance for *D. flavus* in 2000, 2001 and 2002 (Fig. 19) in IGR plots was significantly higher for each year (ANOVA, 2 df; P<0.05). *S. invicta* exhibited a significant reduction following IGR treatment compared to Control plots in 2000 (Fig. 48) (ANOVA, 2 df; P<0.030) and for 2001 (ANOVA, 2 df; P<0.05). Treatment was not applied in 2002. Fire ant density increased in IGR and difference in *S. invicta* density in IGR vs. Control were not found (ANOVA, 2 df; P=0.421). *D. flavus* numbers remained high in IGR plots in 2002 despite increase in *S. invicta* density indicating somewhat resistance to RIFA reinvasion.

Baited Vials. Recruitment behavior recorded by baited vials (Fig. 20) was significantly higher in IGR plots for *D. flavus* in 2000 and 2001 among treatments (ANOVA, 2 df; P<0.05). *S. invicta* showed significant reduction in recruitment to vials (Fig. 49) for 2000 and 2001 and significant differences were found between IGR and the Control (ANOVA, 2 df; P<0.05).

Direct Sampling. *D. flavus* was significantly more active in IGR (Fig. 21) (ANOVA, 2 df; P<0.05). Direct sampling also showed significant differences in *Solenopsis invicta* density (Fig. 50). IGR plots had less fire ants foraging compared to the control (ANOVA, 2 df; P<0.05).

Survey of Ant Colonies. Density of *D. flavus* nests was significantly higher in IGR plots compared to the Control for every sample day in 2000 and 2001 except for 4 October 2001 and 31 July 2002 (Fig. 22). Individual Mann Whitney tests confirmed that

D. flavus was significantly higher in IGR (Mann Whitney Test $P<0.05$). *S. invicta* density was also significantly reduced in IGR (Mann Whitney Test $P<0.05$) for all sample days following IGR application in 2000 (27 June, 25 July and 12 December), and on one sample day in 2001 (4 April) but no differences were found in 2002, when indicating reinfestation by *S. invicta* occurred (Fig. 51). IGR (Extinguish™) appeared to differentially affect RIFA and *D. flavus* colonies.

Analysis of Middens. Careful inspection of *D. flavus* middens showed that more than 98% of identifiable ant remains found consisted of RIFA (6,780 remains) with the remainder consisting of *D. flavus*, *M. minimum*, *Pheidole*, *Paratrechina* and *Pogonomyrmex* (Fig. 64). Accumulation of 1,979 fire ant remains occurred during the 8 day sampling interval (Fig. 65). Intact bodies were most abundant in the initial census and the remains varied little in the follow-up census (Fig. 66). All *D. flavus* middens contained RIFA remains (Fig. 65), strongly indicating the interaction leading to deposition of RIFA remains on the midden was occurring throughout the *D. flavus* population. The density of RIFA remains was positively correlated with midden size (Spearman's coefficient of rank correlation, $R^2 = 0.73$ on 31 July and $R^2 = 0.87$ on 8 August 2003, 8 df, $P<0.05$, $\alpha=0.05$). Midden volume was assumed to be a reflection of both colony size and colony activity. Correlation of RIFA remains with midden volume indicated interactions were occurring across the spectrum commensurate with size and activity, even in smaller or less active colonies.

Video Analyses of *D. flavus* Behavior. Results obtained from 28 hours of recordings taking at intervals from 4 September to 15 October 2003, showed *D. flavus* was frequently bringing presumably dead RIFA and/or their remains into and out of the nest (Fig. 67). Activity varied through time, but the ratio of these two behaviors remained consistent, with more ants (whole body) carried into nests than were removed. Aggressive behaviors from *D. flavus* towards *S. invicta* were recorded twice, when RIFA workers entered the *D. flavus* nest and were then attacked, repelled out of the nest, and then forcibly carried into the nest. The *D. flavus* – RIFA interaction documented here indicates *D. flavus* accumulates RIFA within the nest at a rate of three to five times that of RIFA accumulation on the midden. These results indicate the RIFA densities found on the midden (discussed above) are a gross underestimation of the degree of interaction taking place. The limited documentation of the actual cause of RIFA death bears further examination.

Analysis of Middens with Marked Fire Ants. *D. flavus* middens were found to contain marked RIFA from every area 48 hours after marking (Fig. 68). The rapid appearance of dead RIFA in *D. flavus* middens in every midden surveyed indicates *D. flavus* is actively and widely involved in their demise as opposed to restoring their midden solely with RIFA that have died from other causes. The dead marked RIFA represented about 10% of the total RIFA found in each *D. flavus* midden. This indicates rates of interaction can be examined using capture-recapture methods that would further quantify the rate and degree of interaction.

