IDENTIFICATION, DISTRIBUTION AND CONTROL OF AN INVASIVE PEST ANT, *Paratrechina* SP. (HYMENOPTERA: FORMICIDAE), IN TEXAS

A Dissertation

by

JASON MICHAEL MEYERS

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

DOCTOR OF PHILOSOPHY

August 2008

Major Subject: Entomology

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Approved by:

Committee Chair,	Roger Gold
Committee Members,	Jerry Cook Albert Mulenga Leon Russell Jr. Jim Woolley
Head of Department,	Kevin Heinz

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ABSTRACT

Identification, Distribution and Control of an Invasive Pest Ant,
Paratrechina sp. (Hymenoptera: Formicidae), in Texas. (August 2008)
Jason Michael Meyers, B.S., Southwest Missouri State University;
M.S., University of Arkansas
Chair of Advisory Committee: Dr. Roger Gold

Invasive species are capable of causing considerable damage to natural ecosystems, agricultures and economies throughout the world. These invasive species must be identified and adequate control measures should be investigated to prevent and reduce the negative effects associated with exotic species. A recent introduction of an exotic ant, *Paratrechina* sp. nr. *pubens*, has caused tremendous economic and ecological damage to southern Texas. Morphometric and phylogenetic procedures were used to identify this pest ant, *P*. sp. nr. *pubens*, to Southern Texas. The populations in Texas were found to be slightly different but not discriminating from *P. pubens* populations described in previous literature. Analysis of the distribution and expansion of *P*. sp. nr. *pubens* found numerous geographically discrete populations and moderately expanding territories. These expansion rates were determined to be ~20 and ~30 m per mo for a neighborhood and industrial area, respectively.

Several laboratory and field control strategies were implemented for control of this intensely pestiferous species. Dinotefuran exhibited high laboratory efficacy against

P. sp. nr. *pubens*, while treatments using novaluron were inconclusive. The use of expanded-use Termidor® demonstrated trends in these data that suggest it as the treatment of choice. Other field treatments, such as Termidor and Top Choice®, Termidor and Advance Carpenter Ant Bait™, and Transport® and Talstar® G, did not attain the success found in the expanded-use Termidor treatment. Most treatments examined were determined ineffective against high populations of *P*. sp. nr. *pubens*. Additional and more intensive population management regimes should be investigated. Abating further *P*. sp. nr. *pubens* population proliferation to other regions will only be realized from additional control research supplemented with state and federal interdiction policies.

DEDICATION

I wish to thank my parents, Harlan and Cathy Lupton, for putting up with me and all the times I needed advice on life. Without them this would have truly not been possible. Mom, thank you for always being there for me, loving me, and looking out for me. Dad, thank you for instilling a hard work ethic in me and for always doing what was right. Kelsi, thank you for reminding me to be young and silly; two things I seem to do very well.

I dedicate this dissertation to my family, Harlan, Cathy and Kelsi Lupton.

Proverbs 6:6-8
Go to the ant, O sluggard;
Observe her ways and be wise,
It has no commander,
No overseer or ruler,
Yet it stores its provisions in summer,
And gathers its food at harvest.

Proverbs 30: 24-25
Four things are small on the earth,
But they are exceedingly wise:
The ants are not a strong folk,
But they prepare their food in the summer.

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I thank and acknowledge those who assisted with the data collection and the many hours of hard and difficult labor. I thank Dr. James Austin for his immense help, knowledge, and above all else, friendship. I want to also thank him for his thorough and critical review of this dissertation. I thank Bryan Heintschel when he volunteered to endure the heat and humidity in La Porte and Deer Park during the dead of summer. I thank Dr. Harry Howell, may God rest his soul, for helping me set up my first experiment with this ant. I would like to thank Bill Summerlin who taught me a great deal about ant identification and for being a good friend. I thank Dr. Grady Glenn for putting up with all of my pest control questions, advice for my projects, and how to speak to residents. I also thank the other graduate students in our lab for their advice, support, experience and friendship, including; Barry Furman, Molly Keck, Chris Keefer, Aaron Thompson, and Rachel Wynalda. I greatly thank Dr. Jimmy Olson for all of the great advice and conversations we've had over the years; I will be forever in debt.

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CHAPTER I

INTRODUCTION

Invasive species represent a major threat to the world's biodiversity (Wilcove et al. 1998, UCS 2001), agriculture (Hallman and Schwalbe 2002), economy (Pimental et al. 2000), and unknown impacts on native ecosystems. These non-endemic organisms have caused an estimated $137 billion in damage per year in the United States alone (Pimental et al. 2000). With the current global emphasis on human commerce, it is incumbent upon scientists to increase biological invasion research. Identification of these threats is imperative to prevention and subsequent bioinvasive control of successful events. Literature is replete with articles regarding management of bioinvasions with focal points on prediction (Pimm 1989, Moller et al. 1993, Kolar and Lodge 2001, USDA APHIS 2004) or prevention (Reichard 1997, Leung et al. 2002, Simberloff 2003) of invasive events. Inherently placed at the foundation of such interdiction is identification of non-indigenous species as early as possible. Prevention and control are resources that are capable of decreasing the threat that exotic species pose.

A recent successful introduction of an invasive ant species, *Paratrechina* sp. nr. *pubens* to Texas, U.S.A., has created numerous economic and ecological concerns. Field observations suggest that companion animals have also acted abnormally in the presence

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This dissertation follows the style of Journal of Economic Entomology.

of this ant pest and there have been unknown effects on indigenous arthropod and small vertebrate fauna. Preliminary field observations also indicate that a biotic homogenization of formicid species including undocumented adverse affects to other taxonomic arthropod groups are possible. Similar unicolonial invasive ant species that may exist in high densities, the Argentine ant, *Linepithema humile*, and the yellow crazy ant, *Anoplolepis gracilipes*, have adversely impacted native systems in New Zealand (Harris 2002) and Christmas Island (Abbott and Green 2007), respectively. *L. humile* has caused adverse effects on ant diversity (Human and Gordan 1996, Holway 1999), abundance and diversity of other invertebrates (Cole et al. 1992, Way et al. 1992, Human and Gordon 1997), vertebrate abundance (Suarez et al. 2000), pollination (Buys 1987, Visser et al. 1996), seed dispersal and regeneration (Bond and Slingsby 1984, Giliomee 1986), and decomposition and nutrient cycling (Ward 1987, De Kock 1990, Folgarait 1998).

In certain geographical areas, *Paratrechina* species (Formicidae) possess abilities or characteristics for successful invasions into non-indigenous areas. Therefore, this group can potentially cause more confusion regarding its species composition. Because of morphological similarity (overlap), many taxonomic groups of insects can be indistinct at the species level. Ant taxonomic literature is rife with such occurrences. The state of *Paratrechina* taxonomy was once described as “depressing” (Creighton 1950). The revision of *Paratrechina* from the continental United States (Trager 1984) offered some reprieve from this confusion; however, the morphological ambiguity of *Paratrechina* species continues to sustain confusion among ant taxonomists.

A species-specific identification of *P*. sp. nr. *pubens* populations in Texas remains to be completed. This exotic pest species has successfully invaded an industrial area near Houston, Texas in 2002. Despite efforts from ant taxonomy experts (J. Trager, ant taxonomist, Shaw Nature Reserve; J. Cook, biology professor, Sam Houston State University), morphological and behavioral similarities of *P*. sp. nr. *pubens* populations from Texas, *P*. *pubens*, and *P*. *fulva* have caused an identification stalemate. As a consequence, a study will be conducted to elucidate the specific identification using morphological comparisons of the populations found in Texas and a previous description of *P*. *pubens* (Trager 1984). Preliminary comparisons noted morphometric differences in the populations of Texas and the previously described *P*. *pubens*. However, these findings were inconclusive as to its definitive identification as *P*. *pubens* or an undescribed species. As a result the Texas populations are designated as *P*. sp. nr. *pubens*.

Since its introduction in 2002, P. sp. nr. pubens has spread to numerous locations surrounding the greater Houston, TX area. Preliminary investigations indicate that the spread of *Paratrechina* sp. nr. *pubens* in non-urban areas is likely to occur at higher rates. Territorial expansion of a close taxonomic relative, *P*. *fulva*, has been known to occur at ca. 100 m per mo with rivers as the only geographical barrier to advancement (Zenner-Polania 1990). Expansion of a similar unicolonial ant, *L*. *humile* has been variably reported from 1.3 (Holway 1998b), 5.5 (Fluker and Beardsley 1970), 8.3 (Erickson 1971), 22.8 (Pasfield 1968), to 62.5 m per mo (Krushelnycky et al. 2004). These findings have been greatly dependent upon landscape suitability for *L*. *humile*.

Landscape suitability estimations will help to develop accuracy regarding potential geographical invasions and subsequent economic and ecological damage assessments of *P*. sp. nr. *pubens*.

Invasive social insects can create ecologically devastating results (Moller 1996, Chapman and Bourke 2001, Holway et al. 2002). These same social behaviors of ants create a weakness that can be exploited during the control process. Shared resources, trophallaxis, cannibalism, and grooming are all avenues for behavioral exploitation of active ingredient (AI) treatments. This is particularly evidenced by the horizontal transmission of pesticides, as has been observed in cockroaches (Kopanic and Schal 1999), termites (Ibrahim et al. 2003) and other ants (Soeprono and Rust 2004). Proficient invasions by social insects often encompass large geographical regions, are detrimental to agricultural systems and natural communities, and are expensive to control (Vinson 1986, Vander Meer et al. 1990, Williams 1994). The ease of application of aerially applied control measures is a desirable character for a management program for invasive species. The idea that baits could not only be used exclusively, but also as integration into an overall management program is certainly a reasonable scientific objective. These programs have been historically evaluated (e.g. Mirex against red imported fire ant, *Solenopsis invicta*) and more recently for termites as “Operation Full Stop” for the Formosan subterranean termite, *Coptotermes formosanus*, in New Orleans, Louisiana (Ring et al. 2001).

Containment of an early detected invasive species may afford time for research to conclude successful management or eradication techniques (Krushelnycky et al.

2004). It is imperative that basic science be completed regarding control of *P*. sp. nr. *pubens*.

The use of baits for eradication of ants has been reviewed (Stanley 2004). The use of baits has proven successful against other invasive species behaviorally similar to *P*. sp. nr. *pubens*. Unicolonial ants have been successfully controlled despite high densities, such as with *L. humile* (Krushelnycky et al. 2004) and the yellow crazy ant, *Anoplolepis gracilipes* (Abbott and Green 2007).

Despite repeated informative communications regarding *P*. sp. nr. *pubens*, there are no current federal expansion-preventing measures in place (Tony Koop, pers. comm., botanist, New Pest Advisory Group). Much of these efforts have been hampered by the confusion regarding the species specific-identification of *P*. sp. nr. *pubens*. Although biological control efforts against *P*. sp. nr. *pubens* have been considered, there are risks associated with the potential for permanent ecological change (Simberloff and Stiling 1996). These introduced species intended for biological control of pest species may not have adverse effects exclusively on their intended target (Simberloff 1992). A species taxonomically similar to *P*. sp. nr. *pubens*, *P. fulva*, was introduced to control venomous snakes in Colombia, South America; however, had unintended consequences, creating biotic homogenizations of the arthropod community in addition to economic losses (Zenner-Polania 1990).

This research was intended to answer basic scientific questions regarding identification, distribution and control of *P*. sp. nr. *pubens*. Morphological and phylogenetic identification of *P*. sp. nr. *pubens* was conducted. Distribution and geographical expansion of *P*. sp. nr. *pubens* was completed in South Texas. This research also included laboratory and field evaluations of control strategies involving novel insecticides, baiting, and multiple control tactics. This research will provide a foundation for baseline scientific knowledge of this incredible pest, *P*. sp. nr. *pubens*, with the hopes to propel future intensive studies that will explore and exploit its behavior and biology for containment and possible eradication.

CHAPTER II

MORPHOMETRIC ASSESSMENT OF *Paratrechina* SP. NR. *pubens* (HYMENOPTERA: FORMICIDAE) POPULATIONS IN TEXAS

Introduction

With the current global emphasis on human commerce, it is incumbent upon scientists to increase biological invasion research. Invasive species represent a major threat to U.S. biodiversity (Wilcove et al. 1998, UCS 2001) agricultural industries (Hallman and Schwalbe 2002) and economy (Pimental et al. 2000). Identification of these threats is imperative to prevent bioinvasions or control of successful events. Literature is replete with articles regarding management of bioinvasions with focal points on prediction (Pimm 1989, Moller et al. 1993, Kolar and Lodge 2001, USDA APHIS 2004) or prevention (Reichard 1997, Leung et al. 2002, Simberloff 2003) of invasive events. Inherently placed at the foundation of such interdiction is identification of non-indigenous species as early as possible.

Several *Paratrechina* species have demonstrated invasive behavior within the U.S. including: *P. bourbonica* (Forel), *P. flavipes* (F. Smith), *P. fulva* (Forel), *P. guatamalensis* (Forel), *P. longicornis* (Latreille), *P. pubens* (Forel) (Trager 1984a), and *P. vaga* (Forel) (Wilson and Taylor 1967). *Paratrechina* species are relatively successful tramp ants whose opportunistic behavior allows them to overcome adverse conditions in moist or dry environments. Other species (*P. fulva* and *P. longicornis*) of this genus have aggressively out-competed other non-homopteran insects, resulting in ecological dominance (Zenner-Polania 1994, Wetterer et al. 1999, respectively).

In particular, *P. pubens* invaded the U.S. in southern Florida (Trager 1984a) and now is found throughout the state (Warner and Scheffrahn 2004). *Paratrechina pubens* may have originated from the Caribbean archipelago based on type locality specimens collected from St. Vincent Island, Lesser Antilles (Forel 1893, Trager 1984a). The ant is now located in several other Caribbean island countries and Florida, U.S. Among these are Anguilla, Guadalupe, Puerto Rico (Trager 1984a), Bermuda (Wetterer 2006, 2007), and St. Croix (Wetterer unpublished data). Introduction of *P. pubens* to the United States was first described in 1953 (Trager 1984a) in Coral Gables and Miami, Florida. Potential for further spread is great among other island nations and mainland countries and states within and surrounding the Gulf of Mexico.

Populations of a species of *Paratrechina* nr. *pubens* have been discovered in Texas. The incipient population was found by a pest control operator in 2002 in Pasadena, Texas in an industrial area located ~8 km south of the Port of Houston (Harris County, TX). Immense populations of this problematic ant were found as soon as the following season. In 2005, this ant was observed in many different habitats; including trees, fallen branches, soil cracks, within the soil, and outside and within buildings and structures. Overwhelming numbers of this ant could be found at almost any location on the property. Since this time, new populations have arisen at alarming rates. These new populations were discovered by various pest control operators and Extension agents throughout the region. These populations were found in and around homes, businesses, wooded areas, and grassed fields.

Anecdotal reports have indicated that this ant has caused electrical shortages in a variety of apparatuses in businesses and homes, including, but not encompassing, phone lines, air conditioning units, chemical-pipe valve computers, and sewage lift pump stations. Additional residential complaints have also included rare but painful bites with occasional physiological reaction and abnormal behavior of companion animals in the presence of higher levels of *P*. sp. nr. *pubens* infestation. Residents and businesses alike have resorted to costly and unsuccessful consumer remedial control. These unguided and ineffective control measures by untrained and inexperienced citizens have been ineffective and are of major concern due to their negative environmental impact to the urban ecology of affected areas.

Ecological impacts of *P*. sp. nr. *pubens* are not yet known. However, preliminary field observations minimally suggest a homogenization of ant fauna and/or reduction or displacement of native ant populations, /and the invasive red imported fire ant, *Solenopsis invicta*. As the spread of *P*. sp. nr. *pubens* continues, this pest will likely create more problems in other geographical areas. These problems include further spread outside the currently infested state of Texas creating additional biotic homogenization and/or unknown adverse ecological impacts.

Confusion regarding identification of the populations in Texas has remained despite samples examined by experts (J. Trager, ant taxonomist, Shaw Nature Reserve; J. Cook, professor, Sam Houston State University). Despite established and intermittently dense populations of *P. pubens* in Florida (Warner and Scheffrahn 2004) and Caribbean islands (Wetterer 2006, 2007, unpublished data), this ant has not become established in other areas of the U.S. It is curious as to the reason(s) for lack of previous successful *P. pubens* invasions. This questions the validity of the taxonomic synonymy of the populations of Florida and Texas. Intraspecific variation of *P. pubens* populations from different clinal regions may explain some, if not all, of the morphological and biological differences viewed between earlier (Trager 1984a) and current descriptions of this species. However, these differences may also suggest the current Texas populations are an undescribed species of *P. pubens*, *P. fulva*, or an unknown synonymy of a previously described species.

Despite repeated informative communications regarding *P.* sp. nr. *pubens*, there are no current federal expansion-preventing measures in place (Tony Koop, pers. comm., botanist, New Pest Advisory Group). Much of these efforts have been hampered by the confusion regarding the species specific-identification of *P.* sp. nr. *pubens*.

The use of morphometric differentiation for species identification has been used for social insects such as formicids (Wang and Lester 2004, Steiner et al. 2006) and termites (Hostettler et al. 1995, Heinstchel et al. 2006). This study compares the morphologies of *P.* sp. nr. *pubens* populations in Texas to previously described populations of *P. pubens* (Trager 1984a). Given the morphological similarities and subsequent identification confusion, this study intends to alleviate concern regarding species-specific identification of these populations.

Materials and Methods

Material Examined. Morphometric and morphology data for this study were from specimens of two colonies collected in a grassed field in Pasadena, TX in May 2005. Measurements and morphological observations were made of 16 workers and one male alate from 'Texas 1' colony, while 16 workers constituted the specimens from 'Texas 2' colony. Male alates were rare in previously collected colonies. For this reason, a third colony from Pasadena, TX was collected in February 2008 when males are much more plentiful. Some of the male alates (n = 5) of this population were collected and described (total male alates, n = 6). For the description of this species, several morphological measurements (Table 2.1) were taken per Trager (1984a). Morphometric and indices data for additional populations were taken from a previous study (Trager 1984b). These character data taken for worker and/or male alates included:

1. HL = Head length
2. HW = Head width
3. SL = Scape length
4. EL = Eye length
5. PW = Pronotal width
6. MCL = Longest macrochetae of pronotum
7. WL = Thorax length
8. GL = Gaster length
9. TL = Total length
10. FL = Femur length
11. SM = # scape macrochetae
12. PM = # pronotal macrochetae
13. MM = # mesonotal macrochetae
14. CI = (HWx100)/HL
15. OI = (ELx100)/HL
16. SI = (SLx100)/HL
17. FI = (FLx100)/HL

Full definitions of measurements can be found per Trager (1984a, b).

When statistically analyzing populations of *P. pubens* and *P.* sp. nr. *pubens* worker morphometrics, *P. pubens* data (“Trager”) (Trager 1984b) from Florida populations were combined as one population (n = 14) and data from all other states were combined as one population (n = 20). “Other” states included *P. pubens* collected from New York, Puerto Rico, and Washington D.C. *P.* sp. nr. *pubens* from Texas was treated as a third population (n = 32). Missing values within analyzed individuals were not evaluated in the canonical discriminate statistics.

To compare measurement and index means among the populations, multivariate analysis of variance (MANOVA) was conducted. Post-hoc comparisons were performed using Bonferroni means comparison of worker morphometrics. Wilks’ λ was used to determine significant differences among means of morphometric characters. To determine discriminating characters, an ANOVA was run in discriminant function analysis using Wilks’ λ to test the equality of group means for the worker and male alate characters. To find differences among the groups of worker populations, a canonical discriminant analysis was run. A paired t-test was used to evaluate differences among means between male alate *P.* sp. nr. *pubens* (Texas) and *P. pubens* (“Trager”) (Trager 1984b). All statistical analyses were run using SPSS (SPSS Inc. 2005).

Distribution. A preliminary biogeographical distribution for the purposes of species description of this pest was conducted. Specimens of *P.* sp. nr. *pubens* were discovered by pest control operators, extension agents, or residents. Suspected specimens were mailed to the Center of Urban and Structural Entomology, Texas A&M University and examined by JMM. If these specimens were unable to be mailed, JMM inspected and collected specimens of the potentially infested facility or landscape. Preliminary geographically specific distribution of *P*. sp. nr. *pubens* was conducted using baited area monitor traps.

Results

P. sp. nr. *pubens* were found in grassed and wooded fields, leaf litter, under covered objects such as porous stones or logs, esp. in termite-inhabited logs, trailing in mass on trees and also outside and within buildings. These ants were typically found in remarkable numbers with trails as wide as 10 cm.

Material Examined. All measurements reported in mm.

Worker. A composite description of the Texas colonies is given. HL 0.68-0.77, HW 0.55-0.64, SL 0.75-0.87, EL 0.16-0.20, PW 0.40-0.48, MCL 0.18-0.22, WL 0.84-0.97, FL 0.58-0.69, SM 19-26, PM 8-13, MM 2-3, CI 77.27-85.29, OI 21.74-28.13, SI 103.03-119.70, FI 82.86-98.44, $(n = 32)$ (Table 2.1).

Similar morphological results were confirmed (J. Trager, pers. comm.) from worker and male alate individuals of the colonies described above. These workers are very similar to previous character descriptions of *P. pubens* (Trager 1984b, J. Trager, pers. comm.). Medium-sized, reddish-brown with dense pubescence on thorax and gaster with flexuous, light brown macrochaetae. In regards to *P. pubens*, *P*. sp. nr. *pubens* has a subcordate head, and is not as pubescent on the thorax or gaster. Most *P*. sp. nr. *pubens* measurements were significantly (Wilke's $\lambda = 0.006$, $F = 4.17$, $P < 0.001$) smaller than previously described *P. pubens* (Trager 1984b) (Tables 2.1-3, Fig. 2.1). Morphological

Table 2.1. Multivariate ANOVA of worker morphometrics from *P*. *pubens* and *P*. sp. nr. *pubens* populations.

	Mean (SE ±) worker measurement (mm) of *P*. *pubens* and *P*. sp. nr. *pubens* populations[abc]					
Population	HL	HW	SL	EL	PW	MCL
Washington D.C.[d]	0.78 (0.007) a	0.70 (0.008) a	0.95 (0.008) a	0.20 (0.002) a	0.49 (0.006) b	0.25 (0.004) b
Florida[d]	0.80 (0.006) a	0.71 (0.006) a	0.97 (0.007) a	0.20 (0.002) a	0.54 (0.005) a	0.27 (0.003) a
New York[d]	0.78 (0.011) a	0.66 (0.012) ab	0.95 (0.013) a	0.19 (0.004) ab	0.48 (0.009) bc	0.25 (0.007) b
Puerto Rico[d]	0.81 (0.009) a	0.71 (0.010) a	0.97 (0.011) a	0.20 (0.003) a	0.51 (0.008) b	0.26 (0.005) ab
Texas 1	0.74 (0.006) b	0.61 (0.006) c	0.82 (0.007) b	0.18 (0.002) b	0.45 (0.005) c	0.20 (0.003) c
Texas 2	0.70 (0.006) c	0.57 (0.006) c	0.79 (0.007) b	0.18 (0.002) bc	0.42 (0.005) d	0.20 (0.003) c

Table 2.1. Continued.

	Mean (SE ±) worker measurement (mm) of *P*. *pubens* and *P*. sp. nr. *pubens* populations[abc]					
Population	WL	FL	SM	PM	MM	CI
Washington D.C.[d]	1.02 (0.010) a	0.77 (0.006) a	26.40 (0.79) a	10.70 (0.45) ab	3.80 (0.21) ab	87.20 (0.56) ab
Florida[d]	1.05 (0.008) a	0.79 (0.005) a	29.87 (0.79) a	11.87 (0.37) a	4.45 (0.17) a	88.53 (0.46) a
New York[d]	1.01 (0.015) a	0.75 (0.010) a	24.75 (1.52) ab	11.00 (0.71) ab	3.25 (0.33) bc	84.75 (0.88) bc
Puerto Rico[d]	1.03 (0.013) a	0.78 (0.008) a	21.50 (1.24) b	10.33 (0.58) ab	4.67 (0.27) a	84.83 (0.72) ab
Texas 1	0.91 (0.008) b	0.67 (0.005) b	22.44 (0.76) b	10.44 (0.36) ab	2.13 (0.17) cd	82.44 (0.44) c
Texas 2	0.87 (0.008) c	0.64 (0.005) c	21.44 (0.76) b	10.06 (0.36) b	2.00 (0.17) d	82.06 (0.44) c

Table 2.1. Continued.