D. flavus may use RIFA as a source of food as well as killing them while actively defending their colony. Further analyses of gut contents of brood and workers are needed to explore these observations, however there is a strong indication that *D. flavus*

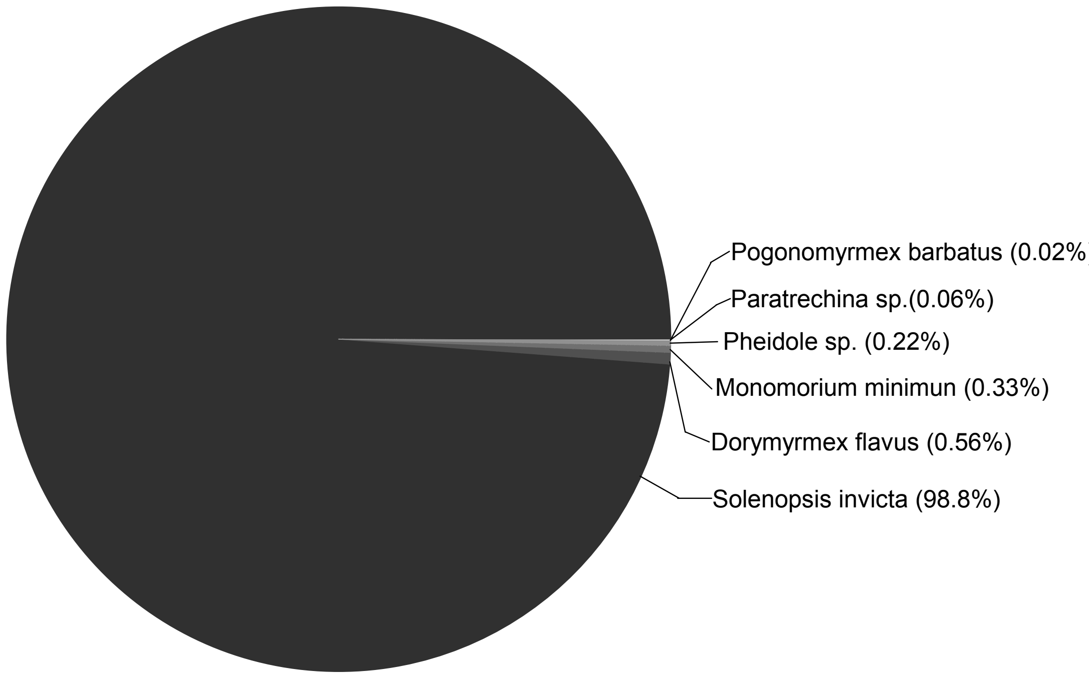

Figure 64. Relative abundance of ant remains found by two inspections without replacement of 10 complete *D. flavus* middens on July 31 and August 8, 2002.

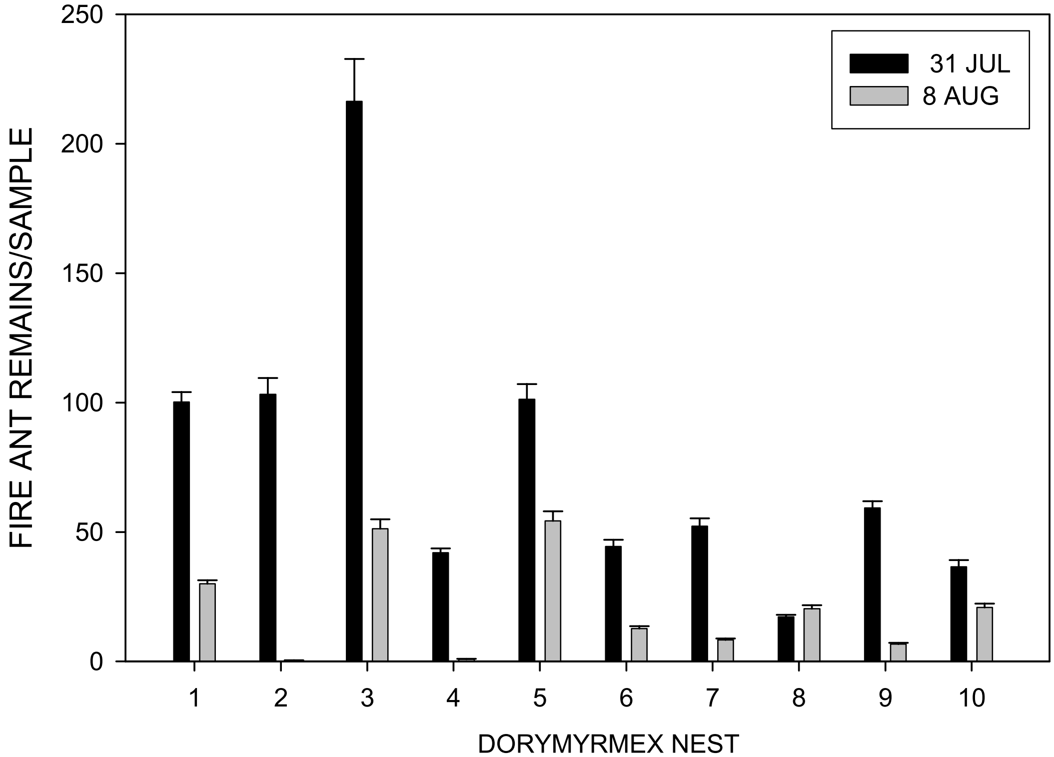

Figure 65 Number and recovery of remains of *Solenopsis invicta* found in 10 *Dorymyrmex flavus* middens in 2002.

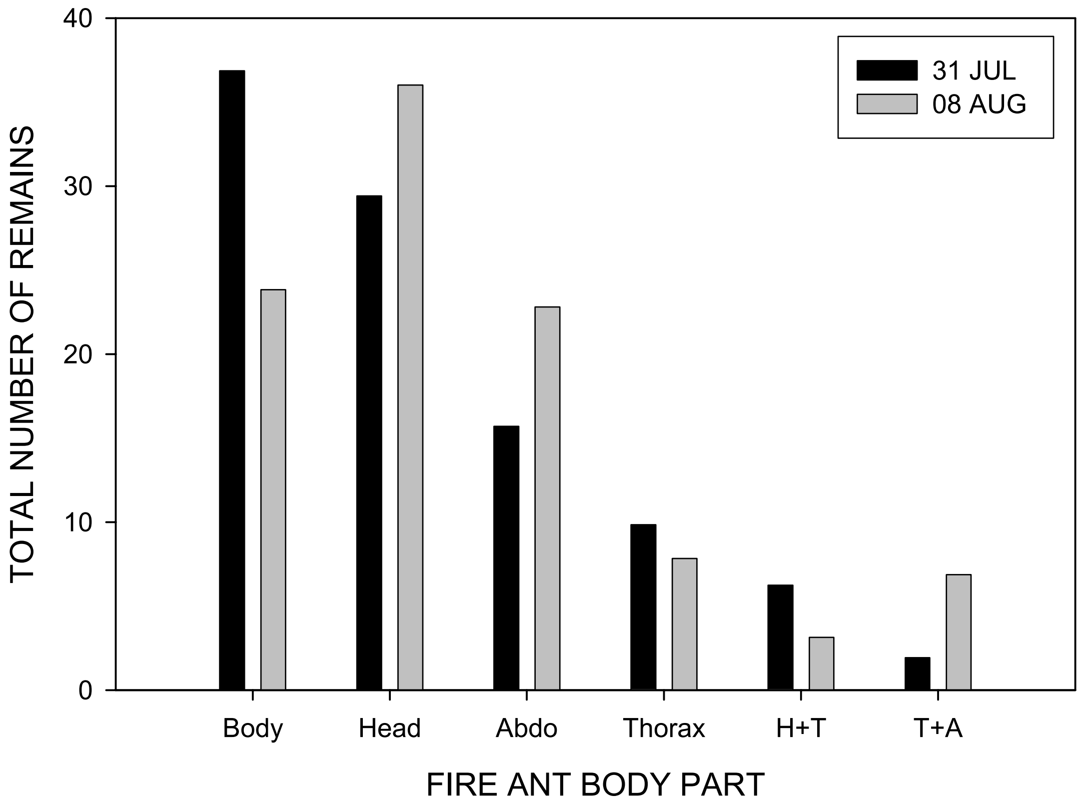

Figure 66. Total numbers of remains of *Solenopsis invicta* recovered in 10 *Dorymyrmex flavus* middens in 2002.