Population	Mean (SE ±) worker measurement (mm) of *P. pubens* and *P.* sp. nr. *pubens* populations[abc]		
	OI	SI	FI
Washington D.C.[d]	25.60 (0.34) a	119.10 (0.97) a	96.30 (0.82) a
Florida[d]	25.00 (0.28) ab	121.13 (0.79) a	97.67 (0.67) a
New York[d]	24.75 (0.54) ab	121.75 (1.54) a	96.25 (1.30) a
Puerto Rico[d]	24.83 (0.44) ab	120.00 (1.26) a	95.67 (1.06) a
Texas 1	24.06 (0.27) b	111.88 (0.77) b	91.19 (0.65) b
Texas 2	24.44 (0.27) ab	112.69 (0.77) b	91.31 (0.65) b

[a] Full measurement definitions can be found per Trager (1984a, b). HL = Head length, HW = Head width, SL = Scape length, EL = Eye length, PW = Pronotal width, MCL = Longest macrochetae of pronotum, WL = Thorax length, FL = Femur length, SM = # scape macrochetae, PM = # pronotal macrochetae, MM = # mesonotal macrochetae, CI = (HWx100)/HL, OI = (ELx100)/HL, SI = (SLx100)/HL, FI = (FLx100)/HL.

[b] Same letters following means within the column are not significantly different (MANOVA, Bonferoni, Wilke's λ, $P = 0.05$).

[c] Wilke's $\lambda = 0.006$, $F = 4.17$, $P < 0.001$.

[d] Data from Trager (1984b).

Table 2.2. Paired t-test of male alate morphometrics from *P*. *pubens* and *P*. sp. nr. *pubens* populations.

	Mean (SE ±) male alate measurement (mm) of *P*. *pubens* and *P*. sp. nr. *pubens* populations[a,b]										
N = 6	HL	HW	SL	EL	WL	GL	SM	CI	OI	SI	TL
Texas	0.59	0.60	0.74	0.26	1.02	0.74	8.67	101.21	44.09	123.76	2.36
	(0.005)	(0.01)	(0.02)	(0.009)	(0.03)	(0.02)	(0.76)	(1.49)	(1.12)	(2.58)	(0.04)
Trager[b]	0.68	0.65	0.96	0.27	1.10	1.19	16.17	95.67	40.83	143.00	2.96
	(0.006)	(0.01)	(0.02)	(0.004)	(0.02)	(0.54)	(1.30)	(0.49)	(0.31)	(0.68)	(0.07)
F value (*P* value)	13.92	5.23	14.99	1.29	4.70	9.75	4.34	4.27	3.39	7.50	16.79
	(0.000)	(0.003)	(0.000)	(0.253)	(0.005)	(0.000)	(0.007)	(0.008)	(0.019)	(0.001)	(0.000)

[a] For measurement definitions see below Table 1.

[b] Data from Trager (1984b).

[c] All analyses are significant at α = 0.05.

Table 2.3. Canonical discriminant analysis of caste morphometrics for *P*. sp. nr. *pubens* and *P*. *pubens* populations.

		Tests of Equality of Group Means		
Character	Caste	Wilks' λ	F	*P*
HL	Worker	0.311	69.91	0.000
	Male	0.078	117.44	0.000
HW	Worker	0.194	130.81	0.000
	Male	0.446	12.41	0.006
SL	Worker	0.116	239.52	0.000
	Male	0.070	133.37	0.000
EL	Worker	0.381	51.28	0.000
	Male	0.911	0.98	0.345
PW	Worker	0.188	136.04	0.000
	Male	-	-	-
MCL	Worker	0.144	187.07	0.000
	Male	-	-	-
WL	Worker	0.189	135.23	0.000
	Male	0.623	6.05	0.034
GL	Worker	-	-	-
	Male	0.139	61.73	0.000
FL	Worker	0.119	233.51	0.000
	Male	-	-	-
TL	Worker	-	-	-
	Male	0.157	53.63	0.000
SM	Worker	0.521	29.00	0.000
	Male	0.288	24.76	0.001
PM	Worker	0.844	5.81	0.005
	Male	-	-	-
MM	Worker	0.297	74.44	0.000
	Male	-	-	-
CI	Worker	0.304	72.12	0.000
	Male	0.445	12.46	0.005
OI	Worker	0.847	5.70	0.005
	Male	0.559	7.90	0.018
SI	Worker	0.343	60.23	0.000
	Male	0.162	51.82	0.000
FI	Worker	0.435	40.91	0.000
	Male	-	-	-

[a] Analyses are significant at $\alpha = 0.05$.

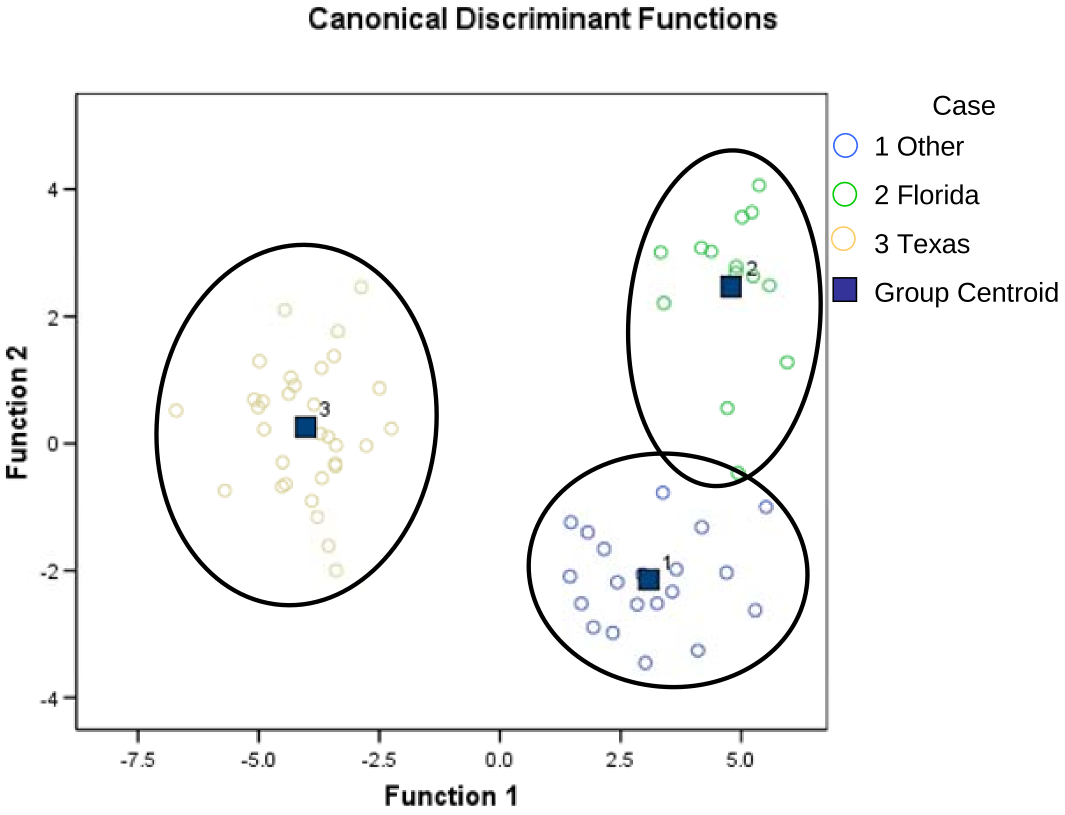


Figure 2.1. Canonical discriminant function analysis of male alate morphometrics for *P. pubens* (Florida and Other) and *P*. sp. nr. *pubens* (Texas) populations. Function 1 (Eigen value = 16.35, % variance = 85.2, Canonical correlation = 0.97; Wilks' $\lambda = 0.02$, $\chi = 235.23$, df = 30, $P < 0.001$), Function 2 (Eigen value = 2.85, % variance = 14.8, Canonical correlation = 0.86; Wilks' $\lambda = 0.26$, $\chi = 75.44$, df = 14, $P < 0.001$).

dissimilarity alone does not necessarily indicate *P*. sp. nr. *pubens* as an undescribed species.

Male. (n = 6) Cuspides ca. ¾ length of aedeagus. Very similar to previous description of *P. pubens* (Trager 1984b).

Male. A composite description of the Texas colonies is given as follows; HL 0.58-0.62, HW 0.56-0.64, SL 0.67-0.79, EL 0.24-0.30, WL 0.90-1.08, GL 0.69-0.79, SM 6-11, CI 96.23-105.56, OI 41.51-48.21, SI 115.09-133.33, (n = 6) (Table 2.2).

These *P*. sp. nr. *pubens* males are either *P. pubens* or very near *P. pubens* (J. Trager, pers. comm.). Males are not decidedly black as those from typical *P. fulva* populations (J. Trager, pers. comm.); however, the setal arrangement on the parameres is dissimilar to *P. pubens* (J. Trager, pers. comm.). These character similarities and dissimilarities suggest that there is some variation among populations of *P. pubens*.

Biology. Along with the morphological findings, the densities demonstrated by P. sp. nr. pubens may also indicate a taxonomic classification of an undescribed species. *Paratrechina pubens* has been previously described as “non-biting” (Warner and Scheffrahn 2004). Anecdotal reports have indicated painful ant bites, generally without swelling, from the Houston populations as well as *P. pubens* populations found at the Jacksonville Zoo and Gardens, Florida (D. Calibeo-Hayes, pers. comm., graduate student, University of Florida).

The paired t-tests of male alate characters revealed significant differences between the means of the two populations (“Trager” and Texas) (Table 2.2). The only character for male alates of the populations that was not significant was EL.

Canonical discriminant analysis identified differences among the groups based on these worker data (Fig. 2.1). These groups found that “Other”, Florida (Trager 1984b), and Texas populations were significantly different (df = 2, 63; Function 1: Eigen value = 16.35, % variance = 85.2, canonical correlation = 0.97; Wilks’ $\lambda = 0.02$, $\chi =$ 235.23, df = 30, $P < 0.001$ and Function 2: Eigen value = 2.85, % variance = 14.8, canonical correlation = 0.86; Wilks’ $\lambda = 0.26$, $\chi = 75.44$, df = 14, $P < 0.001$). No significant difference was found between the “Other” and “Florida” groups of the populations.

The ANOVA tests of equality of group means indicated nearly all characters as significantly discriminating (Table 2.3). The closer the Wilks’ λ value is to 0, the more important the independent variable becomes to the discriminant function. The most discriminating characters for workers were; SL (Wilks’ $\lambda = 0.116$), FL (Wilks’ $\lambda =$ 0.119), and MCL (Wilks’ $\lambda = 0.144$). There were no significantly excluded characters for the workers. The most discriminating characters for male alates were; SL (Wilks’ $\lambda =$ 0.70), HL (Wilks’ $\lambda = 0.078$), and GL (Wilks’ $\lambda = 0.139$). The only significantly excluded character for male alates was EL.

Distribution. *Paratrechina pubens* has spread significantly since its discovery in 2002 and currently encompasses 25 known, geographically discrete populations in five counties (Brazoria, Galveston, Harris, Montgomery, and Wharton). Additional and more detailed results on distribution and spread of *P.* sp. nr. *pubens* are discussed in Meyers and Gold unpublished a.

Discussion

Statistical analysis of the morphometric characters among populations of the composite worker description for *P. pubens* (J. Trager 1984b) result in significant differences among the various populations (Table 2.1). These differences are not as great as the differences found between their composite description and *P*. sp. nr. *pubens*. Intraspecific variation of morphology among *P. pubens* populations may be greater than previously reported. Based on a composite description I do not deem it statistically prudent to differentiate the Texas populations from the previous description of *P. pubens* (J. Trager 1984b). If this statistical procedure was conducted to differentiate species, it would likely raise a few of the *P. pubens* populations from previous findings (J. Trager 1984b) to the species level.

Numbers of mesonotal macrochetae (MM) may offer a diagnosibly distinct character to distinguish between *P. pubens* and *P*. sp. nr. *pubens* populations. MM means were 2.13 and 2.00 in the TX1 and TX2 colonies, respectively, while previously published (Trager 1984b) populations averaged 3.25 - 4.67. Only two (6.25%) individuals from the Texas populations exhibited three MM. Given the additional statistical disparity of the character and relative ease of character identification, this may offer a simple identification character. Although other *Paratrechina* species within the U.S. exhibit this 2-3 MM character, this is a differentiating character between the two species in question. Although the test for equality of group means for MM revealed significant discrimination of the character (Wilk’s $\lambda = 0.297$, $P = 0.000$), it may not be discriminating between the *P*. sp. nr. *pubens* and *P. pubens* populations. Examination of voucher samples from another study (Meyers and Gold unpublished b) of *P. pubens* from Florida, Anguilla, St. Croix, and St. Kitts do not differentiate from the 2-3 MM character found in *P.* sp. nr. *pubens* populations in Texas. This character would easily diagnose the difference between previously described (Trager 1984b) and currently described populations.

According to the analyses of these data, few measurements need to be taken in order to differentiate the populations of *P. pubens* and *P.* sp. nr. *pubens*. Clearly, *P.* sp. nr. *pubens* is smaller than previously described *P. pubens* (Trager 1984b). Even though morphological dissimilarities are present, species differentiation may not be correct or prudent. Intraspecific variation could claim many morphologically discrete species descriptions, especially in insects.

Due to the morphological and biological similarities of *P. fulva* and *P.* sp. nr. *pubens* supplementary research of morphological and biological characters of these populations should be conducted. Despite a previous, inadequate species description for the original concept of *P. pubens* (Forel 1893), morphological evidence alone does not suggest that a new species classification is warranted for the Texas populations. Based on the results of this study, it is currently recommended that our assessments be combined with previous findings (Trager 1984a) until a more comprehensive study is done. This would result in the expansion of the morphometric ranges for *P. pubens*. These results may indicate a geographical intraspecific variation of *P. pubens*, frequently observed in insect species. Given the statistical disparity between the two Texas colonies, more populations should be analyzed to further examine intraspecific variation in this region. It remains a distinct possibility that the Texas populations of *P*. sp. nr. *pubens* are a an undescribed species. There are currently no known or described subspecies within *P*. *pubens* (Bolton et al. 2006).

Many theories of successful invasion events have been described. Identification of the avenue by which this species has invaded may assist in preventing further invasions of this species. The successful invasion of *P*. sp. nr. *pubens* in Texas and extreme densities may indicate an ideal ecosystem for the species. It may also indicate a total enemy release (Keane and Crawley 2002, Mitchell and Power 2003) and/or ideal scenario for biotic release hypothesis (Simberloff 1986, 1989). *Paratrechina pubens* type locality is an island ecosystem (Caribbean archipelago) (Forel 1893). This would place it in a rare circumstance as type locality island species invading and dominating a continental ecosystem. To the contrary, *P*. *fulva* type locality is continental (Brazil) (Mayr 1862) and as such would place it as a continental species invading a continental ecosystem.

This study may represent the first record of *P*. *pubens* in Texas and demonstrates yet another invasive species (the Formosan subterranean termite, *Coptotermes formosanus* and Asian cockroach, *Blattella asahinai*) which has produced negative consequences for the urban areas in which they have established (Dorward 1956, Austin et al. 2007, respectively). The United States is a non-indigenous geographical area of *P*. *pubens* (Forel 1893, Trager 1984a). The Texas populations constitute a new and established geographical location for either *P*. *pubens* or *P*. *fulva*. This study could stand as the initial identification of invasion history for *P*. *pubens* or a renewed invasion of *P*.

fulva in Texas (Trager 1984b). The near exponential spread is unlikely to be abated unless interdiction policies are put in place or significant research is conducted on preventing or slowing their progress.

Results of this study and pers. comm. (J. Trager and J. Cook) may suggest identification of this ant as *P. pubens*, *P. fulva*, or an undescribed species of *Paratrechina* of North America. The need for a cosmopolitan taxonomic key of *Paratrechina* is critical for the identification of this morphologically ambiguous and invasively competent group. More comprehensive comparative morphological analysis of these taxa and type locality *P. pubens* and *P. fulva* populations should be conducted to provide a more competent identification of this ant. Additionally, phylogenetic analysis should be conducted on these populations and other type locality to elucidate identities of populations with indistinct morphologies. This analysis may also reveal these Texas populations as an undescribed species that are morphologically indistinct.

In the most established portions of its range, such as the site of original known infestation, no other ant species were found on or in the traps, including the typically common red imported fire ant, *Solenopsis invicta* Buren. To the contrary, at any trapping site not placed within the strongly established areas of *P.* sp. nr. *pubens*, *S. invicta* was found in nearly every trap. Peripheral (areas without strong *P*. sp. nr. *pubens* establishment) traps often contained a diverse collection of ant species. These results may indicate rapid establishment of ecological dominance in the area of infestation.

Until more comprehensive sampling and/or diagnostic evidence is discovered for its current position; the taxonomic identity of *P*. sp. nr. *pubens* populations in Texas will remain unchanged. More research regarding behavior, mating compatibility, phylogeny, or other analyses of these populations should be conducted before raising the *P*. sp. nr. *pubens* Texas populations to an undescribed species.

The biological and temporal caveats associated with successful invasive populations of social arthropods are quite complex. It is a rare scientific opportunity to follow the incipient biology of an unexpected, invasive, and dominant pest. Information and conclusions gained from this and future studies on *P*. *pubens* populations of Texas may assist research of other impending arthropod invaders, especially social insects.

CHAPTER III

MOLECULAR PHYLOGEOGRAPHY OF AN INVASIVE *Paratrechina* SP. AND OTHER *Paratrechina* SPP. FROM VARIOUS COUNTRIES

Introduction

Many insect species descriptions are based on distinct morphology alone. Ant taxonomic literature is rife with such occurrences. The state of *Paratrechina* taxonomy was once described as "depressing" (Creighton 1950). The revision of *Paratrechina* from the continental United States (Trager 1984a) has offered some reprieve from the confusion, but the morphological ambiguity of *Paratrechina* species continues to sustain confusion among ant taxonomists. Due to their penchant for successful invasions into non-indigenous areas, species of *Paratrechina* can potentially cause more confusion regarding the species present in certain geographical areas.

Since the revision (Trager 1984a), synanthropic behaviors of *Paratrechina* species (Hölldobler and Wilson 1990), have caused numerous invasive events which have occurred into and throughout the U.S. including: *P. bourbonica* (Forel), *P. flavipes* (F. Smith), *P. fulva* (Forel), *P. guatamalensis* (Forel), *P. longicornis* (Latreille), *P. pubens* (Forel), (Trager 1984a) and *P. vaga* (Forel) (Wilson and Taylor 1967). There are likely many more undocumented occurrences of these *Paratrechina* invasive events. This leaves more questions than answers regarding the current state of *Paratrechina* species existing within the continental U.S. To date, a very successful invasion of *P.* sp. nr.

pubens has been discovered in an industrial area in the Houston, Texas area. Within just a few years, this bioinvasion has caused severe economic and ecological harm to the geographical area.

A species-specific identification of *P*. sp. nr. *pubens* populations in Texas remains to be completed. Despite efforts from ant taxonomy experts (J. Trager, ant taxonomist, Shaw Nature Reserve; J. Cook, biology professor, Sam Houston State University), morphological and behavioral similarities of *P*. sp. nr. *pubens* populations from Texas, *P*. *pubens*, and *P*. *fulva* have caused an identification stalemate. *Paratrechina pubens* and *P*. *fulva* are morphologically similar and are in the Complex Fulva of *Paratrechina* (Trager 1984a). As a consequence, a study (Meyers and Gold unpublished b) was conducted to elucidate the specific identification using morphological comparisons of a previous description of *P*. *pubens* (Trager 1984a). These comparisons noted significant differences between morphometric means of the populations of Texas and the previously described *P*. *pubens* (Meyers and Gold unpublished b). However, these findings were inconclusive as to its definitive identification as *P*. *pubens* or an undescribed species and the Texas populations remained *P*. sp. nr. *pubens*. Despite repeated informative communications regarding *P*. sp. nr. *pubens*, there are no current federal expansion-preventing measures in place (Tony Koop, pers. comm., botanist, New Pest Advisory Group). With no current expansion-preventing measures in place for *P*. sp. nr. *pubens* (Tony Koop, pers. comm.), the spread will likely continue into other non-endemic areas. Much of these efforts have been hampered by the confusion regarding the species specific-identification of *P*. sp. nr. *pubens*.

Little research has been conducted in the area of phylogenetic identification of the morphologically ambiguous species in *Paratrechina*. A previous study included the analysis of COI sequences which confirmed two *Paratrechina* morphospecies from a collection in Madagascar (Smith et al. 2005). The current study offers supplemental analyses of the *P*. sp. nr. *pubens* populations in Texas. This study is the first significant attempt to answer population identification of different species causing, in part, the taxonomic problems of *Paratrechina*. The phylogeographic relationships of *P*. sp. nr. *pubens* and *P*. *pubens* populations and other *Paratrechina* species are estimated.

Materials and Methods

In this study, *Paratrechina* spp. workers and alates were collected or donated from various locations in the United States and Caribbean from 2005 to 2007 (Table 3.1, Fig. 3.1). These samples were preserved in alcohol, dried, or frozen at -20ºC. Some samples were morphologically identified based on previous descriptions (Trager 1984a) while others were identified by specimen donors. The voucher specimens are deposited in the Center for Urban and Structural Entomology and Texas A&M University Insect Collection (Voucher #672), Department of Entomology, Texas A&M University, College Station, TX.

Polymerase chain reaction (PCR) of a 708 bp region of the mtDNA COI gene was conducted using the primers LCO1490-F forward: 5’-GGTCAACAAATCATAAAGATATTGG-3’ (Simon et al. 1994) and HCO2198-R reverse: 5’- TAAACTTCAGGGTGACCAAAAAATCA -3’ (Simon et al. 1994). PCR

Table 3.1. Source of formicid samples.

Species	State/Country	County/City	Accession #
Aphaenogaster iberica	Spain	Los Belones	DQ074361
Paratrechina arenivaga	Mississippi	Coila	
P. faisonensis	Mississippi	Winston	
	Louisiana	Clinton	
P. longicornis	Arizona	Oracle	
	Louisiana	Baton Rouge	
	Texas	Baytown	
		Baytown	
		Baytown	
		College Station	
		Pasadena	
		Pasadena	
P. pubens	Anguilla	Brimegin	
		Shoal Bay	
	Florida	Parkland	
	St. Croix	Cruban Gorde	
	St. Kitts	Bermatt	
P. sp. nr. *pubens*	Texas	Deer Park	
		Houston	
		Jacinto Port	
		Manvel	
		Pasadena	
		Pearland	
		Pearland	
P. vividula	Alabama	Lawrence	
P. sp.	Arizona	Marana	
		San Simon	
	Madagascar	Antsiranana	DQ176052
		Antsiranana	DQ176066
		Antsiranana	DQ176124
		Antsiranana	DQ176171
		Antsiranana	DQ176178
	Texas	Bryan	
Tapinoma sessile	Texas	Deer Park	

Figure 3.1. Formicid sample locations from various countries and states. Circle size is proportional to sample size.

reactions consisted of an initial denaturation of two min at 94ºC, followed by 40 cycles of 94ºC for 45 s, 46ºC for 45 s and 72ºC for 60 s, and a final extension of five min at 72ºC. Amplified DNA from individual ants was purified and concentrated with Amicon Microcon PCR centrifugal filter devices (Millipore, Billerica, MA) according to the manufacturer's instructions. Samples were sent to the DNA core sequencing facility at The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Sequences used in this study will be submitted to GenBank. DNA sequences were aligned using ClustalW (Thompson et al. 1994) within BioEdit (Hall 1999). The best-fitting nucleotide substitution model was chosen according to the general time reversible + gamma (GTR+G) model among 64 different models by using the ModelTest version 3.7 (Posada and Crandall 1998) and PAUP* 4.0b10 (Swofford 2001) programs. Bootstrapping was performed using NJ or MP (1,000 replicates) to determine the reliability of the obtained topologies.