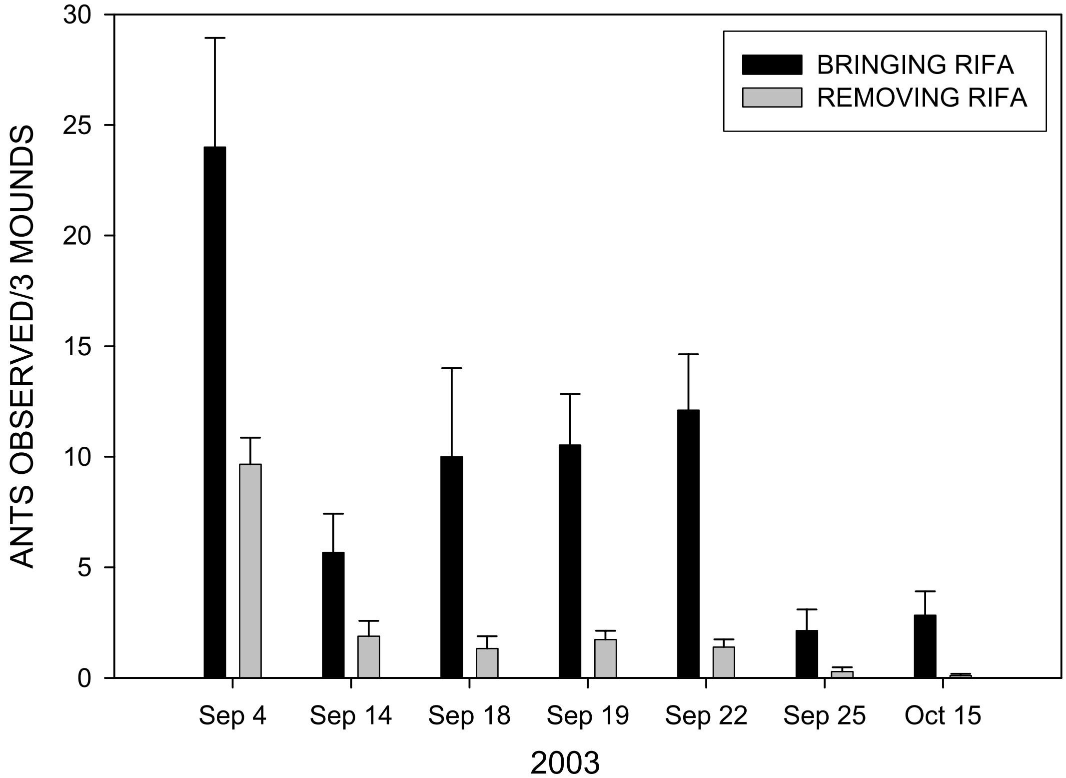

Figure 67. Workers of *D. flavus* recorded carrying bodies of dead fire ants into the nest and removing them from the nest during 28 hours of observation on 7 days during 4 September and 15 October, 2003.

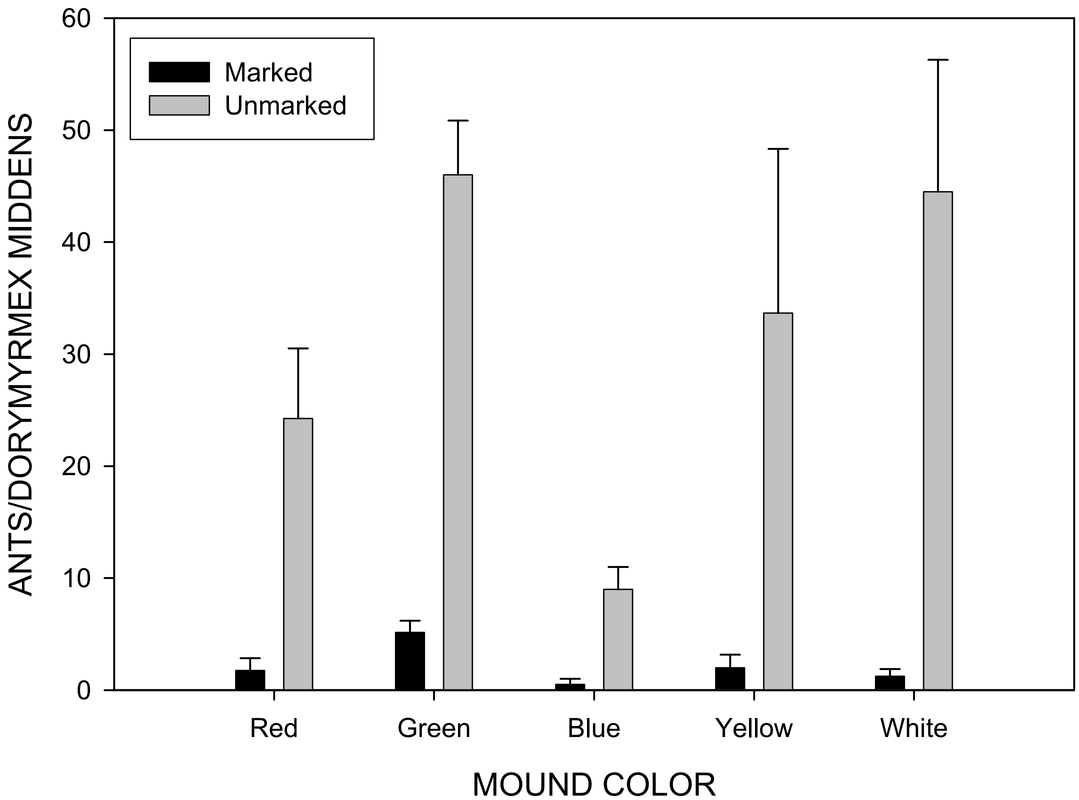

Figure 68. Fire ants recovered in middens of *Dorymyrmex flavus* after 48 hours of being marked.