Mitochondrial COI sequences of *Paratrechina* sp. from Madagascar were added to the dataset along with DNA sequences of *Aphaenogaster iberica* from Spain and *Tapinoma sessile* from Texas which were added to act as outgroup taxa. Maximum likelihood and unweighted parsimony analysis on the alignments was conducted using PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing characters for all analyses. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings using the Heuristic algorithm of PAUP*.

For Bayesian analysis, the best-fitting nucleotide substitution model was chosen as described above. Phylogenetic trees were obtained using Bayesian inference with the GTR+G model using Bayesian Evolutionary Analysis Sampling Trees (BEAST) v1.4.2 software (Drummond and Rambaut 2003). For Bayesian inference, four Markov chains run for 10^6 generations with a burn-in of $2x10^4$ were used to reconstruct the consensus tree.

The *P*. sp. nr. *pubens* and *P*. *pubens* clades were selected for correlative effects of geographic distance and genetic similarity. This correlation was made under the assumption that geographic distance can create genetic differentiation. A linear regression was plotted using the relationship between Kimura 2-parameter (K2P)

(Kimura 1980) genetic distance and geographic distance by using JMP 5.01 (SAS, Cary, NC). All geographical relationships were calculated from *P*. sp. nr. *pubens* Pasadena, TX as a reference point.

Results

Analysis of COI sequences. A 626-bp region of the mtDNA COI gene was sequenced from a total of 34 formicid samples collected (Table 3.1). Of these characters, 185 (29.6%) were variable and 81 (12.9%) were parsimony-informative. Base frequencies were A (29.9%), C (17.7%), G (12.3%), T (40.1%). Neighbor-joining (NJ) analysis (Fig. 3.2) revealed nearly identical clades found in Maximum Parsimony (MP) and Bayesian analyses. MP resulted in one tree with a length of 616, CI = 0.620, RI = 0.853 (Fig. 3.3). Bayesian analysis of the formicid samples revealed several distinct

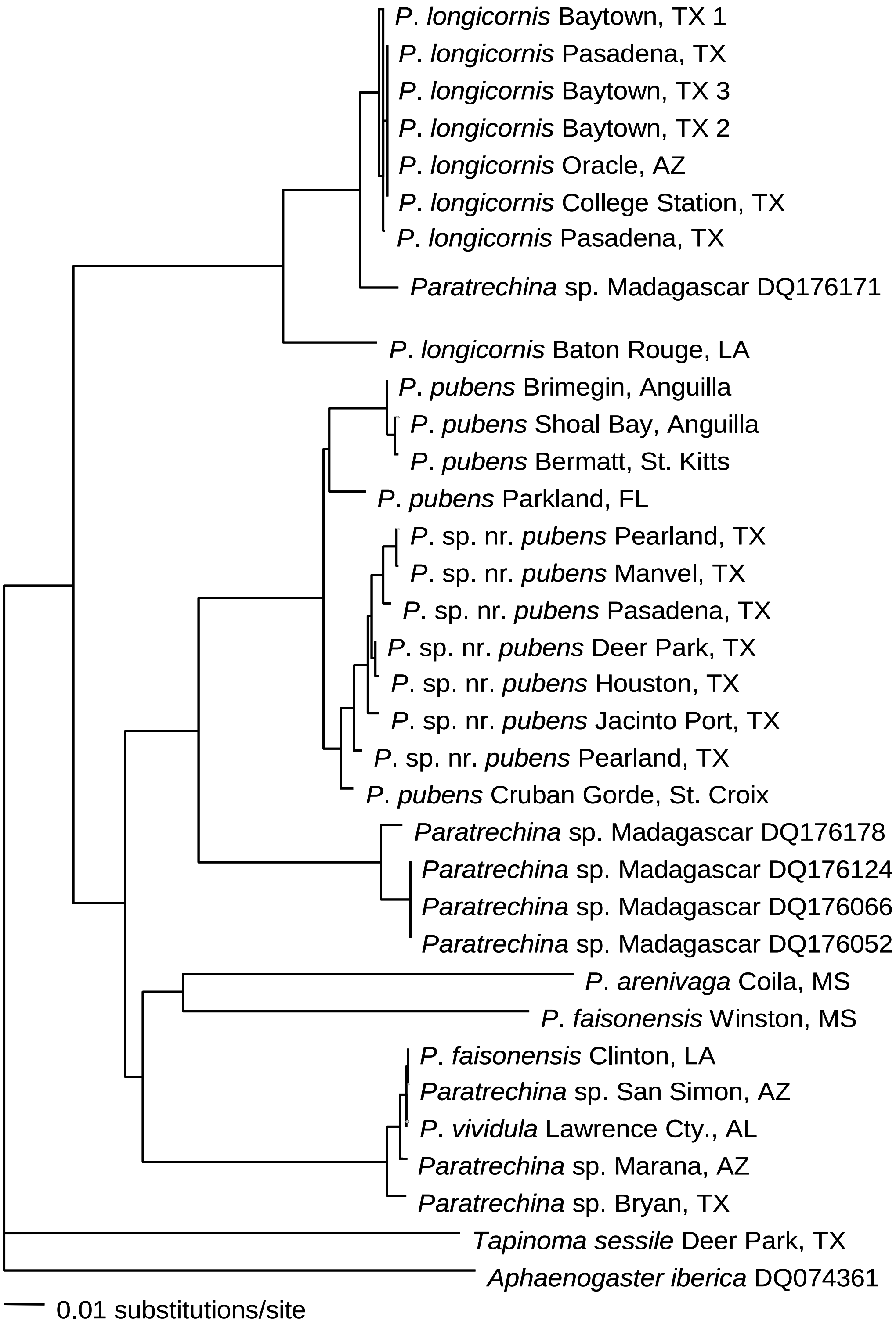


Figure 3.2. Neighbor-joining phylogenetic tree.

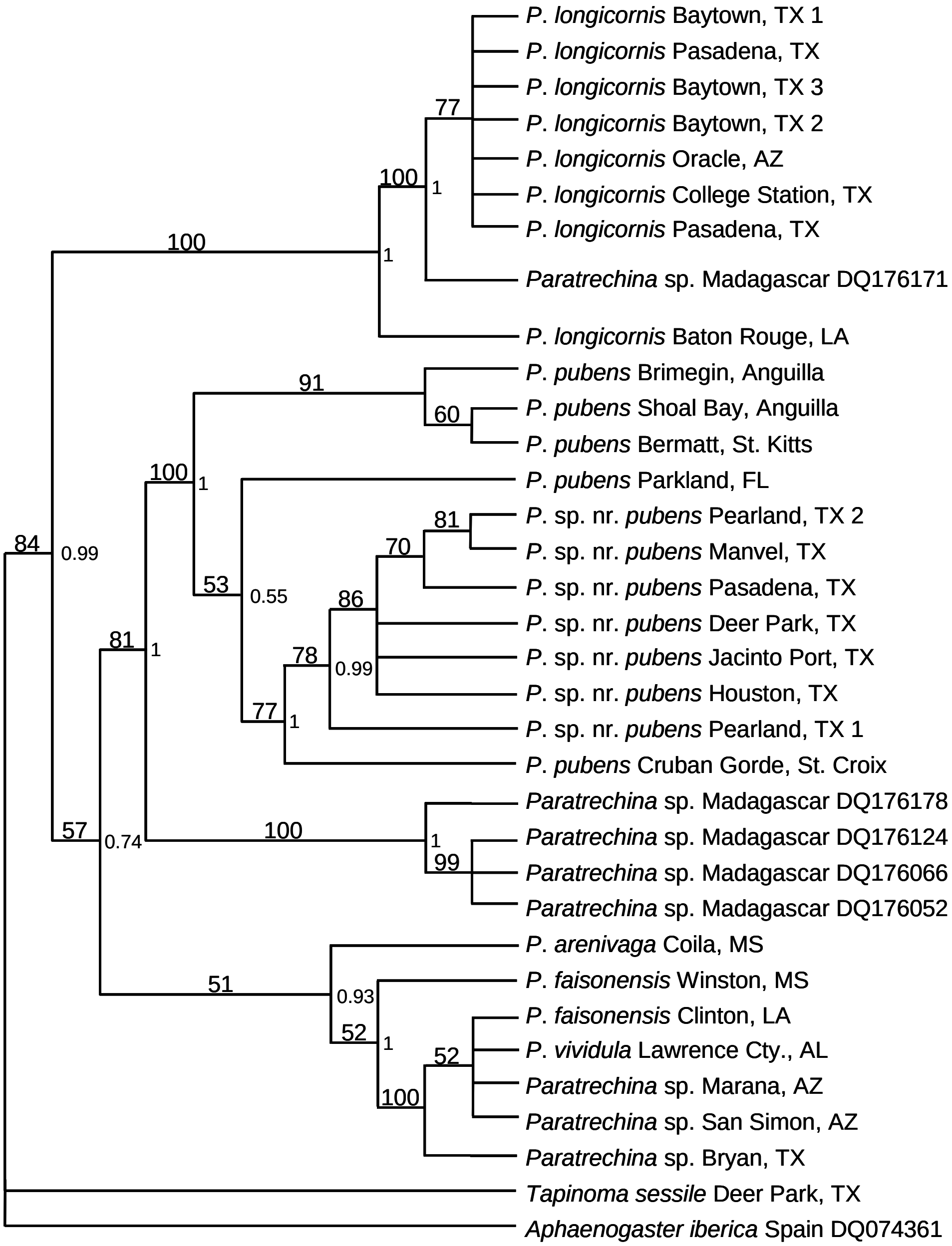


Figure 3.3. Single most parsimonious tree during a heuristic search by using PAUP* (Swofford 2001). Phylogenetic relationship of *P*. sp. nr. *pubens* mtDNA COI to other *Paratrechina* species. Numbers at the tree nodes indicate Bayesian posterior probabilities, and numbers above nodes indicate bootstrap values obtained from 1,000 replicates using MP analysis.

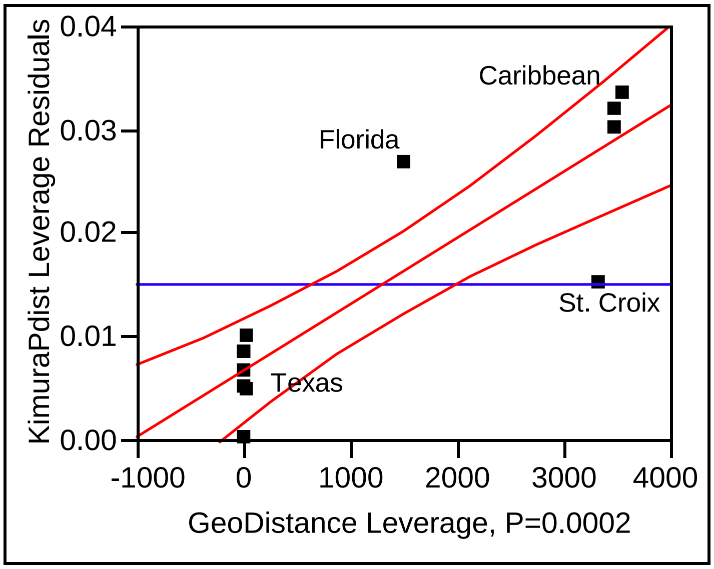


Figure 3.4. The scatter plot shows the relationship between geographic distance and genetic similarity of *P*. sp. nr. *pubens* and *P*. *pubens* populations sequenced for this study (P = 0.0002, see text). The linear regression line is defined as Kimura 2-Parameter genetic distance = 6E – 05 [km] + 0.0067, and accompanied by the corresponding 95% confidence interval (red dotted line) and β_o (blue dotted line).

clades (Fig. 3.3) with high posterior bootstrap values supporting basal clades of MP. Several of the distal clades (scores not shown) were not as highly supported with posterior bootstrap values, indicating a lack of support.

The linear regression analysis of geographical and genetic distance revealed a positive correlation (n = 12, R^2 = 0.768, t = 5.76, P = 0.0002) (Fig. 3.4). The distribution of sampling locations created a distribution of geographic distance within and between populations of *P*. sp. nr. *pubens* and *P*. *pubens*. Genetic similarity among Texas populations of *P*. sp. nr. *pubens* ranged from 0.00658 to 0.00989. Genetic similarity from

P. sp. nr. *pubens* among *P*. *pubens* ranged from 0.01497 to 0.3354. Genetic similarity increased with increasing spatial separation between populations.

Discussion

The analyses of mtDNA COI of *Paratrechina* spp. revealed several robust clades from the various trees. Within these clades are two or three distinct clades of *P*. *pubens* and *P*. sp. nr. *pubens*, including an clade of *P*. *pubens* from Florida. The clade of *P*. sp. nr. *pubens* and *P*. *pubens* from St. Croix may support identification of an undescribed species of *P*. *pubens*. The analysis also demonstrated misidentification(s) of *Paratrechina* sp(p). samples. The phylogenetic trees supported work from a former *Paratrechina* phylogeny reconstruction (Smith et al. 2005). The analysis of genetic and geographic distance supported paleogeographic isolation of the fulva complex and further corroborates a possible source of introduction of *P*. sp. nr. *pubens* to Texas.

The curious phylogenetic identification of *P*. *pubens* of St. Croix within the *P*. sp. nr. *pubens* NJ, MP, and Bayesian clades may aid in narrowing the search for geographic descent from which *P*. sp. nr. *pubens* infestation of Texas arose. This does not necessarily identify St. Croix or another Caribbean island as a point-of-origin, as more thorough studies must be completed that include a geographically broader collection of *P*. *pubens* and *P*. sp. nr. *pubens*. These types of phylogenetic studies have been conducted prior. The origination of invasively destructive alga species, *Caulerpa taxifolia*, of the Mediterranean was identified as an aquarium strain of Australia (Jousson et al. 1998, Wiedenmann et al. 2001). Nuclear DNA analysis of microsatelites can identify single or multiple introductions (Rheindt 2003) and may be able to do so for *P*. sp. nr. *pubens* and could explain colonial affiliation of supposed supercolonies found within geographically distinct populations.

Although no in-depth morphological analyses were conducted (as in Meyers and Gold unpublished b), the voucher specimen examination revealed no morphological dissimilarity between *P*. *pubens* from Florida, Anguilla, St. Kitts, St. Croix, and *P*. sp. nr. *pubens* from Texas. Given their apparent morphological similarity, the robust clade separations resolved in this study suggest that the only way to accurately differentiate populations of *P*. sp. nr. *pubens* and *P*. *pubens*, is through phylogenetic analyses. When viewing specimens of *P*. sp. nr. *pubens*, two slight morphological differences from *P*. *pubens* were noted (by J. Trager pers. comm.). It was noted that the macrochetal hairs of *P*. sp. nr. *pubens* were slightly less curved and males exhibited macrochetal arrangement dissimilarity compared to that of *P*. *pubens*. This study did not evaluate dentition or morphological arrangement of the mandibles. This may reveal unknown characters differentiating the taxonomic groups in question. Similar studies have demonstrated significant differences in labral morphologies in other morphologically ambiguous insect groups (e.g. *Reticulitermes* (Rhinotermitidae)) (Heinstchel et al. 2006). Unless viewed by an expert, these types of characters are unlikely to be consistently and correctly identified.

This study may support the identification of the Texas populations as *P*. *fulva*. Given the pest status and pestiferous behavior of previous infestations of *P*. *fulva* in Colombia (Zenner-Polania 1994) and *P*. *pubens* in St. Croix (Wetterer unpublished), an additional character may be useful when identifying this species. This study may also support the misidentification of *P. fulva* as *pubens* from St. Croix. The biological potential for numerical density in non-indigenous geographic areas may be an unfortunate yet identifying character for this species.

Clearly there are biological differences between the three clades of *P. pubens* and *P.* sp. nr. *pubens*. The *P. pubens* sister clade of Anguilla and St. Kitts and the taxa from Florida were collected from populations without the reported densities found in the populations collected from St. Croix and Texas. Another possible differentiating character is their biting behavior. *Paratrechina pubens* has been previously described as "non-biting" (Warner and Scheffrahn 2004). Reportedly high densities of *P. pubens* in the Jacksonville Zoo and Gardens, Florida have become more aggressive and have bitten zoo personnel and visitors (D. Calibeo-Hayes, graduate student, University of Florida). These behaviors have been previously reported in the Texas populations of *P.* sp. nr. *pubens* (Meyers and Gold unpublished b). Although not collected for this study, given the high densities and biting behavior of *P. pubens* populations at the zoo, the population likely constitutes a synonymy with *P.* sp. nr. *pubens* from Texas and St. Croix. Alternatively, these behaviors may be a product of high densities and within the phenetic range of *P. pubens* and *P.* sp. nr. *pubens*. These and the findings discussed above may support the identification of a cryptic species. Comprehensive sampling of *P. pubens* type locality populations will absolutely confirm the identity of *P.* sp. nr. *pubens* and many other populations of *Paratrechina* species.

Significant genetic variability among the *P*. sp. nr. *pubens* and *P*. *pubens* St. Croix clade (Fig. 3.3) may suggest a broad genetic range enabling successful invasions of non-indigenous geographic areas. This genetic variation within the *P*. sp. nr. *pubens* populations may support genetic plasticity or multiple introductions from discrete populations. This genetic plasticity may also allow for quick adaptations to new environments. This genetic variability may also enhance their ability to avoid a genetic bottleneck in the exotic geography of Texas. The MP and Bayesian node scores within this clade may be in contrast to previous literature that suggests reduced genetic variation of a behaviorally similar ant species, *Linepithema humile*, the Argentine ant (Tsutsui et al. 2000). This reduced genetic variation supports the unicolonial behavior of both *L. humile* and *P*. sp. nr. *pubens* in exotic geographies. Establishment of social insects in non-indigenous areas is a relatively rare event. Thereby, this study may support a rather surprising and unlikely scenario of multiple introductions of *P*. sp. nr. *pubens* into Texas. Aggression studies involving various *P*. sp. nr. *pubens* populations and also *P*. *pubens* may be warranted to resolve this question.

The surprising variability found among populations of *P*. sp. nr. *pubens* of Texas, suggests phenotypic plasticity for this group. What influence these physical adaptations may direct their behavior or ability to survive in foreign environments remain unknown. Social characters can become enhanced in these non-indigenous geographies (Tsutsui et al. 2000). This may help explain their adaptive range for successful invasions of these new environments. This is in stark contrast to the genetically similar *P. longicornis* clade (Figs. 3.2, 3.3).

However, this genetic variability found within this group may also simply be a product of the gene region (COI) that was investigated. This genetic dissimilarity could suggest multiple introductions of different populations during the initial founding of the site of original known infestation (SOOKI). Multiple incipient introductions could create a more adaptive capability of a species in an exotic location as has been observed with other organisms (e.g. the Formosan subterranean termite, *Coptotermes formosanus*).

There must be a minimum viable beachhead population for successful invasions to take place (Moller 1996). This population must be large enough to succeed initially in often inhospitable environments. This population must also contain a genetically variable population that avoids a critical bottleneck. It remains to be seen if *P*. sp. nr. *pubens* populations will undergo this bottleneck. Based on the observed variability within the phylogenetic results (Fig.s 3.1, 3.2) this may be unlikely in the near future. During an invasive event a period of adaptation may occur, during which genetic change could be a result (Tsutsui et al. 2000, Williamson 1989). When regarding social insects, these genetic changes can result in a cessation of intraspecific variation creating large unicolonial populations (e.g. Argentine ants (Tsutsui et al. 2000)).

Paratrechina pubens from Florida represents a clade within the NJ, MP and Bayesian reconstructions. This is indicated by the relatively low bootstrap values observed in both the MP and Bayesian phylogenetic trees. More populations must be collected and examined before any conclusions are made regarding its evolutionary relationship between other near *P. pubens* populations (Texas and Virgin Islands).

This study supports the conclusion of two morphospecies collected during a previous study, including populations of *Paratrechina* species in Antsirañana, Madagascar (Smith et al. 2005). *Paratrechina* sp. DQ176171 inclusion in the *P. longicornis* clade, clearly supports its identification as *P. longicornis*. *Paratrechina* species DQ176052, 176066, 176124, while 176178 likely comprise a distinct clade of an unknown species.

Paratrechina sp. collected from San Simon, Arizona further represents the propensity for *Paratrechina* species to be transported to nonindigenous areas. The sample was collected during a routine check of a commercial truck hauling household goods. The load was likely being shipped to California from the eastern U.S. and discovered mid-shipment as part of routine inspections for quarantinable pests (C. Baptista, pers. comm.).

Examination of the voucher specimens of the *P. vividula*, *P. faisonensis*, and *P. arenivaga* samples, using the *Paratrechina* key (Trager 1984a). revealed *P. faisonensis* from Louisiana as a possible misidentification. This indicates the clade of *P. faisonensis* from Louisiana, *P*. sp. San Simon, Arizona, *P. vividula* Lawrence County, Alabama, *P*. sp. Marana, Louisiana, and *P*. sp. Bryan, TX likely constitutes identification as *P. vividula*. The *P. faisonensis* Winston, Mississippi and *P. arenivaga* Coila, Mississippi clade remain unidentified, as the examined voucher samples were not differentiated after examination. Logical deduction concludes a misidentification of at least one of these samples. These possible misidentifications and unknown species underscore the difficulties of the taxonomy of the genus. It also indicates the need for further research of the taxonomy of this prolifically invasive group.

A positive correlation was found between geographical and genetic distance (Kimura 2-Parameter) when plotted using linear regression analysis (Fig. 3.4). As geographical distance increased, there was a subsequent increase of genetic distance between populations of *P. pubens* and *P.* sp. nr. *pubens*. These findings offer further support of the separation of the clades. This also supports allopatry between populations of the complex Fulva. Similar allopatric results were found when analyzing genetic and geographical distances of *Pogonomyrmex badius* populations in Florida (Strehl and Gadau 2004). This genetic distance caused by geographic distance may be exaggerated when regarding island and continental populations.

These genetic differences may reflect past paleogeographic isolation. Similar studies have demonstrated the clear relationship of genetic and geographic distance (Strehel and Gadau 2004). However, one would expect these genetic distances can be subverted due to unnatural anthropogenic introductions. This may give reason for the genetic similarity of *P. pubens* from St. Croix to *P.* sp. nr. *pubens* and its dissimilarity from *P. pubens*. These differences may also be explained by an exponential increase in genetic variability in populations separated by >100 km (Strehel and Gadau 2004).

This study is the first significant endeavor describing the phylogeny of several *Paratrechina* species. The study found misidentifications of *Paratrechina* samples. The conclusions also may reflect paleogeographic events within the complex Fulva. This procedure has offered further proof a cryptic species causing deleterious ecological and economical effects to Texas and St. Croix.

CHAPTER IV

DISTRIBUTION AND SPREAD OF AN EXOTIC ANT, *Paratrechina* SP. NR. *pubens,* IN TEXAS

Introduction

Exotic insects can have immense deleterious effects ecologically and economically. A great number of these successful introductions are from social insects such as ants, wasps and bees (Moller 1996, McGlynn 1999). *Paratrechina* species exhibit a great propensity of successful invasions (Wilson and Taylor 1967, Trager 1984, Zenner-Polania 1990, Passera 1994, Fellowes 1999, Wetterer et al. 1999, Freitag et al. 2000, Wetterer 2007, Wetterer unpublished data). Some of these invasive events of *Paratrechina* species can also cause great economic and ecological damage (Zenner-Polania 1990, Zenner-Polania 1994, Wetterer 1999, Wetterer 2007, Wetterer unpublished data). *Paratrechina* species can be among the most difficult urban pests to control (Hedges 1998). During the infestations of non-indigenous geographies localized populations of these ants can exhibit immense density (Zenner-Polania 1990, Wetterer 2007) creating increased control difficulties. The Formosan subterranean termite, *Coptotermes formosanus*, exhibits relatively moderate colony size in their native environment, however, when introduced to new geographies (e.g. U.S.), colony size drastically increases. This dramatic biological change has created one of the most severe arthropod pests in the U.S.

In 2002, the Center for Urban and Structural Entomology was notified of a pest ant found in an industrial area of Pasadena, TX. By 2003, *P*. sp. nr. *pubens* infestation was found in overwhelming numbers. It is chiefly a nuisance pest, however, has been known to short-circuit electric apparatuses, causing considerable financial cost to residents and businesses alike. Amazing densities of *P*. sp. nr. *pubens* have been found in the infested areas and preliminary field observations indicate a displacement of the normally populous red imported fire ant, *Solenopsis invicta*. Because of these facts, it is very likely that deleterious ecological effects will occur. There may be other unknown problems associated with this ant species as many risks of invasive species go un-assessed (Simberloff et al. 2005).

Predictive models are an important aspect of invasional biology. Prediction of potentially invasive species can lead to prevention. Geographical estimations of exotic species incursions can assist researchers and governmental personnel to make decisions regarding research and funding opportunities. Many invasions, especially exotic ants, can encompass large geographical areas (Vinson 1986, Vander Meer et al. 1990) and as such create substantial ecological (Ramakrishnan and Vitousek 1989) and economic (Williams 1994) impact of affected areas.

Extensive biological knowledge of potential or recurring invasive species is needed for bioinvasion prevention. This may not be a reliable mechanism for prevention. This method requires considerable biological knowledge of a great many potentially invasive species, many of which may not have even been described by scientists.

However, this study stands as a foundation of that biological knowledge for one of the identified species, *P*. sp. nr. *pubens*.

It is imperative to follow the expansion of *P*. sp. nr. *pubens*, an already ecologically and economically destructive invasive species. Predicting the geographic spread of *P*. sp. nr. *pubens* will assist in the ecological and economic estimations effecting the region. This study followed the distribution and spread of *P*. sp. nr. *pubens* for three years in South Texas.

Materials and Methods

Distribution and spread analyses were chosen for two discrete infestation sites. The first site, in Pasadena, TX (Fig. 4.1a), was chosen because it was the site of original known infestation (SOOKI) of *Paratrechina* sp. nr. *pubens* discovered in 2002. This site is located ~eight km south of the Port of Houston in an industrial zone of Pasadena. Within and surrounding the site were various industrial businesses including biodiesel and a variety of chemical plants amongst scattered fields of grasses or woods. Interlacing the area were train tracks and roads leading to the industrial plants. The second site, in Deer Park, TX (Fig. 4.1d), was chosen because it was the first neighborhood infested with *P*. sp. nr. *pubens* and was discovered in 2005. This site is located ~five km northwest of the Pasadena site and ~two km south of the Port of Houston. The neighborhood contained ~200 houses and was isolated from other such neighborhoods by ≥ 0.4 km on all sides. Distributions for Pasadena and Deer Park were established in 2005 and 2006, respectively, and subsequent spread of infested areas was observed until 2007.

An expansion estimation of *P*. sp. nr. *pubens* was conducted from 2005-2007 in Pasadena and 2006-2007 in Deer Park, TX. These distribution estimations were focused on estimating the rate of spread in two areas where monitoring was hindered by business security and private land. Baited pitfall or above ground area monitor traps (baited area monitor trap (BAMT)) were installed for the study. Baited pitfall traps consisted of a 1 l plastic container including a six by eight cm area monitor (New South Products, Greenville, MS), a hot dog piece, and a 20% honey-water soaked cotton ball. The above ground traps consisted of an area monitor attached to the ground using grade stakes and baited with the same food resource attractants as described above. Above ground BAMTs were used in opposition to the pitfall traps due to time constriction and preliminary results suggesting equal formicid attraction. A total of 72 pitfall traps were placed out within and surrounding the Pasadena site during the spring and summer of 2005. During 2006 and 2007, 70 and 75, respectively, above ground BAMTs, were placed at ground surface. BAMTs were located within and surrounding a targeted zone based on the previous findings of 2005 and 2006.

As expansion of *P*. sp. nr. *pubens* distribution increased, subsequent problems such as electrical shortages increased. According to anecdotal stories from management, these shortages encouraged businesses to bypass security and increased their willingness to take part in the distribution study.

As the distribution of *P*. sp. nr. *pubens* in Pasadena and Deer Park began to expand outside the range of business security and private property, respectively, it became prudent to use transects where possible. Collections for distribution description of 2007 were made using a baited grid sampling system. Distribution estimation of 2007 Pasadena site involved 100 m transects placed within each of 25 plots (0.84 x 0.84 km) within a 4.2 x 4.2 km area (Fig. 4.1c). This area was selected based on previous knowledge of distribution and subsequent expansion rates in 2005 and 2006. Quadrants 7-9, 12-14, and 17-19 were considered infested based on the previous studies and field visual identification of *P*. sp. nr. *pubens* supplanted baited transects. Distribution estimation of 2007 Deer Park site involved 100 m transects placed within each of 25 plots (0.25 x 0.25 km) within a 0.75 x 1.25 km area (Fig. 4.1e). This area was selected based on previous knowledge of distribution of 2006. Quadrants 5, 8, and 11 were considered infested based on the previous studies and field visual identification of *P*. sp. nr. *pubens* supplanted baited transects. Each directional transect contained five BAMT placed, where possible, along a randomly selected direction (East-to-West or North-to-South). Some of the areas were inaccessible due to business security or construction (Fig. 4.1c, e).

A broader distribution analysis was conducted that considered only the identification of incipient, discrete populations of *P*. sp. nr. *pubens* throughout Texas. This distribution was also supplemented with a targeted analysis that included mailed sample vials alerting pest control operators of the new ant species. These pest control operators were located within the surrounding southwest Houston area during 2006. Also, during statewide entomology Extension and urban pest control conferences, Extension agents and pest control operators were notified of the study. These agents and controllers became an integral part of this study by mailing suspected samples and subsequent identification was conducted by JMM.

Identification of ant species was completed using formicid taxonomic keys (Creighton 1950). Identification to species was often difficult due to the collection method utilizing glue area monitor traps.

Results

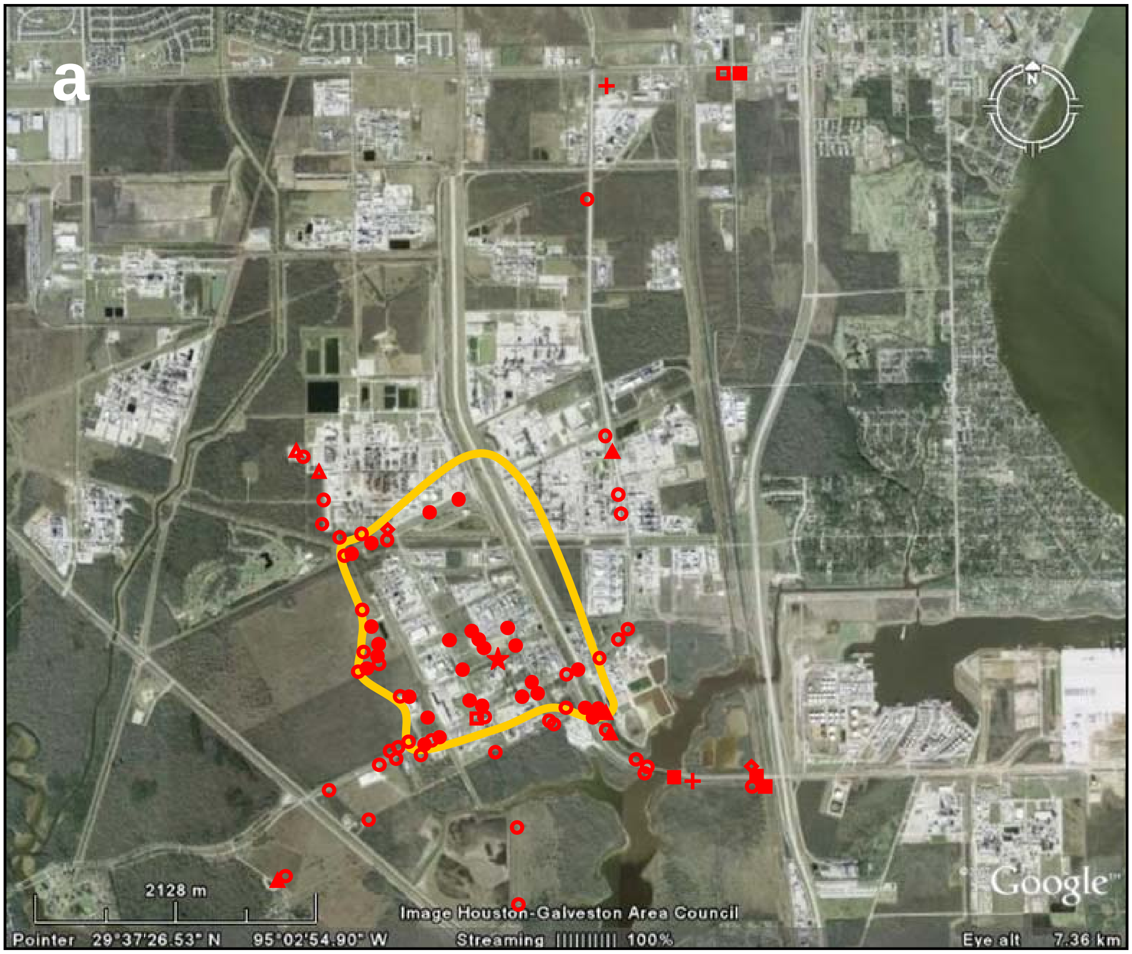
During the distribution studies in Pasadena and Deer Park, 12 ant species were collected (Table 4.1). The number of species collected at either site within a year ranged from 3-8. The number of species collected in the neighborhood was less (n = 7) than the number collected in the industrial area (n = 10). Only 1 out of 294 (0.3%) of the BAMTs resulted in a collection of zero ant species. A majority (93.6%) of the ant species collected were *Solenopsis invicta* or *Paratrechina* sp. nr. *pubens*. Numerous BAMTs (n = 36, 12.2%) collected multiple ant species.

2005 Pasadena, TX. P. sp. nr. *pubens* were collected a mean distance of 0.78 km from SOOKI. An analysis of six different locations along the estimated distribution line (Fig. 4.1a) revealed a mean distance of 1.08 km from site of original known infestation (SOOKI). The mean estimated distance of expansion for *P*. sp. nr. *pubens* in Pasadena from 2002-2005 was 360 m per year (30 m per mo). A more precise estimation of the eastern portion of the distribution of 2005 Pasadena site (Fig. 4.1a) was hindered by

Table 4.1. Yearly collection percentages of formicid species during *Paratrechina* sp. nr. *pubens* distribution estimations from 2005-2007 in Pasadena and Deer Park, TX.

	Yearly collection percentages of formicid species (total BAMTs)					
	Pasadena			Deer Park		
Species	2005 (72)	2006 (70)	2007 (75)	2006 (22)	2007 (55)	Total (294)
Solenopsis invicta	58.3 (42)	40.0 (28)	92.0 (69)	54.5 (12)	45.4 (25)	59.9 (176)
P. sp. nr. *pubens*	37.5 (27)	57.1 (40)	6.7 (5)	45.4 (10)	61.8 (34)	39.5 (116)
Monomorium minimum	5.6 (4)	10.0 (7)	2.7 (2)	18.2 (4)	-	5.8 (17)
Crematogaster sp.	6.9 (5)	-	-	4.5 (1)	-	2.0 (6)
Camponotus sp.	2.8 (2)	1.4 (1)	2.7 (2)	-	-	1.7 (5)
Cyphomyrmex rimosus	2.8 (2)	1.4 (1)	1.3 (1)	-	-	1.3 (4)
Brachymyrmex depilis	-	2.9 (2)	1.3 (1)	-	-	1.0 (3)
Dorymyrmex sp.	-	-	-	-	5.4 (3)	1.0 (3)
P. longicornis	-	4.3 (3)	-	-	-	1.0 (3)
Pheidole sp.	1.4 (1)	1.4 (1)	1.3 (1)	-	-	1.0 (3)
Tapinoma sessile	1.4 (1)	-	-	9.1 (2)	-	1.0 (3)
Aphaenogaster texana	-	-	-	4.5 (1)	-	0.3 (1)
None	1.4 (1)	-	-	-	-	0.3 (1)

Figure 4.1 a-f. Distribution maps of *P*. sp. nr. *pubens* and various ant species collected in Pasadena and Deer Park, Texas. (a) 2005 Pasadena distribution, (b) 2006 Pasadena distribution, (c) 2007 Pasadena distribution, (d) 2006 Deer Park distribution, (e) 2007 Deer Park distribution, (f) ant species key for a-e. The gold line represents the estimated distribution of *P*. sp. nr. *pubens* based on BAMT data and visual inspections. The blue line represents areas of construction or inaccessible land. The star represents the site of original known infestation. The northern portion of the line (a) represents an estimate based on employee observations and complaints from two industrial complexes. The author was not allowed to collect on either of these properties.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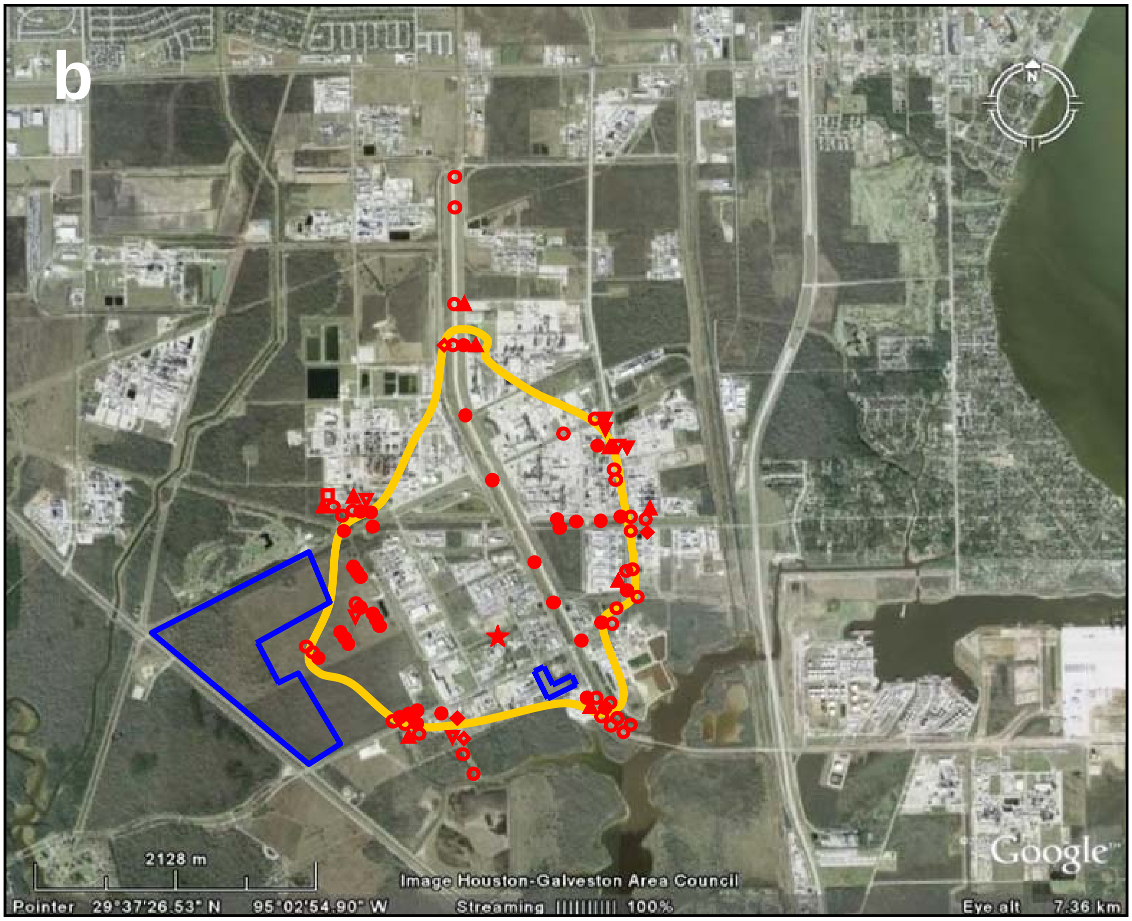

Figure 4.1. a-f. Continued.

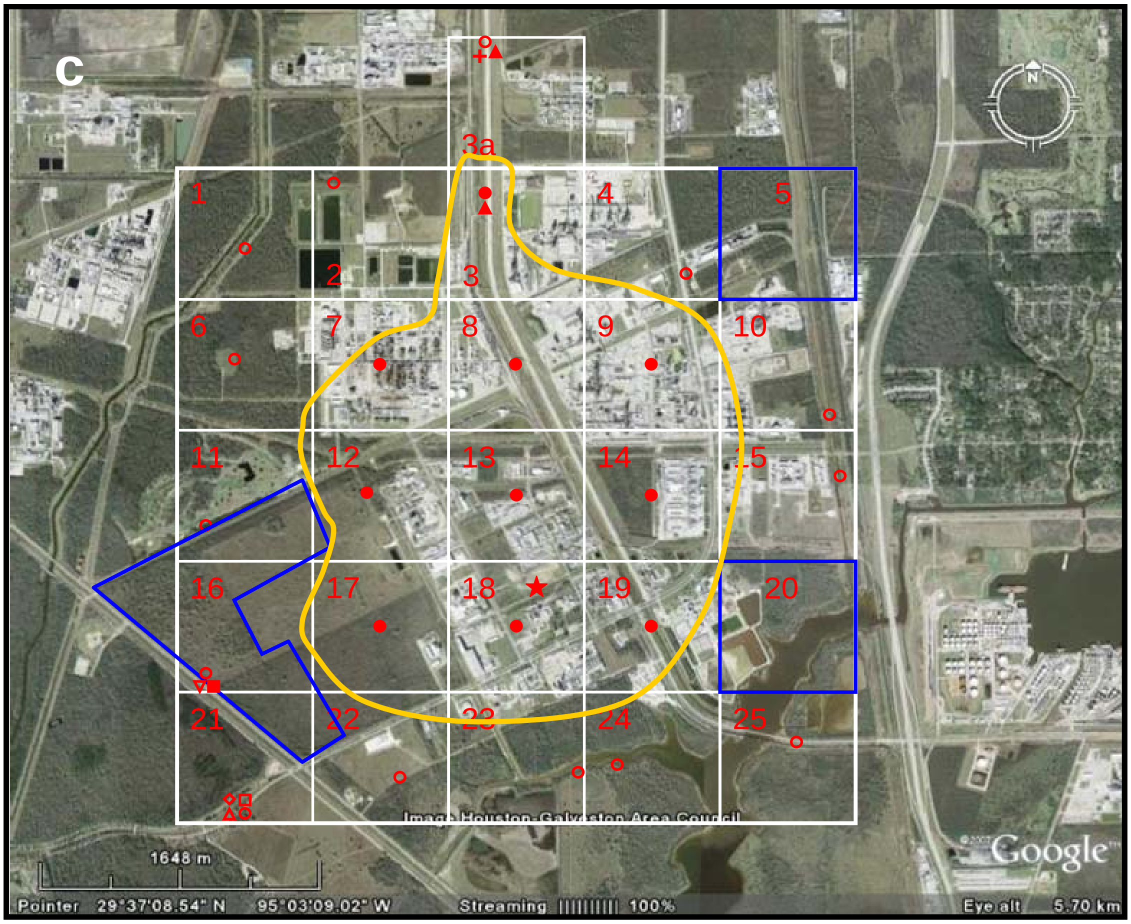

Figure 4.1. a-f. Continued.

Figure 4.1. a-f. Continued.

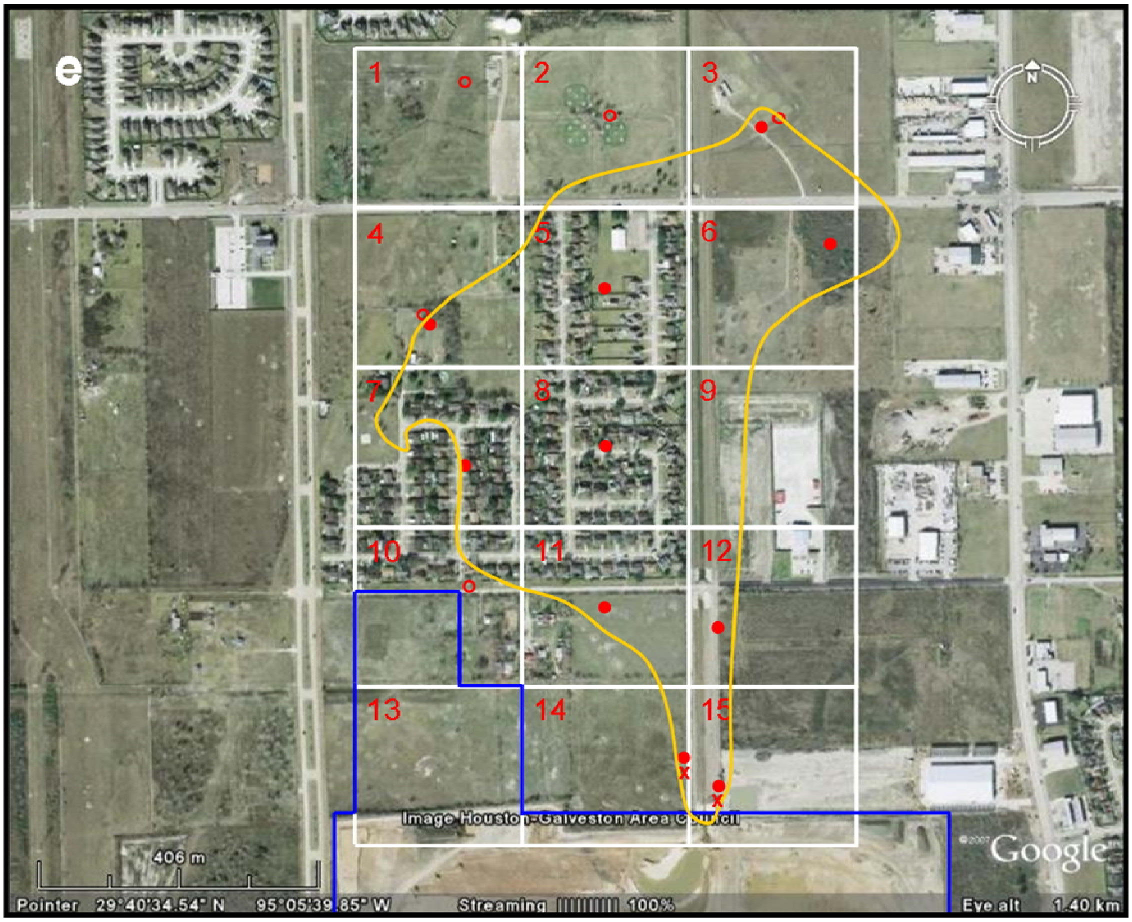

Figure 4.1. a-f. Continued.

f

Key to collected ant species

●	*Paratrechina* sp. nr. *pubens*	▼	*Paratrechina longicornis*
○	*Solenopsis invicta*	▽	*Brachymyrmex depilis*
▲	*Monomorium minimum*	◇	*Cyphomyrmex rimosus*
△	*Camponotus* sp.	◆	*Tapinoma sessile*
■	*Crematogaster* sp.	*	*Aphaenogaster texana*
□	*Pheidole* sp.	X	*Dorymyrmex* sp.
□	Area of construction	+	No ant species collected
◯	Area of estimated distribution	★	Site of original known infestation

Figure 4.1. a-f. Continued.