is attacking and possibly preying upon fire ants. Video documentation of *D. flavus* carrying of live RIFA into the nest and removal of dead, often disarticulated ants from the nest is strong circumstantial evidence for their use as food.

***D. flavus* Nest Density after *S. invicta* Reinfestation.** IGR treated and Control plots were inspected during the first two weeks of September 2003, two years after the last IGR treatment was applied on 21 June 2001. Fire ants were abundant in both plot types and mound densities were not significantly different between these two (Mann Whitney Test, $P>0.05$). *D. flavus* was abundant in the previously treated IGR plots and colony densities were significantly greater when compared to the Control (Mann Whitney Test $P<0.05$) (Figs. 69, 70). This difference may indicate that *D. flavus* has biological and ecological features that makes this species a good competitor when densities of RIFA are low, and that can resist some degrees of RIFA reinvasion, therefore allowing them to coexist in this environment.

D. flavus may play an important role considering new efforts by the USDA to control and reduced RIFA combining several methods. This method includes biological control of RIFA with parasitic phorid flies and pathogens, and a prophylactic use of insecticide baits (Pereira 2003). *D. flavus* may help dispersing of a great numbers of dead RIFA and therefore promoting the dispersion of these biological control.

Summary *of D. flavus-S. invicta* interaction. From the six species in IGR treatments that showed positive response, *D. flavus* showed the greatest. *D. flavus* has been reported to attack newly mated RIFA queens of fire ants and to destroy up to 97% of the would-be founders in its territory (Whitcomb et al. 1973, Nickerson et 1975) as some other species do. However *D. flavus* has also been reported nesting at the edge of fire ant mounds (Wilson 1971), as observed in this study. Few observations of the antagonistic behavior of *D. flavus* towards RIFA were reported by Warriner (1998) exposing colonies of *D. flavus* to RIFA colonies under lab conditions and observing their interactions. However, these experiments observed under lab conditions were not replicated and lacked additional field observations and experimentation. The remains of *S. invicta* in *D. flavus* middens, the regular movement of dead RIFA into nests and removal of remains, the rapid appearance of marked live RIFA as remains in *D. flavus* middens and persistence of *D. flavus* in areas being reinvaded by RIFA, indicate *D. flavus* interaction with RIFA may reduce RIFA foraging efficiency. These results indicate that further study of *D. flavus* is needed to determine how its microhabitat may be enhanced to increase its value as a buffer species to retard domination of the ant assemblage by RIFA.