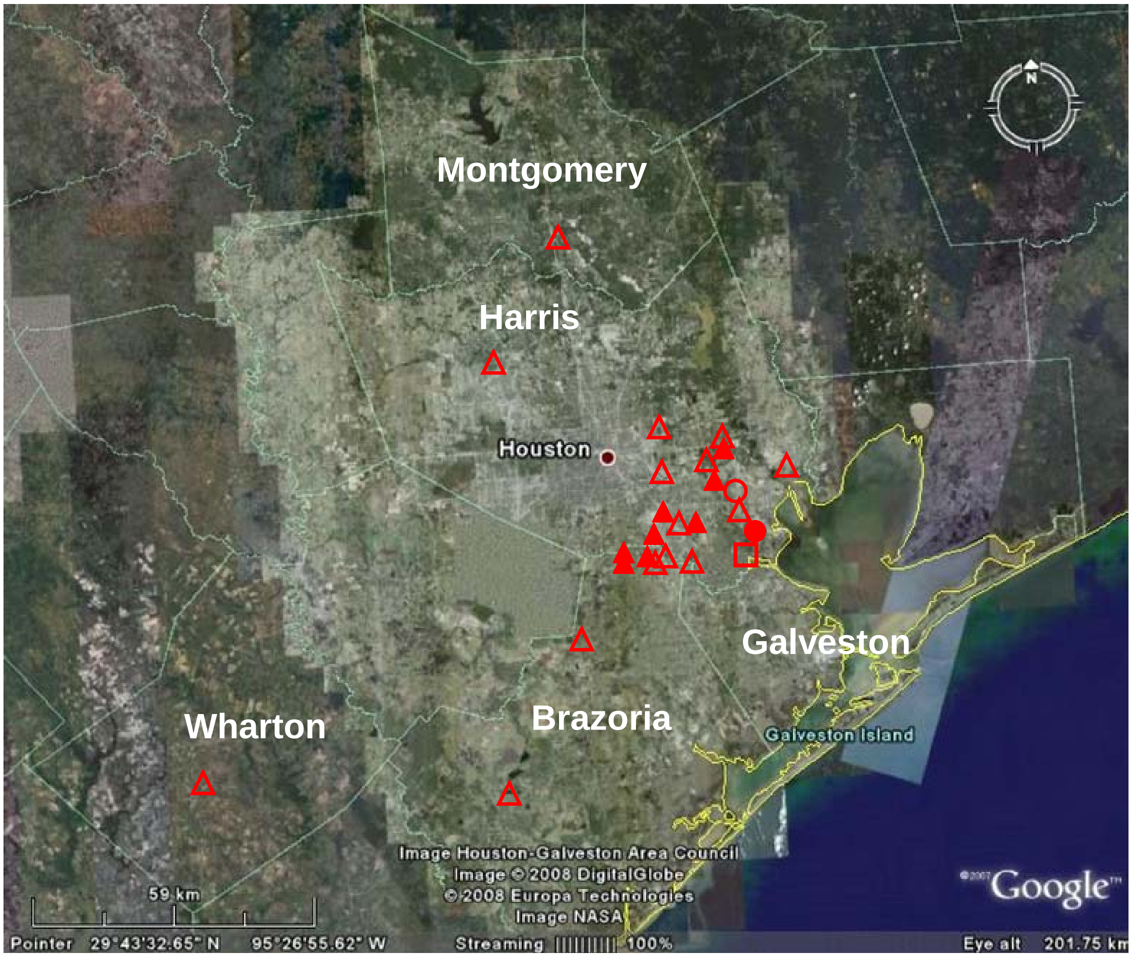

Figure 4.2. The overall distribution of *P*. sp. nr. *pubens* discrete populations in Texas. The closed circle represents the site of original known infestation of 2002. The open circle represents the second known infestation of 2005. Closed triangles represent infestations of 2006. Open triangles represent infestations of 2007. The open square represents an infestation of 2008.

business security. The low estimated expansion rate of 2006 may reflect this bias. As such, the most distal discovery along the eastern portion of *P*. sp. nr. *pubens* infestation was used when assessing the mean distance spread.

2006 Pasadena, TX. P. sp. nr. *pubens* were collected a mean distance of 1.13 (SE ± 0.72) km from SOOKI. An analysis of seven locations along the estimated distribution line (Fig. 4.1b) revealed a mean distance of 1.46 (SE ± 0.19) km from SOOKI. The mean estimated distance of expansion for *P*. sp. nr. *pubens* in Pasadena from 2002-2006 was 364 m per year (30 m per mo). Access to a portion of the southern distribution was limited by construction (Fig. 4.1b).

2007 Pasadena, TX. The mean distance of new *P*. sp. nr. *pubens* infestations in the 2007 Pasadena site within quadrants 3 and 3a and was 1.66 km (SE ± 0.21) (Fig. 4.1c) from SOOKI. An analysis of six locations along the estimated distribution line (Fig. 4.1c) revealed a mean distance of 1.67 (SE ± 0.26) km from SOOKI. The mean estimated distance of expansion for *P*. sp. nr. *pubens* in Pasadena from 2002-2006 was 334 m per year (28 m per mo).

2006 Deer Park, TX. P. sp. nr. *pubens* were collected a mean distance of 0.28 (SE ± 0.04) km from SOOKI. An analysis of five locations along the estimated distribution line (Fig. 4.1d) revealed a mean distance of 0.38 (SE ± 0.07) km from SOOKI. The mean estimated distance of expansion for *P*. sp. nr. *pubens* in Pasadena from 2002-2006 was 280 m per year (24 m per mo).

2007 Deer Park, TX. P. sp. nr. *pubens* were collected a mean distance of 0.38 (SE ± 0.55) km from SOOKI. An analysis of six locations along the estimated distribution line (Fig. 4.1e) revealed a mean distance of 0.46 (SE ± 0.69) km from SOOKI. The mean estimated distance of expansion for *P*. sp. nr. *pubens* in Pasadena from 2002-2006 was 229 m per year (19 m per mo).

Discrete populations in Texas. P. sp. nr. *pubens* were collected or identified from 2005 to 2008 from 25 new, discrete populations at a mean distance of 29.24 km (SE ± 5.46) from SOOKI (Fig. 4.2).

Discussion

Field observations and distribution studies of Pasadena and Deer Park, TX using BAMTs suggested a definite homogenization of ant species within the more established areas infested by *Paratrechina* sp. nr. *pubens*. This distinct majority of ants collected were *Solenopsis invicta* and *P*. sp. nr. *pubens*. The collection of these two species supports their ability to aggressively find and allocate food resources quickly. This also suggests their dominance within the local ecosystems of the Pasadena and Deer Park sites. This dominance is certainly creating a decrease in ant diversity within these established environments. The greater diversity of ant species and expansion rate of *P*. sp. nr. *pubens* found in the industrial area of Pasadena may be a result of a variety of factors. The industrial area is not a conducive environment for ant activity due to the increased concrete and lack of moisture and humidity availability. The within and surrounding areas of the industrial area include wooded and grassed environments that may have greatly increased the density and expansive rate of *P*. sp. nr. *pubens*. The general use of insecticides by homeowners in the neighborhood may decrease the density and resulting expansive capabilities of *P*. sp. nr. *pubens*.

More precise *P*. sp. nr. *pubens* distribution estimations were limited because of construction, business security, and physical inaccessibility. Construction during 2006-07 likely hindered the 2007 advancement of *P*. sp. nr. *pubens* in quadrants 11, 16, 21, and 22. The construction may have caused the decrease in expansion rate found in the Pasadena site in 2007. As such, the Eastern portion of the distribution line, in part, reflects field observation estimation (Fig. 4.1c). Heavy equipment and construction precluded a more thorough distribution study of Pasadena during 2006 and 2007 (Fig. 4.1b, c). Most of these areas were denuded and as such, death of formicid colonies or drastic population reductions likely occurred. Some quadrants (5 and 20) included areas of both construction and security concerns of the businesses and were, in turn, precluded from the 2007 Pasadena study (Fig. 4.1c). Based on the conclusions of this study, these quadrants would not have likely yielded any distribution information regarding *P*. sp. nr. *pubens*.

The transects of 2007 using 0.84 km^2 quadrants did not offer as precise an estimation as warranted for this particular study. This lack of precise estimation does not allow for well defined expansion estimation for *P*. sp. nr. *pubens* at this stage of distribution. Because *P*. sp. nr. *pubens* was found in grid ‘3’, this procedure did allow for the discovery of an infested area beyond the estimated distribution. The quadrant system did allow for quick response outside of the infested area (grid ‘3a’) (Fig. 4.1c) due to the physical limitations of a pre-determined quadrant size. Quadrants 19, 14, 13, 8, 3 and 3a all include a waterway associated with nearby Taylor Lake of Taylor Lake Village, TX. This unexpected expansion discovery may indicate that proximity to a water source or underground water availability allows *P*. sp. nr. *pubens* to spread at a quicker rate than other areas not endowed with this factor. This may also indicate the disparity between landscape types (riparian vs. industrial) and difficult expansion by *P*. sp. nr. *pubens* when associated with industrial areas.

The estimated expansion is much lower in Pasadena, TX than previous estimations of a study on closely related, exotic *P. fulva* in Colombia (100 m per mo) (Zenner-Polania 1990). This lower expansion rate may be caused by two factors. Business security and construction hindered access to likely infested areas, decreasing the precision of the estimations of spread. The industrial environment may prevent ideal conditions for expansion. Much of the area consists of concrete, inappropriate structures, and inadequate food and water resources that may hinder conducive factors needed for ideal *P*. sp. nr. *pubens* density and spread. When *P*. sp. nr. *pubens* reaches non-urban areas the expansive rate will likely greatly increase. Other similar behaving invasive ant species have spread at 62.5 m per mo (the Argentine ant, *Linepithema humile*) (Krushelnycky et al. 2004) and 15 m per mo (the yellow crazy ant, *Anoplolepis gracilipes*) (Abbott 2006).

Field observation suggested a clear homogenization of ant fauna in and around the Deer Park site. This clear decrease in ant species diversity at the Deer Park site was demonstrated with the reduction in species from six to three in 2006 to 2007, although it was not observed from collections at the Pasadena site (eight to seven), (Table 4.1).

In Deer Park 2007, east of quadrants 3 and 6 was inaccessible for the distribution study (Fig. 4.1e). Construction south of the grid prevented further distribution estimation; however, visual field observations suggested a finite range of *P*. sp. nr. *pubens* had been found at the southern periphery of quadrants 14 and 15.

Due to successful invasive events, a disproportionate amount of negatively (70%) and positively (10%) affected bee, wasp, and ant species could be estimated (McKinney and Lockwood 1999). Other examples have demonstrated a restructuring of invasion-receiving communities (Howarth 1985, Thomas et al. 1989, Wojcik 1994). Despite concerns regarding introduced species, 79.4% do not have any effect on the indigenous community (Simberloff 1981), while only 8.3% caused extinction events. And of those, extinction driving occurrences, 77.5% are caused in island species. These extinction events may not be the case despite seemingly overwhelming numbers of *P*. sp. nr. *pubens*. Field observations suggest the distinct possibility that other formicid species subsist in smaller numbers than prior to the *P*. sp. nr. *pubens* introduction.

The collection percentages (Table 4.1) are necessarily biased as the focus of the study was to find *P*. sp. nr. *pubens* distributions. I devoted 90% effort for discovery in likely areas of infestation and 10% effort for discovery in unlikely areas, thus optimizing time and resources to achieve the search goals (R. Gold, professor Urban Entomology, Texas A&M University, pers. comm., 2008). Additionally, when an aggressive and dominant ant species is present in a community, the use of a variety of food resources can facilitate a more accurate assessment of the ant fauna (Sarty et al. 2007). The use of only two food resources may have limited the conclusions of this study.

The conclusions of this study may also be biased because of the ability of *P*. sp. nr. *pubens* to find and allocate resources more quickly than other ant species (Wilson and Hölldobler 1990). This may further hamper the ability of other ant species to search for resources in the immediate area when in the presence of uncommon interspecific formicid densities. Other species may be currently surviving in more established areas, however, in very low numbers. The high densities of *P*. sp. nr. *pubens* may simply cause other ant species to become less active and not necessarily displaced or locally extinct. Normal ranges of activity would logically occur within normal ranges of interspecific formicid activity. It may be that other formicid species exist even in the well established areas of *P*. sp. nr. *pubens* distributions. Some native ant species may have become displaced or undergone temporary microgeographic extinction and/or extreme isolation. Although not collected in well established areas, other formicid species may be unable to find and allocate a resource as quickly or readily as *P*. sp. nr. *pubens*. These factors may all lead to under estimates of populations of other ant species. Once a food resource (e.g. pitfall trap bait) has been located, the quick allocation of workers may overwhelm the proximal area surrounding the resource.

The displacement of the red imported fire ant (RIFA), *Solenopsis invicta*, by *P*. sp. nr. *pubens* is a concern when regarding the dichotomy with which *S. invicta* and *P*. sp. nr. *pubens* can be controlled. *Paratrechina* sp. has been found coexisting in the presence of *S. invicta* (Sanchez 2005). *Paratrechina* species are 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). The displacement of *S. invicta* implicates the strength of ecological influence that *P*. sp. nr. *pubens* is creating in south Texas. This notable influence on a previously ecologically dominant species in the region, *S. invicta*, may signify the possible effects *P*. sp. nr. *pubens* may be having on less influential fauna of the local ecosystem.

Paratrechina sp. nr. *pubens* has invaded numerous locations in Texas that comprise geographically discrete populations (Fig. 2). These locations include industrial areas, schools, neighborhoods, landfills and areas with amplified environmental, ecological, and human concerns. One currently substantiated location is the National Aeronautics and Space Administration (NASA) Johnson Space Center (JSC) in Houston, TX. This infestation was discovered in March of 2008. This infestation is a concern for two reasons. *P*. sp. nr. *pubens* propensity for electrical shortages is alarming when considering such a large facility with direct scientific and human implications. Because of the unknown ecological effects from *P*. sp. nr. *pubens*, endangered species become a concern. Found only on the prairie coastal areas, the endangered, ground-nesting Attwater's prairie chicken, *Tympanuchus cupido attwateri*, has particular habitat requirements (TPWD, 2007). One of the few locations being utilized for raising the bird is the grounds of NASA JSC. Numerous reports of *S. invicta* predating on ground nesting birds have been published (Drees 1994, Allen et al. 1995, Lockley 1995, Wojcik 2001). Therefore it is a concern when another ecologically dominant ant species is encroaching the habitat of *T. c. attwateri* on NASA property.

Other potential locations of concern not yet realized by advancement of the *P*. sp. nr. *pubens* populations include Hobby Airport and the Armand Bayou Nature Center. An infestation of *P*. sp. nr. *pubens* at a Houston middle school is located less than 1.3 km from Hobby Airport. The SOOKI for *P*. sp. nr. *pubens* population expansion has spread to within 0.5 km from the Armand Bayou Nature Center. *P*. sp. nr. *pubens* may inhibit the endemic fauna and disrupt the natural ecology.

Whatever the means; the invasive pathway niche (IPN) exploited by various species has resulted in numerous successful exploitations of the bioinvasive process (Simberloff et al. 2005). *Paratrechina* sp. nr. *pubens* is currently exploiting several IPNs (e.g. commercial trucking, vehicles, train, potted plants, garbage, landfill, or construction). The broad distribution expansion of *P*. sp. nr. *pubens* may have been assisted by the transportation opportunities afforded in an industrial area. During several field studies on *P*. sp. nr. *pubens*, anecdotal stories from numerous individuals suggest periodic infestation of colonies into motor vehicles, boats and other similar equipment. These events typically occurred during or proceeding rain events. As such, the Pasadena site likely acted as a point-of-origin for further anthropogenic introductions into and surrounding Harris county. During the dispersal events, minimum viable population size does not seem to be a limiting factor for *P*. sp. nr. *pubens*. Preliminary laboratory examinations have shown that *P*. sp. nr. *pubens* can still have living caste members, including queens, after six wk of starvation and four wk without water. In whatever capacity, *P*. sp. nr. *pubens* may be spread, with the exclusion of landfills, field observations suggest they are likely being spread as a small event with a single satellite colony.

Although beyond the scope of the current study, future metapopulation analyses should be conducted for predictive estimations of additional geographic infestations and ecological diversity effects from *P*. sp. nr. *pubens*. Metapopulation analyses can lead to identification of influential environmental factors that increase the likelihood for ant diversity (Morrison 1998). Knowledge of island biogeography (MacArthur and Wilson 1963, 1967) may enhance the potential for learning about invasive events and the potential adverse effects from an ecologically dominant species. Although intended for island population dynamics, converse lessons may be learned from island biogeography regarding continental invasions, such as *P*. sp. nr. *pubens* to Texas. Artificial landscapes created by urban environments (Davis and Glick 1978, Niemelä 1999, Yamaguchi 2005) may closely ally with island biogeography. Spatial or metapopulation modeling of this infestation could be infused into LANDIS (landscape dynamics program), RAMAS (metapopulation modeling program) (Akçakaya 2001), or other environmental spatial modelers, to better estimate spread of *P*. sp. nr. *pubens* in an urban landscape that includes patchy or fragmented habitats, respectively. Certain caveats of metapopulation analysis may be elucidated indicating reasoning for such low isolation among populations of *P*. sp. nr. *pubens*. Flow or movement of *P*. sp. nr. *pubens* may be enhanced or restricted from one quadrant to another by suitable or unsuitable habitats. More precise information may be gained by a predictive probability model that may include information gained from this study.

In the same regards, predicting the impacts on native ecosystems could become useful. Invasive prediction models (Byers et al. 2002) and quantitative predictive impact models (Ricciardi 2003) may be used to forecast ecological effects of *P*. sp. nr. *pubens*, utilizing the information gained from this study. The dynamic invasion geographies of *P*. sp. nr. *pubens* populations are key to predictive estimations for spatial scales (Ricciardi 2003). Although held in suspicion of success (Gilpin 1990), prediction models for invasive species, such as *P*. sp. nr. *pubens*, will likely need a great deal of biological data. These prediction models do not necessarily need to predict individual events, but the effects such events may have on the native ecology. This study may be able to offer baseline data for a predictive model for *P*. sp. nr. *pubens*. RAMAS can offer accurate estimations of extinction probabilities of native fauna (Akçakaya 2001) in the *P*. sp. nr. *pubens* effected areas. This ecological risk assessment (Akçakaya 2001) is an important aspect when determining the impact of this and other invasive species.

Given the establishment and synanthropic behavior of *P*. sp. nr. *pubens*, continued introductions are unavoidable without local, state and/or federal expansion-preventing measures in place. Should this ant spread to further locations outside of the Houston area or Texas, it may become a regionally exotic species with remarkable deleterious consequences.

CHAPTER V

LABORATORY EVALUATION OF DINOTEFURAN IN LIQUID ANT BAIT AGAINST *Paratrechina* SP. NR. *pubens*

Introduction

Neonicotinoids comprise a class of insecticide that is very effective against a great variety of insects. Neonicotinoids demonstrate agonistic activity on arthropod postsynaptic nicotinic acetylcholine receptor sites (Tomizawa and Yamamoto 1993, Miyagi et al. 2006). Dinotefuran, *N*-methyl-N'nitro[*N*"-[(tetrahydro-3-furanyl)methyl]guanidine, has insecticidal activity that includes both neuron-excitatory and neuron-blocking mechanisms (Kiriyama and Nishimura 2002). Dinotefuran is a 3rd generation neonicotinoid with broad spectrum activity against insects (Wakita et al. 2003). Typically known as and used in agricultural products (Elbert et al. 1998), neonicotinoids usefulness has been further expanded to the control of urban insect pests (e.g. Premise®, Maxforce® Granular Fly Bait, and Advantage® (imidacloprid) for control of termites, flies, and fleas and ticks, respectively). Dinotefuran insecticidal activity has previously been demonstrated across a few insect groups including houseflies, *Musca domestica* (Kiriyama et al. 2003), mosquitoes (Corbel et al. 2004) and cockroaches (Mori et al. 2001, Kiriyama and Nishimura 2002, Miyagi et al. 2006). Regarding human safety, neonicotinoids have proven to have a low toxicity to mammals (Kiriyama and Nishimura 2002, Corbel et al. 2004).

There is currently no published research concerning dinotefuran efficacy against ants. A recent introduction of a very troublesome invasive ant, *Paratrechina* sp. nr. *pubens* has warranted research involving control of this pest. This invasive species has caused numerous electrical shortages and become an immense nuisance due to their densities. Since its introduction, this tramp ant has spread to 25 geographically distinct locations in five Texas counties. Typical control tactics for urban ant pest population management have been inadequate due to remarkable population densities of *P*. sp. nr. *pubens*. Novel control measures should be evaluated as to their efficacy towards population management of *P*. sp. nr. *pubens*. Successful novel control research tactics will likely be integrated into an overall management program for *P*. sp. nr. *pubens* control or eradication.

Invasive social insects can create ecologically devastating results (Moller 1996, Chapman and Bourke 2001, Holway et al. 2002). Social behaviors of ants create a weakness that can be exploited during the control process. Shared resources, trophallaxis, cannibalism, and grooming are all avenues for an increase in treatment efficacy. This is particularly evidenced by the horizontal transmission of active ingredients (AI's), as has been observed in cockroaches (Kopanic and Schal 1999), termites (Ibrahim et al. 2003) and other ants (Soeprono and Rust 2004). Invasions by social insects often encompass large geographical regions, are detrimental to agricultural systems and natural communities, and are expensive to control (Vinson 1986, Vander Meer et al. 1990, Williams 1994). The ease of application of aerially applied control measures is a desirable character for a management program for invasive species. Baits could be integrated into an overall management program. These programs have been historically evaluated (e.g. Mirex against the red imported fire ant, *Solenopsis invicta* (Banks et al. 1973)) and more recently for termites as "Operation Full Stop" for the Formosan subterranean termite, *Coptotermes formosanus*, in New Orleans, Louisiana (Ring et al. 2001).

The use of baits for eradication of ants has been reviewed (Stanley 2004). The use of baits has proven successful against other invasive species behaviorally similar to *P*. sp. nr. *pubens*. Unicolonial ants, such as the Argentine ant, *Linepithema humile* (Krushelnycky et al. 2004) and the yellow crazy ant, *Anoplolepis gracilipes* (Abbott and Green 2007), have been successfully controlled despite high densities. Containment of an early detected invasive species may afford time for research to conclude successful management or eradication techniques (Krushelnycky et al. 2004).

The objective for this study was to determine mortality ratios of *P*. sp. nr. *pubens* at various concentrations of dinotefuran amended into a liquid bait. This study constitutes an initial effort to find control alternatives for *P*. sp. nr. *pubens* in Texas.

Materials and Methods

Each of thirty plastic boxes, 9 cm high x 15 x 30, coated with fluon, contained 100 *Paratrechina* sp. nr. *pubens* workers collected from Pasadena, TX (29˚ 36.748 N, 95˚ 03.313 W). Workers were collected from laboratory maintained queenright colonies of moderate size containing brood. Glass tubes, 1.6 cm d x 15 cm were placed in each box, containing deionized water with a cotton plug. Tubes were covered with solid color construction paper for darkening purposes. Five replications at each of five concentrations of dinotefuran at 0.00006, 0.00012, 0.00025, 0.0005 and 0.001% were used, along with five replications of the product with no AI (blank). Concentrations were selected based on the suggestions given by the manufacturer. The insecticide was provided in aqueous solution at 0.001%. All dilutions were made using 20% sucrose in deionized water. Ants were starved for 24 h prior to exposure. Two ml droplets of dinotefuran or blank were placed on the bottom of each box. Observations were made at 1, 2, 3, 4, 5, 24, 48, 72, and 168 h after application, and moribund ants were counted. The number of live ants was counted as opposed to the number of dead, as it soon became apparent that the ants were cannibalistic.

Counts of dead or live ants were made after the 24 h starvation period and statistical analysis was conducted accordingly. For statistical purposes, an outlier replication within the 0.001% dose was excluded.

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean percent mortality in treatments. Means were separated using Tukey-

Kramer HSD test. LD_{50} and LD_{90} values of mortality response to treatments were analyzed using PROC PROBIT (SAS Institute 2000, Cary, North Carolina).

Results

Mean percent mortality of *P*. sp. nr. *pubens* was typically higher as the concentration increased at both three ($F = 7.28$; df = 28; $P < 0.001$) and seven ($F = 7.28$; df = 28; $P < 0.001$) d post-treatments (Table 5.1). There were no significant differences between the four highest concentrations for both post-treatment observations. Three d observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven days post treatment (Table 5.2) showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; P < 0.01, df = 1; $\chi^2 = 7.09$; P < 0.01, respectively).

Discussion

Dinotefuran caused more mortality in *P*. sp. nr. *pubens* than did controls. With the relatively low LD_{90} values, these data indicate high efficacy of dinotefuran to control *P*. sp. nr. *pubens*. These data also indicated that dinotefuran caused sufficient mortality to warrant further testing in both the laboratory and field; however, the delivery system of dinotefuran will need modification for field tests. Including this bait with corn grit or other delivery product may decrease evaporation and crystallization rate, along with increasing the likelihood that workers will be able to allocate the bait to remaining colony members.

Table 5.1. Mean dinotefuran-treated *P*. sp. nr. *pubens* mortality rates with doses using five replications of 100 ants per arena.

Concentration (%)	Mean % mortality in five replications @ 3dat[ab]	Mean % mortality in five replications @ 7dat[ac]
0.001[d]	78.82 a	89.17 a
0.0005	63.36 a	82.51 ab
0.00025	58.61 ab	88.62 a
0.00012	44.82 abc	87.61 a
0.00006	16.47 bc	57.15 b
Blank	3.31 c	4.18 c

[a] Means in the same column followed by the same letter are not significantly different ($P < 0.05$; Tukey-Kramer HSD). [b] $F = 7.28$; df = 28; $P < 0.001$, [c] $F = 26.57$; df = 28; $P < 0.0001$

[d] For statistical purposes, this dose had only four replications.

dat = days after treatment.

Table 5.2. Probit regression of mortality data to dinotefuran-treated *P*. sp. nr. *pubens* workers at different time intervals with LD values in percent active ingredient.

# replications	DAT	Slope ± SE	LD_{50} (95% FL)	LD_{90} (95% FL)	χ^2
30	3	0.99 (0.37)	0.0003 (0.00008 – 0.0008)	0.005 (0.001 – 137.75)	7.20
30	7	0.84 (0.32)	1.67×10^{-5} (3.08×10^{-9} – 5.53×10^{-5})	0.00055 (0.00025 – 0.037)	7.09

DAT = Days after treatment.

High survival ratio within the control replications suggests an unbiased analysis of the experiment. However, extraneous factors such as crystallization (Fig. 5.1) of dinotefuran and cannibalism may have affected mortality in this no-choice test. Crystallization of the bait may not have allowed for continued feeding past ca. 48 h (Fig. 5.1). Some individuals became adhered to the product and therefore died *in situ*, which may have adversely affected spread of the insecticide throughout the remaining workers. Crystallization may alternatively create a differential availability of dinotefuran within the formulation. The primary dissipation route for dinotefuran may be through aqueous photolysis (~1.3 d). Sorting and separation of the dead individuals from the living group of workers would not have allowed for the opportunity of cannibalism. This cannibalistic behavior towards exposed individuals of social insects increases the transmission of an insecticide throughout the population (Kopanic and Schal 1999, Ibrahim et al. 2003, Soeprono and Rust 2004). Given the relative stability of dinotefuran, this is likely the case regarding its interaction with *P.* sp. nr. *pubens* both physiologically and behaviorally. It is unknown whether *P.* sp. nr. *pubens* workers were cannibalistic toward healthy or moribund workers or simply consume cadavers as part of a normal behavioral assemblage. Although no counts were taken of major body parts (head, thorax, or abdomen), my observations indicate that consumption of the head was considerably less than the thorax or abdomen. Further studies on horizontal transfer of insecticide through cadaver maintenance or cannibalism should be investigated in *P.* sp. nr. *pubens*. Metabolic dissipation pathways of dinotefuran should also be investigated. These findings may indicate the reasoning for high horizontal transmission through behaviors (trophollaxis, grooming or other) or cannibalistic insects.

The relative success of this laboratory study warrants further laboratory evaluations and initial field efficacy investigations. These findings may assist pest control operators during their efforts to control the numerically superior pest.

Figure 5.1. *P*. sp. nr. *pubens* workers adhered to a crystallized mass of dinotefuran.

CHAPTER VI

LABORATORY EFFICACY OF INSECT GROWTH REGULATOR, NOVALURON, FOR *Paratrechina* SP. NR. *pubens* CONTROL

Introduction

Novaluron, 1-[3-cloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, an insect growth regulator (IGR), has been used against a variety of arthropods (Ishaaya et al. 2003, Su et al. 2003, Cabrera et al. 2005), however, this control agent has not been used against any formicid species. Although IGRs have adverse affects against other ant species in the laboratory (Banks et al. 1983, Kabashima et al. 2007), field control of ants using (IGR) baits can be difficult due to their temporally dynamic nutritional needs. Sustainable amounts of an IGR must be maintained within the colony brood and available in an effective dose during molt. These difficulties are compounded by the inactivity of IGRs on worker and alate castes. Colony death occurs when lack of worker replacement and natural death of adult castes take place (Banks et al. 1983).

An invasive ant species, *Paratrechina* sp. nr. *pubens*, has created numerous problems in and around the vicinity of Houston, TX since 2002. These problems created by *P*. sp. nr. *pubens* include status as an immense nuisance, electrical shortages of a variety of apparatuses, ecological dominance, and companion animal avoidance of outdoors. According to field observations from pest control operators and preliminary laboratory studies, very few bait matrices are attractive to *P*. sp. nr. *pubens*. It may become imperative to discover attractive and successful bait matrices as part of a temporally comprehensive control strategy for the management or eradication of *P*. sp. nr. *pubens*.

This study intended to evaluate the biological activity of novaluron against the invasive ant, *P*. sp. nr. *pubens*. Advance Carpenter Ant Bait (ACAB) with no active ingredient (AI) was amended with novaluron. Various concentrations of this synthesized material were used to determine novaluron efficacy against *P*. sp. nr. *pubens* in the laboratory.

Materials and Methods

Novaluron was administered at various concentrations to *P*. sp. nr. *pubens* in granular form using ACAB matrix (0.1, 0.25, 0.5, and 0.0% AI). *Paratrechina* sp. nr. *pubens* were starved for 24 h pre-treatment. The colonies were allowed to feed on the bait for 1 wk, after which the bait container was removed. Throughout the length of the experiment, *P*. sp. nr. *pubens* were offered 25% honey-water and crickets. Each replicate consisted of 100 workers and 50 brood (small egg clusters, larvae, and/or pupae). Two colonies were field-caught and laboratory-raised. All replicates were placed in plastic boxes, 9 cm high x 15 x 30, coated with fluon and provided glass containers fitted with water-wicks (Fig. 6.1). Colonies were exposed to CO_2 until movement was such that individual workers and brood could easily be counted and removed using a camel-hair paint brush. Colonies were placed in clear Petri dishes (3.5 x 1.0 cm) containing dental stone

Figure 6.1. This picture demonstrates the provisioning of bait and subsequent fungal growth associated with the high humidity and the clustering behavior of *P*. sp. nr. *pubens*. The discoloring (yellowing) of the wick seen here is typical of all field-collected colonies maintained in the laboratory.

substrate for observational purposes and moisture retention. The top of each Petri dish, two holes were made for worker movement. Post-treatment observations of worker and brood numbers, including abnormal behaviors, were made at each time interval. Deviation from the original colony numbers were used to determine efficacy of novaluron concentrations. Each concentration consisted of 7 replicates. Based on former *P*. sp. nr. *pubens* colony maintenance, replications for this study were maintained in a growth chamber at ~29.5ºC and ~64.5% humidity. All treatments and replicates were Completely Randomized Block Design (CRBD) between colonies.

Post-treatment counts were conducted by exposing *P*. sp. nr. *pubens* to CO_2 until rapid movements ceased and workers and brood were counted. Workers were observed until CO_2 recovery. To determine efficacy of novaluron, observations were made 3, 7, 14, and 28 d post-treatment. Efficacy was determined from the colony reduction of post-treatment counts from pre-treatment counts. Temperature and humidity data were taken every hour throughout the experiment using a HOBO Data Logger (Onset Computer, Bourn, MA).

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean mortality (workers) and survival (larvae) in treatments (JMP, SAS Institute, Cary, North Carolina). Means were separated using Tukey's HSD test.

Results

One-Way ANOVA was conducted to observe any bias in replication placement within the growth chamber. This analysis found no bias within replications (*F* = 0.38, df = 6, 101, *P* = 0.89).

There were no statistical differences found between treatments throughout time. No statistical differences were found between means of dead workers by treatment throughout time (Table 6.1, Fig. 6.2). At 14 d post-treatment, the only statistically significant differences (P = 0.028) were found between treatments of live larvae (Table 6.2, Fig. 6.3). However, the results did not differentiate the control means from two of the AI treatments (0.1 and 0.5%). The results for both dead workers and live larvae were inconclusive.

Discussion

Despite, a supposed ideal environment of temperature and humidity (29.45ºC ± 0.007, 64.57% ± 0.09, respectively), workers and brood of replications began dying at a surprising rate. Because of this, the original experiment was cancelled and performed again. The initial experiment was run under the same parameters (with exception of 100 brood rather than 50) and was considered a failure. Statistical analyses were conducted on the truncated data and no apparent biases were found within the experiment. We believe that this demonstrated the difficulties in maintaining *P*. sp. nr. *pubens* in colony-form with such low numbers and without the presence of queens.

Table 6.1. Mean # of dead *P*. sp. nr. *pubens* workers throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of dead workers throughout time (d)[a]			
Treatment (AI%)	3[b]	7[c]	14[d]	28[e]
0.10	9.14 (3.13) a	21.14 (3.26) a	32.29 (5.68) a	55.14 (4.74) a
0.25	6.86 (3.13) a	14.43 (3.26) a	22.00 (3.35) a	41.14 (5.49) a
0.50	4.57 (0.95) a	20.86 (5.39) a	32.00 (6.39) a	53.43 (6.3) a
0.0 (Control)	9.00 (3.18) a	22.29 (5.81) a	32.57 (5.37) a	59.00 (8.03) a

[a] Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).

[b] $F = 0.818$, df = 3, 27, $P = 0.497$

[c] $F = 0.644$, df = 3, 27, $P = 0.595$

[d] $F = 0.937$, df = 3, 27, $P = 0.438$

[e] $F = 1.504$, df = 3, 27, $P = 0.239$

Table 6.2. Mean # live *P*. sp. nr. *pubens* larvae throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of live larvae throughout time (d)[a]			
Treatment (AI %)	3[b]	7[c]	14[d]	28[e]
0.10	20.71 (1.52) a	6.71 (1.29) a	3.86 (1.18) ab	0.17 (0.17) a
0.25	23.29 (1.69) a	7.57 (1.09) a	1.57 (0.65) b	0.00 (0) a
0.50	20.43 (1.88) a	9.00 (1.42) a	5.86 (1.96) ab	1.00 (0.45) a
0.00 (Control)	27.00 (2.04) a	8.71 (1.51) a	7.29 (1.11) a	0.83 (0.65) a

[a] Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).

[b] $F = 2.894$, df = 3, 27, $P = 0.056$

[c] $F = 0.628$, df = 3, 27, $P = 0.604$

[d] $F = 3.589$, df = 3, 27, $P = 0.028$

[e] $F = 1.620$, df = 3, 27, $P = 0.216$

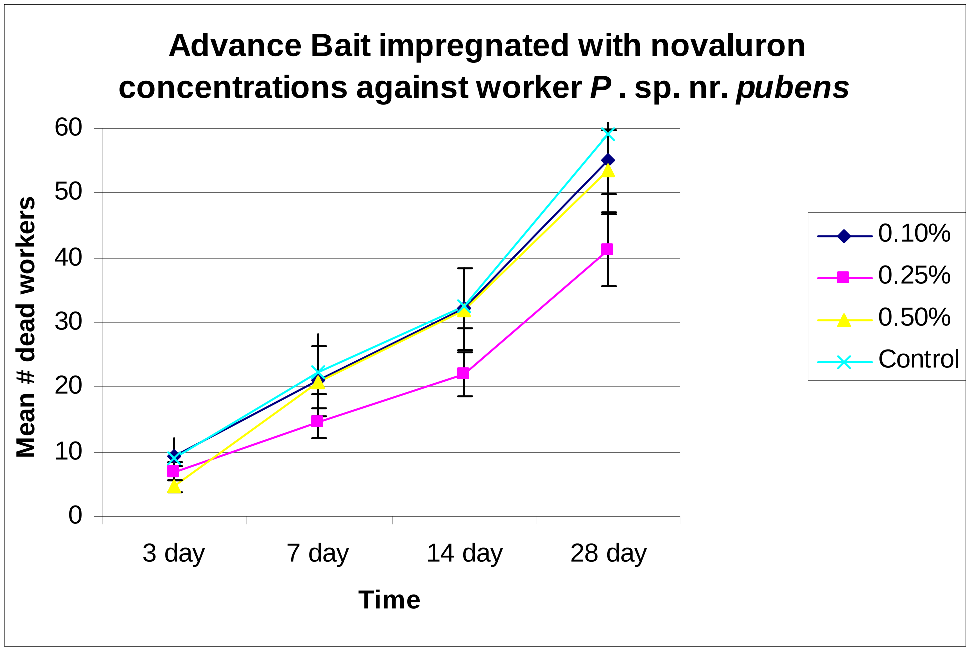

Figure 6.2. Mean number of dead *P*. sp. nr. *pubens* workers exposed to Advance Carpenter Ant Bait amended with various novaluron concentrations.

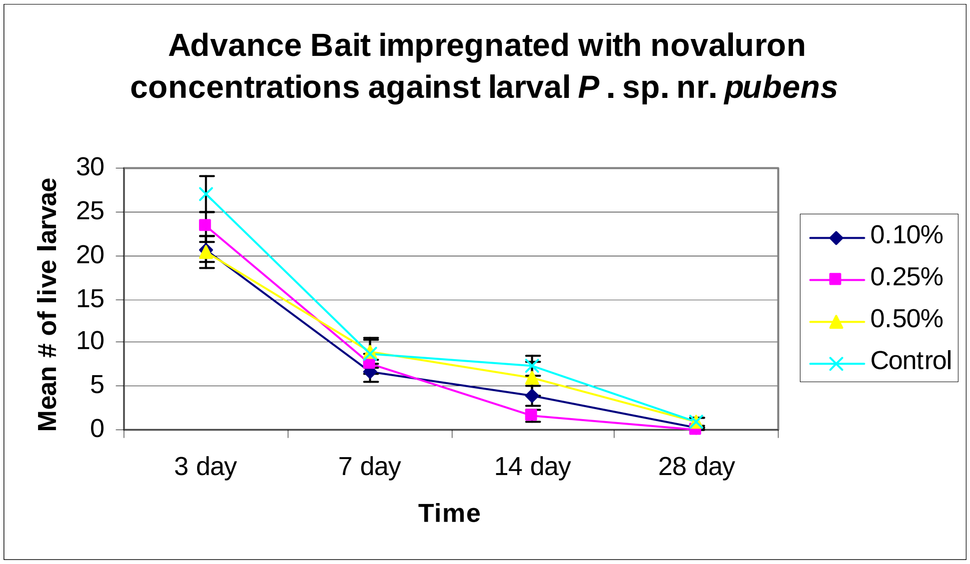

Figure 6.3. Mean number of live *P*. sp. nr. *pubens* larvae exposed to Advance Carpenter Ant Bait amended with various novaluron concentrations.

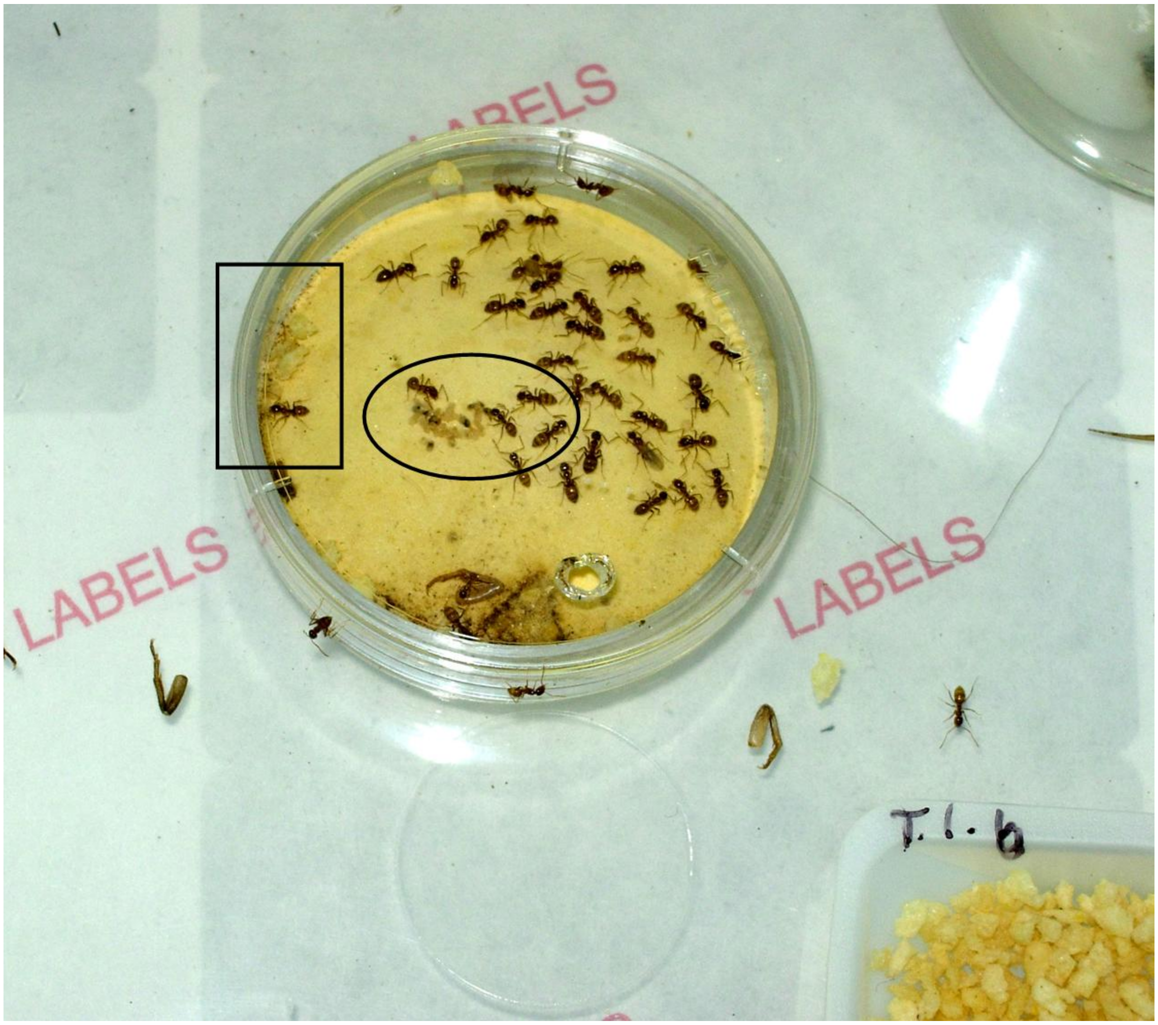

Figure 6.4. This picture demonstrates the provisioning of the bait inside the Petri dish. The square shows provisioned bait granules for 0.1% AI treatment. The circle shows workers tending several larvae.

Paratrechina sp. nr. *pubens* provisioned the bait granules (both control and AI) (Fig. 6.4). The bait was often placed inside the Petri dish or upon and around the water-wick. This created an ideal environment (high moisture) for fungal growth. It also created temporally increased contact with the AI. These facts may have hampered the ability to perform a more informative test. However, if statistical differences were to be found, they would have likely occurred at greater than 28 d post-treatment.

Formicid species often demonstrate temporal fluctuation of food resource consumption. This is not an ideal situation for IGR efficacy experiments. For efficacy of IGRs, there needs to be enough AI titer at a specific given interval (i.e. during larval molt). Because formicids select alternative food resources throughout time, administering an IGR can be a difficult task. Nevertheless, based on these results, this product cannot be recommended nor condemned for the control of *P*. sp. nr. *pubens*. Further laboratory studies should include whole colony tests with natural ratios of brood workers and queens. If used in the field, it would likely be most effective to broadcast large quantities of the bait during early spring as large numbers of brood are maturing.

Although not supported from these results, there remains the possibility that novaluron is ineffective against *P*. sp. nr. *pubens*. Another study conducted during control experiments for red imported fire ant, *Solenopsis invicta*, found results that did not support the use of another IGR against another *Paratrechina* sp. (Sanchez 2005). An increase in *Paratrechina terricola* populations were collected in trees located in areas treated with methoprene.

A previous experiment (Meyers et al., unpublished data), field observations, and communication with various pest control operators with clientele affected by *P*. sp. nr. *pubens* suggest the current label rate for ACAB (abamectin) is not effective. The currently recommended rate of 1.5 lbs per acre is unlikely to create or sustain control of the numerically dense *P*. sp. nr. *pubens* populations. If an additional AI was integrated into the product or an increase in the current broadcast rate, the efficacy of ACAB may increase. If additional bait amount is used, the efficacy of the product will likely increase substantially. The field effectiveness of this product at current label and expanded usage should be assessed against *P*. sp. nr. *pubens* in early spring.

P. sp. nr. *pubens* are considerably attracted to the ACAB matrix in the laboratory and field. It is therefore recommended that ACAB with novaluron be tested against large laboratory colonies (with a full compliment of castes). Field observations suggest an immense increase in numbers of *P*. sp. nr. *pubens* brood and worker members during early spring. During this period foraging for food sources high in protein is needed for brood production. ACAB contains a marine lipid based attractant. Therefore, this product may be a viable option as part of a temporally dynamic control program against *P*. sp. nr. *pubens*.

The failure of this laboratory study underscores the difficulties of maintaining relatively small, queenless colonies of *P*. sp. nr. *pubens* in the laboratory. Although it is not known whether the lack of queens adversely affected the outcome of the study, it could be one of the contributing factors.

CHAPTER VII

FIELD EFFECTIVENESS OF ADVANCE™ CARPENTER ANT BAIT AMENDED WITH DINOTEFURAN FOR CONTROL OF *Paratrechina* SP. NR. *pubens*

Introduction

Dinotefuran, *N*-methyl-N'nitro[*N*"-[(tetrahydro-3-furanyl)methyl]guanidine, acts as a neuron-excitatory and neuron-blocking mechanisms in a variety of insects (Kiriyama and Nishimura 2002). Neonicotinoids are a relatively safe group of pesticides (Kiriyama and Nishimura 2002, Corbel et al. 2004) that can be used against a broad-spectrum of insect groups (Mori et al. 2001, Kiriyama and Nishimura 2002, Kiriyama et al. 2003, Corbel et al. 2004, Miyagi et al. 2006). However, there has been no published research regarding dinotefuran's use against pest ants.

A recent successful introduction of an invasive ant species, *Paratrechina* sp. nr. *pubens*, to Texas, U.S.A., has created numerous economic and ecological concerns. Spread of this species has occurred at ~30 m per mo in urban areas (Meyers and Gold unpublished a). Spread of *P*. sp. nr. *pubens* in non-urban areas is likely to occur at high rates. Territorial expansion of a close taxonomic relative, *P*. *fulva*, has been known to occur at ca. 100 m per mo with rivers as the only geographical barrier to advancement (Zenner-Polania 1990). Expansion of a similar unicolonial ant, the Argentine ant, *Linepithema humile* has been variably reported from 1.3 (Holway 1998b), to 5.5 (Fluker and Beardsley 1970), to 8.3 (Erickson 1971), 22.8 (Pasfield 1968) to 62.5 m per mo (Krushelnycky et al. 2004). These findings have been greatly dependent upon landscape suitability for *L. humile*. Landscape suitability estimations will help to develop accuracy regarding potential geographical invasions and subsequent economic and ecological damage assessments of *P*. sp. nr. *pubens*. It is imperative that basic science be completed regarding control of *P*. sp. nr. *pubens*.