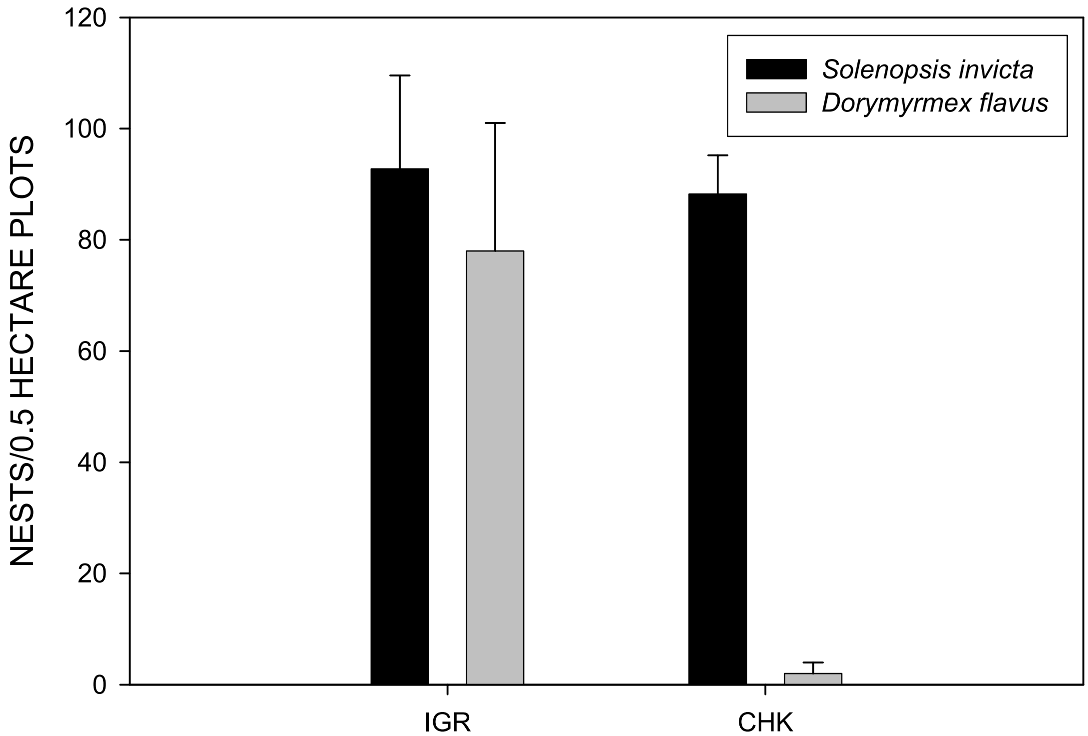

Figure 69. Density of *D. flavus* and *S. invicta* during the fall 2003 in plots treated with IGR compared to the Control plots.

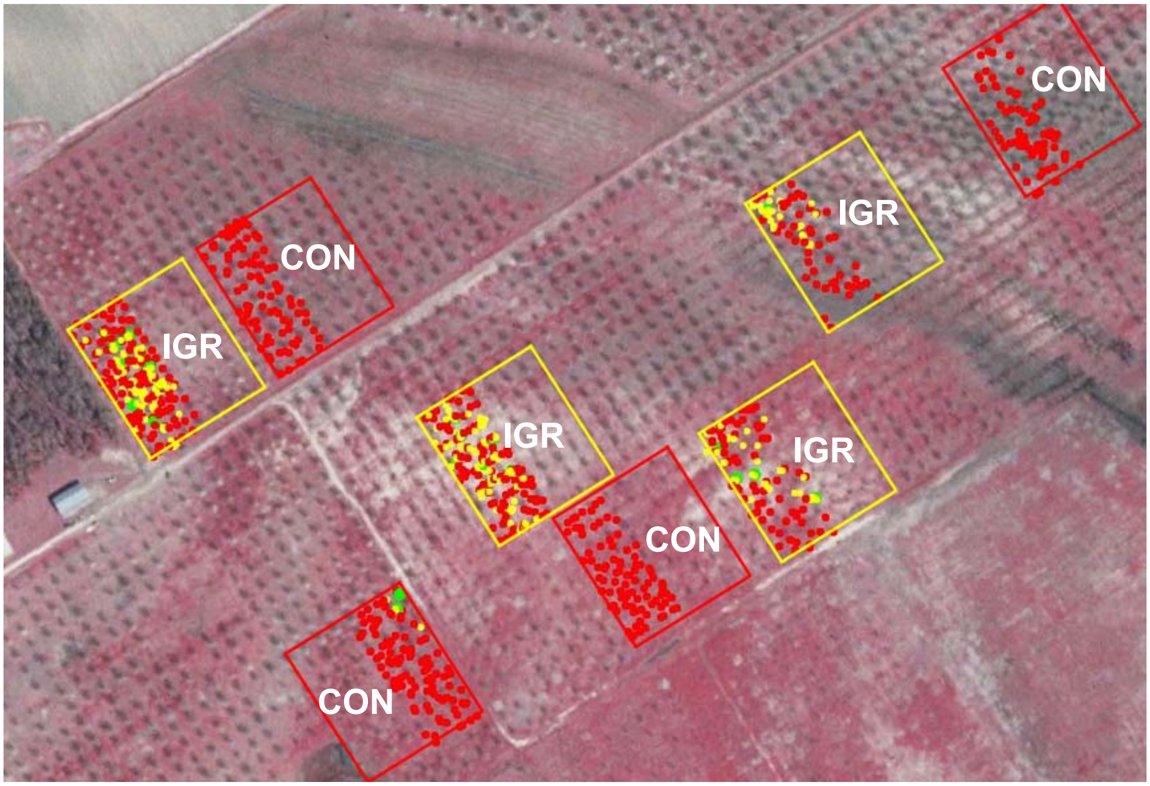

Figure 70. Density of *D. flavus* (yellow) and *S. invicta* (red) during the fall 2003 in areas where fire ants were treated with IGR and Control plots (CON).