Control of aggressively expanding territories of the unicolonial ant, *L. humile* has been implemented using broadcast baiting over large geographical areas (Kruchelnycky et al. 2004). Control efforts of other behaviorally similar and high density invasive ant species using a solitary control measure or incorporation of multiple management strategies should be investigated for *P*. sp. nr. *pubens*.

A laboratory study found successful control using dinotefuran in liquid bait formulation against *P*. sp. nr. *pubens* (Meyers and Gold unpublished c). This promising study suggested field effectiveness trials using a different bait matrix should be investigated.

Advance™ Carpenter Ant Bait (ACAB) is prescribed for use against a wide variety of ants including: *Paratrechina longicornis*, *Camponotus* spp., *Solenopsis invicta*, *Crematogaster* spp., *L. humile*, among others. ACAB has been successfully used against a turf pest ant, *Lasius neoniger* Emery (Lopez et al. 2000). Using the behaviors (trophollaxis, grooming, communication efforts, etc.) of this social ant to its detriment may assist in whole colony elimination. The use of delayed-action insecticides, such as baits, may offer a more effective treatment against *P*. sp. nr. *pubens* than other control methods such as direct sprays or repellents. Whole colony elimination will be difficult because of the lack of interspecific aggression between *P*. sp. nr. *pubens* colonies, creating unicolonial situations with extremely high densities.

Baiting techniques are a targeted form of control that is relatively harmless to non-target species (Pimental 1995). It has been estimated that < 0.1% of pesticides find their target (Pimental 1995). However, not all pesticides are intended to reach their destination (e.g. repellants, pheromones, etc.), and not all pesticide dissipation events are environmental (e.g. photolysis, non-specific binding, volatilization, microbial, etc.). Further evaluations of ACAB containing dinotefuran should be conducted to corroborate baiting techniques for *P*. sp. nr. *pubens*.

There have been no published studies involving field efficacy of ACAB with dinotefuran against pest insects. This study evaluated the biological activity of dinotefuran field treatments against *P*. sp. nr. *pubens*. Amending dinotefuran into ACAB blanks (ACAB without active ingredient (AI)) was tested as a solitary control measure against *P*. sp. nr. *pubens*. The current experiment evaluated the use of ACAB with dinotefuran over small geographical areas against *P*. sp. nr. *pubens*. This study provides needed field effectiveness data for dinotefuran baiting as a potential control for numerically dense *P*. sp. nr. *pubens*.

Materials and Methods

The level of infestation and efficacy of outdoor treatments for the control of *Paratrechina* sp. nr. *pubens* were evaluated on 30 plots in Harris County, TX from August to September 2006. Plots consisted of mostly grasses with intermittently dispersed trees of variable size (Fig. 7.1a). The northeastern part of the field was separated by railroad tracks which physically separated some treatments. Food attraction activity was used as an indication of population density. Food attraction activity was measured using baited vials placed inside the plots to determine the extent of *P*. sp. nr. *pubens* infestations within plots. Each bait station included two glass screw top vials (6 cm length x 1.7 cm diameter; 8 ml); containing a ~1 cm^3 piece of hotdog (as a protein source) or ~1 cm^3 20% honey-water (as a carbohydrate source) soaked cotton, respectively. Each vial was placed within a poly-vinyl pipe (10 cm length x 2.5 cm diameter) to avoid overheating of the vials. Bait stations were placed at the corners (x4) and at the center (x1) of each plot. To avoid additional overheating of vials, bait stations were placed in shaded areas where possible.

Bait stations were placed out ~06:00-09:00 CST or ~19:30-22:00 CST for 30-45 min. Vials were then collected, capped, labeled and brought back to the lab for counts and analyses. Pre-treatment counts were conducted in the morning (06:00-9:00 CST) of treatment prior to application.

Technical grade dinotefuran (99.5%) was diluted in deionized water. Dinotefuran was added at a rate of 0.1 or 0.001 ml active ingredient (AI) per 100 g unamended (ACAB) with intermittent spraying and agitation inside a Plexiglas raffle drum (54.6 w x

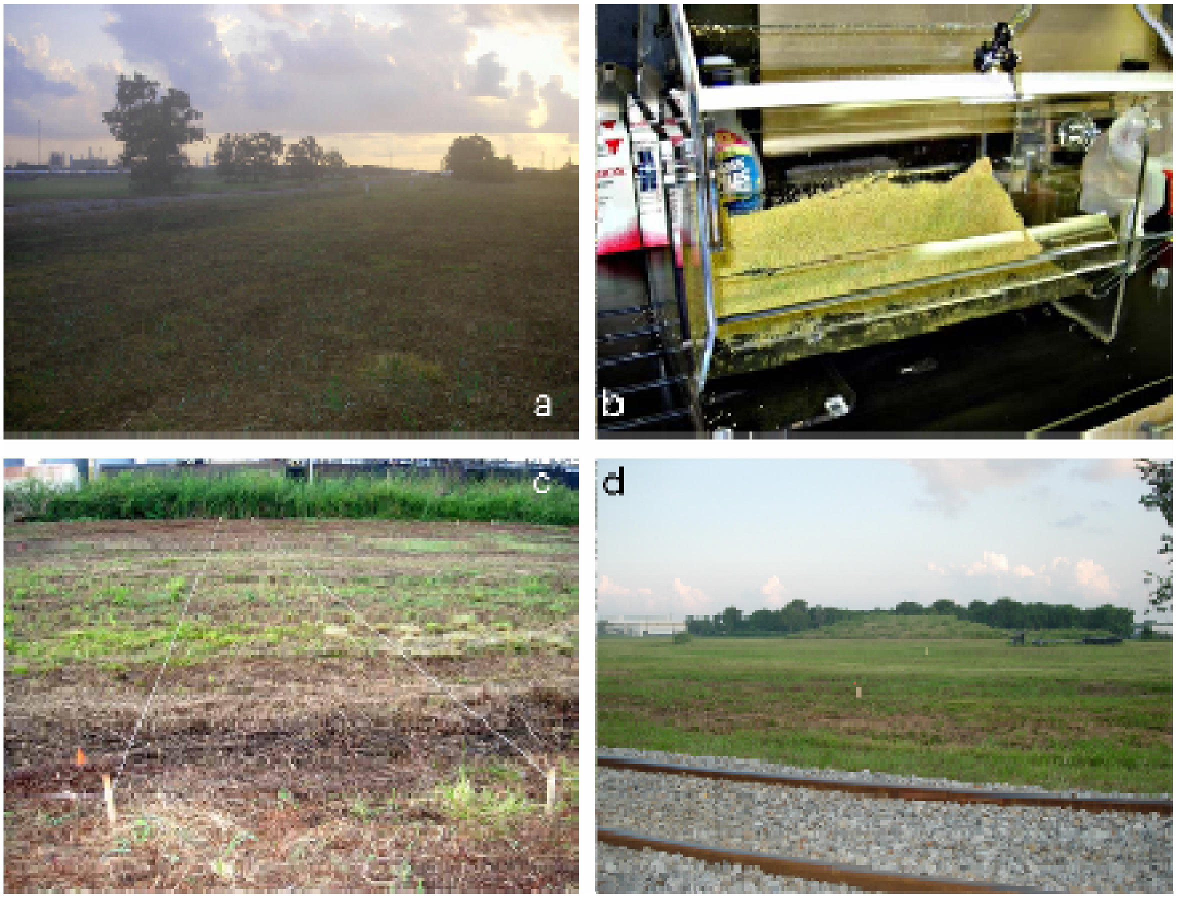

Figure 7.1. a) Overview of field plots with Easterly field plot region in near view. b) Westerly field plot area. c) This figure demonstrates the distance between each plot. Field plot example with view of Easterly field plots and adjacent vegetation structure. d) Raffle drum used to agitate and aerate the dinotefuran-bait mixture.

43.2 d x 47.6 h cm) (Fig. 7.1b) to ensure thorough mixing of AI to bait matrix. This procedure was preliminarily tested for dinotefuran-bait mixture using dyed water to ensure consistent spray distribution. The new dinotefuran bait was then allowed to dry to avoid fungal growth or decomposition of the bait contents.

Dinotefuran bait treatments included 0.1, 0.001, and 0.0% AI at the rates of 1.5 and 3.0 lb per acre. The applications were broadcast over 30.48 x 10.97 m (334.37 m^2) plots. Each of the plot was separated by a distance of ~1.52 m (Fig. 7.1c). Pythagorean's Theorem was used to ensure 90° angles of the plots. Treatments were applied using a Completely Randomized Design (CRD). A total of 16 of the 30 plots were on the eastern side of the field (Fig. 7.1c). These plots were easterly adjacent to an unkempt area that contained high amounts of vegetation structure. The remaining plots (n = 14) were located on the western side of the railroad tracks (Fig. 7.1d). These plots were in a more exposed area of the field receiving more sunlight than the other group and contained fewer, smaller trees. The field of grasses was cut to 15-20 cm prior to treatment.

Data were analyzed using SPSS® software (SPSS Inc. 2005). Univariate ANOVA was conducted to determine significant differences among means of treatment, position among plots, and time. Tukey's HSD was used for means separation. ANOVA was conducted on these data to determine significant differences among bait station position. Paired t-tests were conducted to determine differences among the food resource and plot position.

Results

There were significant differences found between *Paratrechina* sp. nr. *pubens* means of treatments throughout time ($F = 4.37$, df = 20, 1299, $P < 0.001$) (Table 7.1, Fig. 7.2). When comparing significant differences between control and AI treatments, differences in food resource allocation throughout time were clearly observed. Paired t-tests demonstrated significant difference between the food resources regardless of dinotefuran or control treatments ($t = 8.81$, df = 649, $P < 0.001$) (Table 7.2). Paired t-test among control treatments also indicate significant differences in food resource allocation ($t = 5.35$, df = 224, $P < 0.001$) (Table 7.2). This finding may demonstrate a strong relationship between protein:carbohydrate allocations among healthy *P*. sp. nr. *pubens* populations. Paired t-test among treatments also suggested significant differences in food resource allocation ($t = 7.00$, df = 424, $P < 0.001$) (Table 7.2).

Multivariate analysis of mean number of *P*. sp. nr. *pubens* per vial during pre- and post-treatment counts revealed significance differences between food resource over time ($F = 38.71$, df = 4, 645, $P < 0.001$; $F = 63.19$, df = 4, 645, $P < 0.001$) (Table 7.3, Fig. 7.3). There was also a statistical difference between the position of bait stations ($F = 9.15$, df = 4, $P < 0.001$) (Table 7.4).

A total of four plots were excluded from the experiment due to increased sunlight exposure during bait station retrieval and resultant inactivity of *P*. sp. nr. *pubens*. Other data that were possibly biased due to vegetation proximity and sunlight exposure were isolated and further data analyses were conducted. ANOVA determined that vials located on the easterly side of the plots split by the railway East (16 plots) and West (10 plots) had significantly higher means of *P*. sp. nr. *pubens* ($F = 41.78$, df = 1, 518, $P < 0.001$) (Table 7.5).

Table 7.1. ANOVA of mean number (± SE) of *P*. sp. nr. *pubens* per vial over all time by treatment.

Treatment	Number of vials sampled	Mean (± SE)[abc]
5 (0.1% at 1.5 lb)	200	54.41 (3.52) a
6 (0.1% at 3.0 lb)	250	59.56 (3.15) a
2 (Control at 3.0 lb)	250	74.39 (3.15) b
3 (0.001% at 1.5 lb)	200	76.06 (3.52) b
4 (0.001% at 3.0 lb)	200	91.50 (3.52) c
1 (Control at 1.5 lb)	200	92.78 (3.52) c

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD).

[b] $F = 4.37$, df = 20, 1299, $P < .001$

[c] SE results found using harmonic mean

Table 7.2. Paired t-test of mean number (± SE) of *P*. sp. nr. *pubens* by food resource for control and treated plots.

	Food resource	
Treatment	Hot dog	Honey water
Control[a]	97.45 (4.26)	67.67 (3.78)
Dinotefuran[b]	83.06 (3.27)	56.43 (2.66)
All[c]	88.04 (2.61)	60.32 (2.18)

[a] $t = 5.35$, df = 224, $P < 0.001$
[b] $t = 7.00$, df = 424, $P < 0.001$
[c] $t = 8.81$, df = 649, $P < 0.001$

Table 7.3. Multivariate ANOVA analysis of mean number (± SE) of *P*. sp. nr. *pubens* per vial over time by food resource.

	Food resource[ab]	
Time	Honey water[c]	Hot dog[d]
Pre-count	92.85 (±4.40) a	92.29 (±4.96) c
Day 3	73.34 (±4.40) b	52.64 (±4.96) d
Week 1	70.85 (±4.40) b	47.28 (±4.96) d
Week 2	45.11 (±4.40) c	110.97 (±4.96) b
Week 4	22.30 (±4.40) d	140.19 (±4.96) a

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD).
[b] SE results found using harmonic mean
[c] $F = 38.71$, df = 4, 645, $P < .001$
[d] $F = 63.19$, df = 4, 645, $P < .001$

Table 7.4. Mean number (± SE) of *P*. sp. nr. *pubens* per vial by position around structures.

Position	Mean number of *P*. sp. nr. *pubens*[abc]
Center	60.92 (3.11) a
Southwest	69.81 (3.11) ab
Northwest	76.94 (3.11) bc
Southeast	82.78 (3.11) c
Northeast	83.29 (3.11) c

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD).
[b] $F = 9.15$, df = 4, $P < .001$
[c] SE results found using harmonic mean open

Table 7.5. A paired t-test of mean number (± SE) of *P*. sp. nr. *pubens* of easterly and westerly located by plot position (separated by the railroad tracks).

Plot position	Mean number of *P*. sp. nr. *pubens* of vial location[a]
Western	59.89 (4.25)
Eastern	96.37 (3.58)

[a] $F = 41.78$, df = 1, 518, $P < .001$

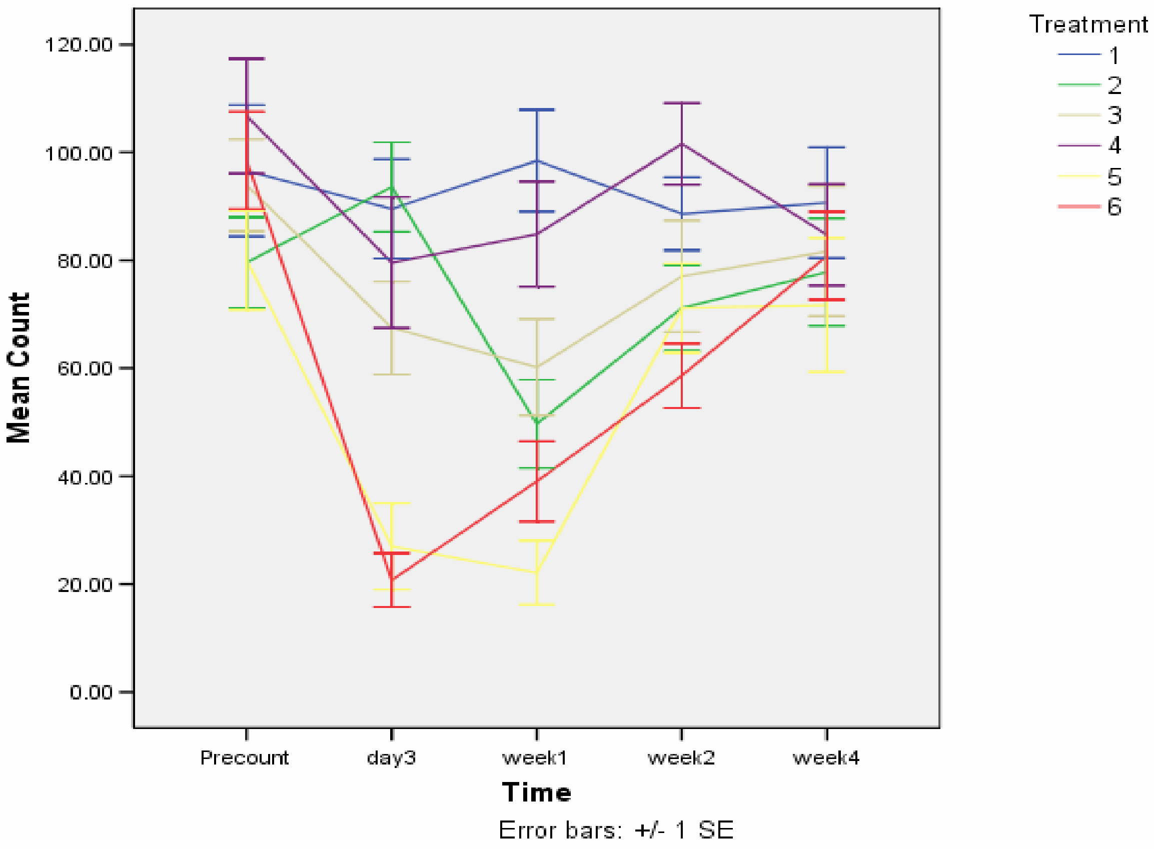

Figure 7.2. Mean number of *P*. sp. nr. *pubens* by time and treatment.

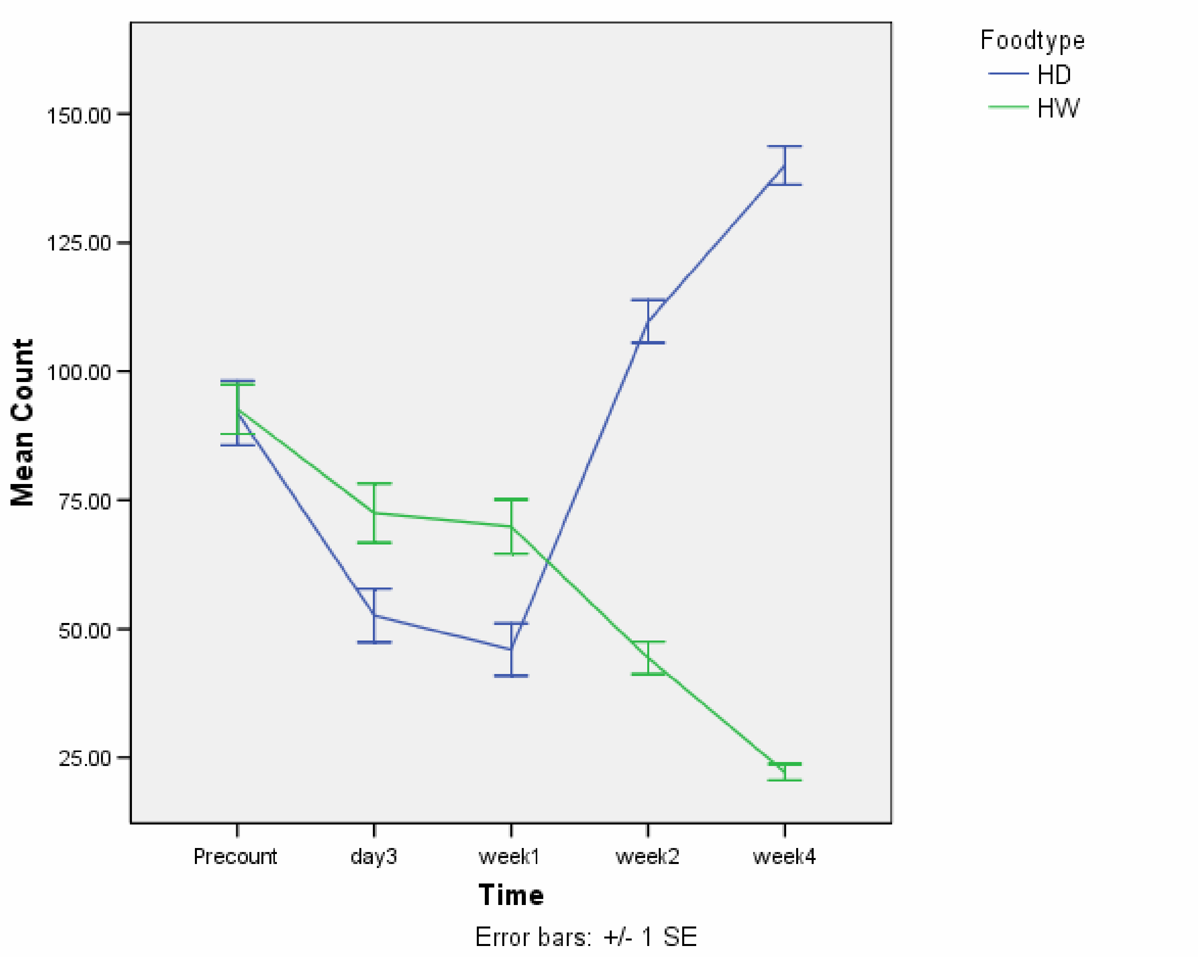

Figure 7.3. Mean number of *P*. sp. nr. *pubens* by time and food resource.

Discussion

The experiment was concluded at four wk, prior to the intended study length of 12 wk. At the beginning of the treatments, all plots were 15-20 cm grass height. At the time of the conclusion of the experiment (four wk) the height of the grasses was ca. chest height (~ 1.5 m). This vegetative growth impeded the researcher (JM) from re-attaining placed bait stations and slowed bait station recovery during the allotted time period.

Field observations also made it apparent that the treatments were not having the desired control effect. For these reasons, the experiment was concluded at four wk.

Limitations of count time intervals were caused by extreme heat in the treated areas that restricted foraging by *Paratrechina* sp. nr. *pubens*. During the four wk study, temperatures ranged from 23.9 – 37.1°C (28.4 ± 0.3). These time limitations decreased replications of some treatments during pre- and post-treatment counts. These extreme temperatures occurred during the late morning (~ 09:00) until evening (~ 19:00). According to field observations (by JM), the PVC-pipe surrounding the vials did not completely prevent extreme temperatures that were obviously outside the range of foraging behavior for *P*. sp. nr. *pubens*. Although unlikely to decrease all of the extreme temperatures, future field studies should include use of more light reflective colors such as sky blue or tan for the PVC-pipes which may reduce temperatures within and around the vials. These colors have been known to capture less heat due to their respective activation energies from color saturation with visible light intensity.

All treatment rates of the bait were above the currently described rate on the label for ACAB. The rates were intended to surpass the currently prescribed rates due to the immense numbers of *P*. sp. nr. *pubens* observed in the field and the ineffective control found by various Pest Control Operators (PCOs) in the field using the current label rate.

Throughout time, means of treatments five and six were significantly less than all other treatments (Table 7.1). Despite treatments five and six being significantly less than all other treatments (1-4) (Table 7.1, Fig. 7.2), it does not indicate successful or satisfactory control of *P*. sp. nr. *pubens* using treatments five or six. More likely, this indicates the remarkable numbers and fecund capabilities of *P*. sp. nr. *pubens* that overcame the treatments during pre- and post-application of the treatments. This also implicates the population pressure created from outside the treated plots that foraged or re-colonized treated plots. Treatments five and six contained the highest concentration (0.1% AI) of dinotefuran of all treatments. These findings warrant further investigations into higher dinotefuran concentrations and/or bait quantity. However, higher concentrations of dinotefuran may negatively influence bait attraction. No studies have been conducted to investigate the repellency of *P*. sp. nr. *pubens* to dinotefuran concentrations. Uses of other corncob grit bait mediums (other than ACAB) are less likely to be successful. Field observations from various PCOs do not support many other bait matrices as an attraction alternative for *P*. sp. nr. *pubens* control. According to some PCOs, all other currently available baits in the market known to attract *P*. sp. nr. *pubens* include sugar-based gels. The gel baits may be unlikely control measures given the densities of *P*. sp. nr. *pubens* and apparent product quantities that would likely include repeated treatments for adequate control. More sugar-based gel bait research efforts are needed to support or refute this assessment based on field observations and suppositions from PCOs. Results from treatments three and four were not statistically different from control treatments (one and two) (Table 7.1). Treatments three and four (0.001% AI) are not viable options for control of *P*. sp. nr. *pubens*, nor are these treatments at concentrations that need further investigation at the rates (1.5 or 3.0 lb per acre) evaluated in this study.