CHAPTER IV

CONCLUSIONS

The ant assemblage (alpha diversity) is reported here for the first time in pecan in central Texas. The wide variety of life strategies of species in the ant assemblage indicates this system is particularly suitable to host a moderate diversity. Many of these species may affect other arthropod densities negatively or positively. The conservation and use of ants for biological control has been underestimated by pest managers for decades (Perfecto and Castineiras 1998) (although the first recorded example of biological control occurred 2000 years ago when Chinese provided bamboo poles between citrus canopies to allow predatory ants tree to tree access to predate their feeders (Bottrell 1979). The negative reputation of ants is related to a lack of understanding of their ecological role in agroecosystems. We need to better understand their ecology in managed systems. The occurrence of an ant mosaic in pecans with a dominant species is now confirmed for pecans, with *S. invicta* being the dominant species surrounded by other species, less competitive, but apparently with more flexibility to exploit this environment and coexist and to avoiding competition (changing foraging patters, switching from food item to another, placing nest out of range of RIFA, etc) . The frequent disturbance of this agroecosystem may reduce the development of exclusive territories, although this needs further investigation.

The use of IGR bait Extinguish™ provided a 77% control of RIFA for the years 2000 and 2001, accompanied by an increase in density and distribution of several native ant species. *D. flavus* appeared to be particularly favored by the IGR treatment. Native ant numbers were observed to increase in treated plots and were maintained in 2002. The mosaic of ant assemblages in 2002 remained similar to the previous year, indicating they sustained density levels achieved following the IGR treatment. Ant diversity remains similar between treated and untreated areas but abundance greatly differs when RIFA is reduced. However, the number of species among treatments was maintained indicating they may coexist in pecans. The complexity and the diversity of trophic levels in this pecan orchard make this crop particularly rich in diversity and highly suitable to hold a moderate diversity of ant species in central Texas. The ant assemblage in pecan in central Texas determined by pitfall traps, bait vials, direct sampling, and colony surveys, consists of a minimum of 16 species, dominated by *S. invicta* under natural pecan growing conditions (Figs. 3-11). Periodic trunk treatment with chlorpyrifos sprays and broadcast application of methoprene poison bait had significant effects on the ant densities observed using sampling methods noted earlier (Figs. 15, 16, 17, 18).

The chlorpyrifos trunk treatment had limited effect on *S. invicta*, and *Pheidole* and *Pogonomyrmex* increased in density in pitfalls, *Tapinoma* and *Paratrechina* increased in density in baited vials, *Paratrechina* increased in direct sampling, and *Pogonomyrmex* increased in colony surveys; remaining species were unaffected or had mixed effects or, were reduced in density. The trunk treatment appeared to have a limited effect on the ant assemblage in pecan. The poison bait (IGR) had the greatest effect on *S. invicta* based on density reduction recorded by every sampling method (Figs. 15, 16, 17, 18). Anecdotal reports have previously indicated that treatments directed at and that substantially reduced *S. invicta*, also had draconian effect on remaining species in the ant assemblage (Phillips et al. 1986, Thompson and Zakharov 1995, Zakharov and Thompson 1998).

However, data gathered in this study show that density increased for *D. flavus*, *Pheidole*, *Diplorhoptrum*, *Pseudomyrmex*, *Strumygenys* and *Pogonomyrmex* in all sampling methods, increased in bait vial, direct sampling and colony survey for *Paratrechina*, and in bait vial and direct sampling for *M. minimum* and *Tapinoma*, and that remaining effects either fluctuated among year or reduced densities of the remaining species in the IGR less than for *S. invicta* (Figs. 15, 16, 17, 18). The use of Extinguish™ seemed to had the greatest adverse effect upon *S. invicta*, while benefiting six (all sampling methods), six (bait vials), seven (direct sampling) and, four (colony survey) of the remaining species; *Smithistruma*, *Myrmecina* and *Brachymyrmex* were all reduced in density in the pitfalls and *Forelius* appeared on balance to be negatively affected as well by IGR. These results show the IGR treatments primarily reduced *S. invicta* and to a lesser extent four other species, while providing a generally increased density for the remaining 11 species in the ant assemblage. This shows the pecan ant assemblage is sensitive to the density of *S. invicta* and remaining species are capable of rapidly responding to a change in density for sustained periods.

Pitfall traps provided a better estimate of the alpha ant diversity within this orchard in Mumford, central Texas. Pitfall traps require little time to place and operate, they are inexpensive and can be tailored to each environment or target specific groups just by changing the size of the trap. Most epigeic ants were represented in pitfall traps as were some hypogean and arboreal ant species. Relative densities of theses species may be biased by differences in life styles (i.e. locomotion) (Greenslade 1973), habitat preferences, etc., so sampling methods should include several techniques (Bestelmeyer et al. 2000) like the proposed ALL protocol (Agosti and Alonso 2000) that combines transects, leaf litter collections, direct sampling and baited vials.

Based upon these findings *D. flavus* appears capable of inhibiting RIFA re-invasion of IGR treated areas and may continue occupation and survival there for many months, or even years, *D. flavus* indicate a territorial behavior and in many instances may actually prey upon fire ants. These findings demonstrate that the species density mix of the ant assemblage may be manipulated through selective elimination of RIFA.

More studies are needed to understand the dynamics of these assemblages, what drives their populations, and to determine the role of many of these species as biological control agents in this important agroecosystem of Texas and the United States.

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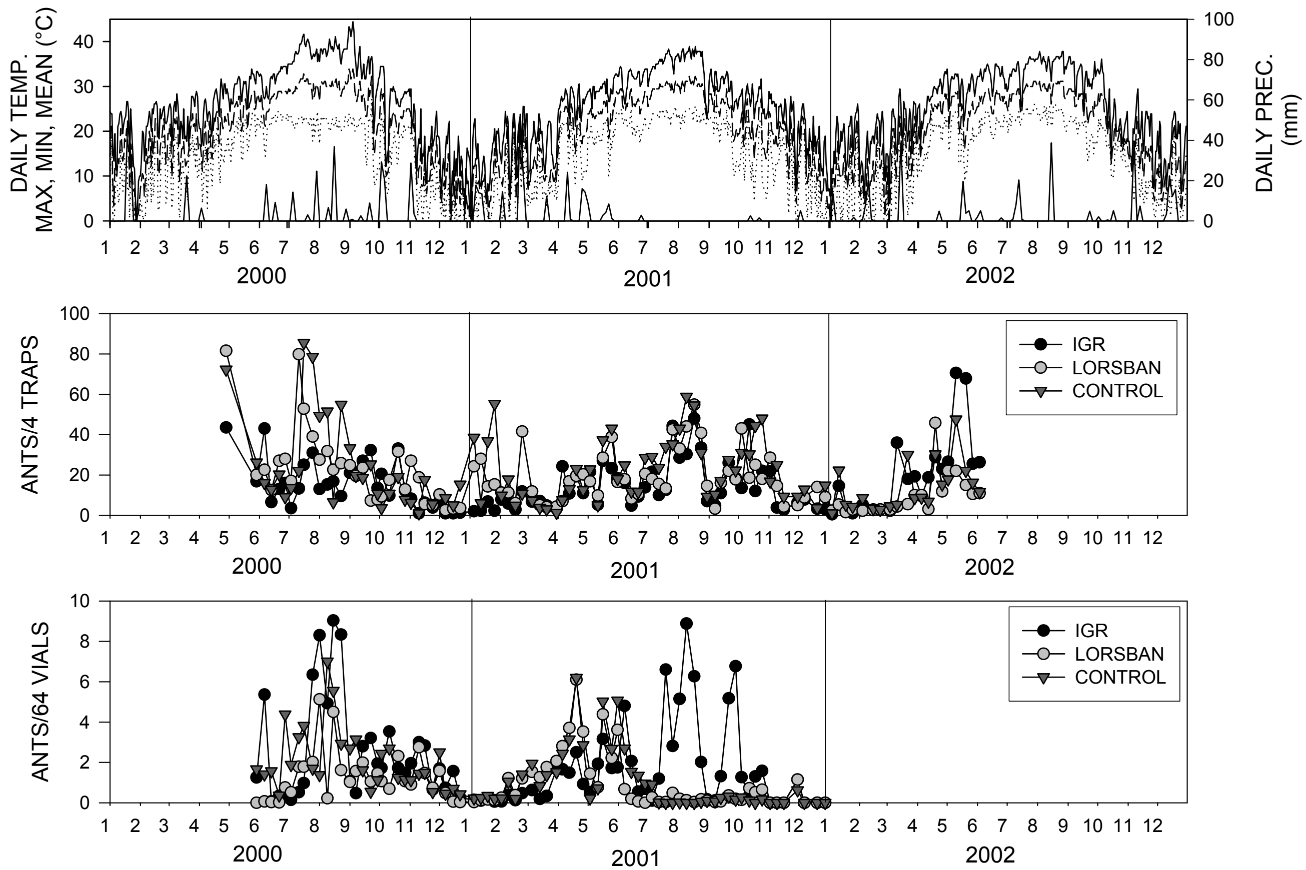
APPENDIX



Appendix. Annual temperature (max, min and mean) and precipitation recorded for Roberson Co., TX for the years 2000, 2001 and 2002, and seasonality of Formicidae among treatments throughout the years in two different methods.

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