Seasonal fluctuation of nutritional needs for insects (especially ants) can inhibit experimental results and conclusions, especially in the field. There was a statistical similarity between food resource means during pre-counts. However, there was significant difference found between food resource means during the 4 wk count. The sudden temporal differentiation found between food resource means of pre-count and 3 d may indicate an immediate behavioral response to the treatment resulting in deviation from normal food resource allocation ratios (Table 7.3). This offers further evidence that food resource allocation of protein:carbohydrate ratios change over time for *P*. sp. nr. *pubens*, resulting in a higher protein intake during late summer. This could however, partially be explained by a change in seasonal nutritional requirements. Table 7.3 and figure 7.3 demonstrates a significant divergence in food resource allocation of protein:carbohydrate ratios. These investigations may need to consider a temporally dynamic baiting system to optimize bait intake throughout the season. Further investigations will need to be conducted to elucidate a conclusion regarding the significance of treatment and seasonal nutrition effects on *P*. sp. nr. *pubens*. Further studies should also include a year-round investigation of temporal changes of protein:carbohydrate ratios. These findings will have direct implications on further laboratory and field oriented research of *P*. sp. nr. *pubens* biological estimations.

Statistical differences found by multivariate analyses (Table 7.3) between treatments may imply that the treatments caused a shift in protein:carbohydrate ratio attraction. This occurrence could be explained as a negative reaction to the insecticide causing abnormal food resource attraction ratios. More investigations regarding behavioral changes and resultant nutritional deficits post-dinotefuran contact and/or ingestion are advised. However, the likely explanation is food resource allocation ratios naturally change over time as control treatments alone demonstrated statistical differences. The nutritional impact of non-AI treatments on pest insects may influence food resource allocation. The no AI ACAB matrix contains powdered sugar (5%), shrimp powder (15%), and soy oil (10%). These food resources may influence the outcome of control treatments for *P*. sp. nr. *pubens* and subsequent seasonal food resource allocation. Although not investigated, the impact for this study is likely negligible. The significant increase in means near vegetative overgrowth indicates that vegetation must be controlled to increase treatment efficacy of *P*. sp. nr. *pubens*.

Significant differences were found in the mean number of *P*. sp. nr. *pubens* by position (Table 7.4). The eastern portions of the plots had the highest means regarding positioning within the plots. This outcome may have been influenced by the vegetative overgrowth found near the eastern side of the property adjacent to several of the plots (Eastern plots). This vegetative overgrowth likely encourages higher densities of *P*. sp. nr. *pubens*, influencing the population influx of the eastern positions. Edge effects may have been a factor for the difference established between the center and outer positions. This conclusion may have restrictions in urban environments, as this scenario is more applicable as the center could constitute a house or structure.

This experiment does not indicate that these treatments are a viable option for control of *P*. sp. nr. *pubens* at the rates and conditions of the current study. There may be several reasons for lack of success: First, the treatment should include a broadcast treatment covering the surrounding areas of the desired control area. Population influx of *P*. sp. nr. *pubens* from surrounding treated and untreated areas near the treatment replications may have influenced results. Secondly, the lack of success may also be attributed to the high environmental temperatures and photodegredation of the AI (dinotefuran). Time constraints for the experimental treatments only allowed for mid-morning application, during which, temperatures ranged 32.2 - 33.3°C. These temperatures and the preceding day temperature high of 36.1°C may have increased dinotefuran and/or bait photodegredation. This is unlikely given the high densities of *P*. sp. nr. *pubens* and the likelihood that most if not all of the bait was discovered and allocated prior to the proceeding day. Thirdly, the treatments may not have included enough AI or product quantity. An increase in the amount of dinotefuran concentration may offer a more viable option for control. As discussed above, the amount of dinotefuran concentration that causes repellency of *P*. sp. nr. *pubens* has not been investigated. However, increasing the product application rates > 0.1% concentration may adequately cause the desired efficacy against *P*. sp. nr. *pubens*. Lastly, the day of treatment was followed by 1.93" of rain the next day (as reported form Houston/Hobby Airport, TX). This may have caused an adverse affect on the efficacy of the treatment. Dinotefuran has a 1.8 d Aqueous Photolysis Half-Life (APHL) (EPA 2004). APHL was an unlikely mode of dissipation for dinotefuran during this experiment as the product was applied during mid-day when no aqueous condensation was apparent and likely allocated prior to the rain event. This adverse outcome is unlikely to have caused poor insecticidal performance because of the persisting temporal statistical differences demonstrated in the treatments (Table 7.1). The exact scope and nature of the poor efficacy of these treatments remains undetermined.

The design regarding the bait station positions enabled an evaluation applicable to a typical treatment for ants surrounding or infesting a structure. Therefore, these negative results may be applicable to a control situation regarding a structural treatment. It may therefore be concluded that the presented treatments should not be used as a "stand-alone", however, may be an excellent choice as a supplementary control application for a field or structure. If used as the solitary control measure against *P*. sp. nr. *pubens* infestations, ACAB with dinotefuran should be applied at considerably larger rates and concentrations than suggested by the current label guidelines. Current maximum label rates, mandated by the EPA, of potential control products are inadequate regarding the biology and densities of *Linepithema humile* (Silverman and Brightwell 2008). Use of ACAB with dinotefuran as an exclusive management tool against *P*. sp. nr. *pubens* should also be broadcast on a large geographical scale for control within desired areas. Similar conclusions have been found regarding control and resurgence origin research of large infestations of the Argentine ant, *L. humile* (Vega and Rust 2003). Historical evaluations of baiting control techniques of similar invasive species, *L. humile* (Kruchelnycky 2004) and the yellow crazy ant, *Anoplolepis gracilipes*, (Abbott and Green 2007) may be applicable to *P*. sp. nr. *pubens* control efforts. It has been proposed that more aggressive and comprehensive control strategies should be employed regarding large populations of the unicolonial *L. humile* (Silverman and Brightwell 2008).

The current study represents the only field efficacy evaluation against insects using ACAB with dinotefuran to date. This field experiment demonstrated initial success despite overwhelming numbers of *P*. sp. nr. *pubens*. The lack of consistent temporal *P*. sp. nr. *pubens* control indicates the need for larger concentrations and/or rates of ACAB with dinotefuran. Additional temporal applications of ACAB with dinotefuran against *P*. sp. nr. *pubens* should be evaluated. Also, an integration of an ACAB with dinotefuran treatment into a temporally comprehensive control strategy should be investigated. The likely application of ACAB with dinotefuran may be best within a more comprehensive control plan for the numerically superior pest, *P*. sp. nr. *pubens*.

CHAPTER VIII

FIELD EFFECTIVENESS OF CURRENT AND EXPANDED LABEL TREATMENTS AGAINST AN INVASIVE ANT PEST, *Paratrechina* SP. NR. *pubens* (HYMENOPTERA: FORMICIDAE), OF TEXAS

Introduction

Fipronil is a broad spectrum insecticide that which has been used with great success. Fipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazol-3-carbonitrile, is a phenylpyrazole, a class of insecticides that act at the γ-aminobutyric acid (GABA)-gated chloride channel (Kidd and James 1991, Cole et al. 1993). Although environmental fate concerns about fipronil have been raised (Chaton et al. 2002), its soil binding capacity, ($K_{OW} = 4.01$ and $K_{OC} = 803$), and environmental instability (low vapor pressure, aqueous photolysis = 4.1 h, very susceptible to photolysis, significant microbial degradation) (Connelly 2001) are high, making fipronil more environmentally acceptable. For this reason, fipronil has been utilized against insects in a number of ways. Successful control has been demonstrated after fipronil soil treatments against a unicolonial tramp ant, the Argentine ant, *Linepithema humile* (Costa and Rust 1998). Significant reductions have been found when using bait integrated with fipronil against *L. humile* and the red imported fire ant, *Solenopsis invicta* (Klotz et al. 2003b, Collins and Callcott 1998, respectively). Barrier treatments using fipronil against *L. humile* have resulted in successful population control (Soeprono and Rust 2004).

Chlorfenapyr, 4-bromo-2-(4-clorohenyl)-1(ethoxymethyl)-5-(trifluoromethyl) pyrrole-3-carbonitrile, is a pyrrole used as a contact and stomach poison insecticide and miticide (Thomson 2001). Successful control of a variety of arthropod structural pests has been obtained utilizing the insecticide chlorfenapyr. These insect groups, for which chlorfenapyr is efficacious, include; cockroaches (Ameen et al. 2000), beetles (Arthur 2008), ants (Buczkowski et al. 2005), and at high concentrations, termites (Rust and Saran 2006).

Abamectin is an avermectin effective against a number of arthropods. Avermectins block GABA transmission resulting in inhibition and excitation of neurons (Lasota and Dybas 1991) resulting in death. Although use of abamectin resulted in survival of queens and workers, significant population reduction of *L. humile* has been achieved (Hooper-Bui and Rust 2000). Control of *Haematobia irritans* has been demonstrated during abamectin evaluations of cattle applications (Doherty et al. 1994, Guglielmone 1999). Use of abamectin for agricultural pests has been demonstrated on the citrus leafminer, *Phyllocnistis citrella* Stainton (Rae et al. 1996). Abamectin has also been formulated into a granular bait product, Advance^{TM} Carpenter Ant Bait (ACAB). There have been several successful population management strategies involving this product. Successful control of turf grass pests such as mound-building ant, *Lasius neoniger* Emery (Lopez et al. 2000, Shetlar 2003). ACAB has been used against American and German cockroaches, *Periplaneta americana* and *Blattella germanica*, respectively, with significant success (Appel et al. 2005). This and the aforementioned AIs, given their varied uses against other pest ant species across the U.S., provide compelling possibilities as potential control candidates for remedial applications to arrest a new exotic ant species introduced to Texas.

Paratrechina sp. nr. *pubens* is a recently introduced ant pest to southern Texas, originating from Pasadena, TX. It is a tramp ant whose unicolonial behavior is caused by a lack of aggression between colonies. This lack of aggression toward conspecifics allows for unicolonial behavior attaining high densities (Hölldobler and Wilson 1977, Porter and Savignano 1990, Macom and Porter 1996, Holway 1998a). Since its introduction, *P*. sp. nr. *pubens* has become an immense pest due to its remarkable numbers (Fig. 8.1a) and ability to cause electrical shortages in a variety of equipment such as outlets (Fig. 8.1b), sewage lift pump stations, air conditioning units, computers, pool pumps, etc.. Residents of a Deer Park, TX neighborhood have reported household incursions by *P*. sp. nr. *pubens* and even organized a neighborhood meeting regarding their concerns. These complaints include kitchen, faucet, and bath infestations, telephone and electrical box shortages (Fig. 8.1c), and ant bites. Their densities have lead Pest Control Operators (PCOs) and consumers alike to undertake costly and unsuccessful control attempts.

Observations of companion animals acting abnormally in the presence of this ant pest and undocumented effects on the indigenous arthropod and small vertebrate fauna, including a biotic homogenization of formicid species (Meyers and Gold unpublished a) and likely more adverse affects to other taxonomic arthropod groups. A similar invasive, unicolonial ant in high densities, *L. humile*, has adversely impacted native systems in New Zealand (Harris 2002). *L. humile* has caused adverse effects on ant diversity (Human and Gordan 1996, Holway 1999), abundance and diversity of other invertebrates (Cole et al. 1992, Way et al. 1992, Human and Gordon 1997), vertebrate abundance (Suarez et al. 2000), pollination (Buys 1987, Visser et al. 1996), seed dispersal and regeneration (Bond and Slingsby 1984, Giliomee 1986), and decomposition and nutrient cycling (Ward 1987, De Kock 1990, Folgarait 1998).

Currently no wide scale control programs for *P.* sp. nr. *pubens* have been instituted or proposed (R. Gold, pers. comm., Professor of Entomology, Texas A&M University; Tony Koop, pers. comm., botanist, New Pest Advisory Group). Although biological control efforts against *P.* sp. nr. *pubens* have certainly been considered, there is a certain risk associated with the potential for permanent ecological change (Simberloff and Stiling 1996). These introduced species intended for biological control of pest species may not have adverse effects exclusively on their intended target (Simberloff 1992). A species taxonomically similar to *P.* sp. nr. *pubens*, *P. fulva*, was introduced to control venomous snakes in Colombia, South America; however it had unintended consequences, causing biotic homogenizations of the arthropod community in addition to economic losses (Zenner-Polania 1990).

It has been recommended that similar pest situations (*P. fulva* in Colombia) implement multiple control methods for success (Zenner-Polania 1994). This study evaluated the biological activity of various multiple-strategy treatments using Termidor®

SC (fipronil), Phantom® SC (chlorfenapyr) and Advance Carpenter Ant Bait G (abamectin) against *P*. sp. nr. *pubens* residential infestations.

Materials and Methods

The level of infestation and effectiveness of treatments for the control of *Paratrechina* sp. nr. *pubens* were evaluated on 40-single family homes in Harris County, TX from July to October 2006. To determine the extent of *P*. sp. nr. *pubens* infestations at the homes, we measured food attraction activity using baited vials placed both around and within the homes. Each bait station included two glass screw top vials (6 cm length x 1.7 cm diameter; 8 ml); containing a ~1 cm^3 piece of hotdog (protein) and ~1 cm^3 20% honey-water (carbohydrate) soaked cotton, respectively. Each vial was placed within a poly-vinyl pipe (10 cm length x 2.5 cm diameter) to avoid overheating of the vials. To avoid additional overheating of outdoor vials, bait stations were placed in shaded areas where possible. Outdoor bait stations were placed on the ground adjacent (within ~1 m of the foundation) to the corners and front porch of the homes. Indoor bait stations were placed in the kitchen and one bathroom during pretreatment counts.

Bait stations were placed out at 06:00-10:30 CST for 1-2 hr. Vials were then collected, capped, labeled and brought back to the laboratory for counts and analyses. Pre-treatment counts were conducted 0 - 10 d prior to treatment. Although 76.5% of the residents complained of indoor *P*. sp. nr. *pubens* infestations, field observations based on initial counts and further conversations with residents indicated an exceedingly random nature of *P*. sp. nr. *pubens* activity indoors. Indoor accessibility issues also became apparent with 40 homes after the initial counts. After initial counts the unapparent relationship between ant activity, both indoor and out, justified the elimination of interior bait stations from the experiment. Post-treatment counts were then conducted as described above at ca. 3 d, 1, 2, 4, 8, and 12 wk.

Treatments. To evaluate the biological activity of Termidor® SC, Phantom® SC and Advance Carpenter Ant Bait™ G (ACAB) against *P*. sp. nr. *pubens* infestations, treatments were conducted on 40 homes in Deer Park, TX. Treatments evaluated efficacy of 0.06 and 0.5% active ingredient (AI), fipronil, Termidor SC and chlorfenapyr, Phantom SC, respectively, applied at label rates. For this study, 104 residents were contacted regarding possible study inclusion and 51 responded positively. Of these 51 residents, 40 were randomly chosen for the study based on the infestation level in the yard or home. Samples of *P*. sp. nr. *pubens* were taken from each residence and maintained in 95% ETOH.

In all treatments, volume was manually approximated by using pressure, broadcast volume, and travel rate in a manner consistent with turf applications of these products. Treatment A was applied to eight houses and included Termidor SC at 0.06% AI applied to exterior, per present label for nuisance ant control, and Phantom SC at 0.5% AI applied to interior areas per present label. There were ~5.7-9.5 l (1.5-2.5 gallons) of Termidor SC applied per house using a 190-l (50 gal.) gasoline powered sprayer equipped with a JD-9 gun 1.76-2.46 kscm (25-35 psi). There were ~1.9 l (0.5 gal.) Phantom SC applied per house using a 3.78 l (1-gal.). B&G sprayer. Treatment B was applied to nine houses as described in Treatment A; however, with expansion of

Termidor SC spray area to 1 m up and 3 m out from the foundation. There were ~18.9-28.4 liters (5 - 7.5 gal.) of Termidor SC applied per house. Treatment C was applied to nine houses as described in Treatment A; however, with broadcast treatment of yard with ACAB at 0.011% AI Bait was broadcast at 0.45 kg (1 lb) per acre rate using a hand-held Lawncrafter 45-0276 (Agri-Fab, Sullivan, IL). For treatments A, B, and C, any apparent *P*. sp. nr. *pubens* outdoor nesting sites proximal to applied areas were directly treated with Termidor SC spray. However, the unapparent nest building behavior of *P*. sp. nr. *pubens* allowed for only rare instances of direct spray. Treatment D included non-treated controls of 11 houses and sprayed with water containing no AI Despite yard infestations, some homes had obvious insecticidal treatments prior to or during the experiment that adversely affected *P*. sp. nr. *pubens* activity. Of the original 40 homes, three of the house data sets were discarded because the residents attempted significant remediation attempts that involved insecticide treatments. Residents were surveyed regarding their satisfaction of the treatments pre- and post-experiment.

Statistics. Data were analyzed using SPSS® software (SPSS 2005). A paired t-test was conducted to conclude differences among food resources. Univariate ANOVA was conducted on these data to determine significant differences among treatments, food types, position surrounding the house, between houses, and time. Tukey's HSD was used for means separation at the $\alpha = 0.05$ level.

Results

Food resource means were compared using paired t-test and demonstrated significant statistical difference ($t = 4.84$; df = 1249; $P < 0.01$) (Table 8.1). Results for isolating each of the food resources by treatment over time demonstrate identical statistical conclusions regarding treatment ($P < 0.05$; Tukey's HSD) (Table 8.2). There was no statistical difference among food resource means of *P*. sp. nr. *pubens* means regarding time ($P < 0.05$, Tukey's HSD) (Table 8.3, Fig. 8.2). Significant statistical differences were found between AI treatments (A, B, and C) and the control treatment (D) ($F = 113.24$, df = 3, 2499, $P < 0.001$) (Table 8.4). Statistical differences were found between treatments when isolating pre and post-treatment means ($P < 0.05$; Tukey's HSD) (Table 8.5). There were significant differences among *P*. sp. nr. *pubens* means over time when regarding all treatments ($F = 39.35$, df = 6, 2499, $P < 0.001$) (Table 8.6). A percent of population reduction from the pre-treatment counts and subsequent post-treatment counts is presented (Table 8.7).

Table 8.1. Paired t-test of mean (±SE) number of *P*. sp. nr. *pubens* per vial by food resource.

Food resource	Mean (±SE)
Hot dog	20.22 (1.23)
Honey water	14.07 (0.92)

$^a t = 4.84$; df = 1249; $P < 0.01$

Table 8.2. ANOVA of mean (±SE) number of *P*. sp. nr. *pubens* per vial by food resource by treatment over all time counts.

Treatment	Mean (±SE) # for both food resources[ab]	Mean (±SE) # for protein[ac]	Mean (±SE) # for carbohydrate[ad]
A (Phantom, Termidor)	9.13 (1.42) a	10.86 (2.10) a	6.31 (1.66) a
B (Phantom, Termidor-expanded)	8.59 (1.34) a	11.32 (2.23) a	6.94 (1.76) a
C (Phantom, Termidor, ACAB)	10.41 (1.35) a	10.90 (2.11) a	9.92 (1.67) a
D (no AI)	36.86 (1.27) b	43.98 (1.99) b	29.73 (1.57) b

[a] Means with same letter in a column are not significantly different (P < 0.05; Tukey's HSD).
[b] $F = 113.24$, df = 3, 2499, $P < 0.001$
[c] $F = 65.96$, df = 3, 1249, $P < 0.001$
[d] $F = 47.98$, df = 3, 1249, $P < 0.001$

Table 8.3. ANOVA of mean (±SE) number of *P*. sp. nr. *pubens* per vial by food resource and all time counts.

Time	Means (±SE) both food resources[ab]	Means (±SE) protein food resource only[ac]	Means (±SE) carbohydrate food resource only[ad]
Pre-count	40.23 (1.76) c	54.07 (2.77) c	26.39 (2.18) d
Day 3	8.65 (1.76) a	10.91 (2.76) a	6.39 (2.18) ab
Week 1	11.48 (1.77) ab	7.88 (2.77) a	15.07 (2.19) cd
Week 2	10.89 (1.77) ab	10.54 (2.77) a	11.25 (2.19) abc
Week 4	9.52 (1.78) a	8.67 (2.79) a	10.38 (2.20) abc
Week 8	14.94 (1.80) ab	13.75 (2.83) a	16.13 (2.23) bc
Week 12	18.00 (1.81) b	29.04 (2.84) b	6.96 (2.24) a

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD).

[b] $F = 14.23$, df = 6, 2499, $P < 0.001$

[c] $F = 37.36$, df = 6, 1249, $P < 0.001$

[d] $F = 9.81$, df = 6, 1249, $P < 0.001$

Table 8.4. ANOVA of mean (±SE) number of *P*. sp. nr. *pubens* per vial by treatment over all time counts.

Treatment	Mean (±SE) # *P*. sp. nr. *pubens* per vial[a]
A (Phantom, Termidor)	8.59 (1.34) a
B (Phantom, Termidor-expanded)	9.13 (1.42) a
C (Phantom, Termidor, ACAB)	10.41 (1.35) a
D (no AI)	36.86 (1.27) b

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD). $F = 113.24$, df = 3, 2499, $P < 0.001$

Table 8.5. ANOVA of mean (±SE) number of *P*. sp. nr. *pubens* per vial by treatment for pre-treatment only, and both pre-treatment and 12 wk post-treatment counts.

Treatment	Mean (±SE) # for pre-treatment only[ab]	Mean (±SE) # for 12 wk post-treatment only[ac]	Mean (±SE) # for both pre-treatment and 12 wk post-treatment[ad]
A (Phantom, Termidor)	37.92 (5.46) ab	11.03 (3.38) a	24.30 (3.24) a
B (Phantom, Termidor-expanded)	49.71 (5.07) a	3.37 (3.09) a	27.24 (3.07) ab
C (Phantom, Termidor, ACAB)	42.57 (5.07) ab	14.43 (3.19) a	28.50 (3.03) ab
D (no AI)	31.17 (4.68) b	42.78 (3.27) b	36.57 (2.95) b

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD).
[b] $F = 2.64$, df = 3, 363, $P < 0.05$
[c] $F = 28.50$, df = 3, 351, $P < 0.001$
[c] $F = 3.17$, df = 3, 706, $P = 0.024$

Table 8.6. ANOVA of mean number of *P*. sp. nr. *pubens* per vial over time for all treatments.

Time	Mean (±SE) # *P*. sp. nr. *pubens* per vial[a]
Pre-count	40.23 (1.76) c
Day 3	8.65 (1.76) a
Week 1	11.48 (1.77) ab
Week 2	10.89 (1.77) ab
Week 4	9.52 (1.78) a
Week 8	14.94 (1.80) ab
Week 12	18.00 (1.81) b

[a] Means with same letter in a column are not significantly different ($P < 0.05$; Tukey's HSD). $F = 39.35$, df = 6, 2499, $P < 0.001$

Table 8.7. Percent reduction of *P*. sp. nr. *pubens* populations over time post-treatment.

	Time (%)					
Treatment	3 d	1 wk	2 wk	4 wk	8 wk	12 wk
A (Phantom, Termidor)	100.0	95.6	90.1	96.0	78.8	70.2
B (Phantom, Termidor-expanded)	99.9	98.9	100.0	96.6	93.4	93.8
C (Phantom, Termidor, ACAB)	99.2	99.6	92.0	90.6	83.4	66.1
D (no AI)	-13.4	-41.4	-18.4	2.3	-16.9	-15.8

Figure 8.1. These pictures represent the large numbers surrounding structures and the potential electrical damage of *P*. sp. nr. *pubens*. A) Taken in June, this picture demonstrates the remarkable numbers of a graveyard of *P*. sp. nr. *pubens* workers prior to a treatment. B) *P*. sp. nr. *pubens* workers have shorted out this outlet. C) *P*. sp. nr. *pubens* have shorted out this electrical unit located in a the backyard in the Deer Park, TX neighborhood. These shortages had created several power outages for whole streets in the neighborhood.

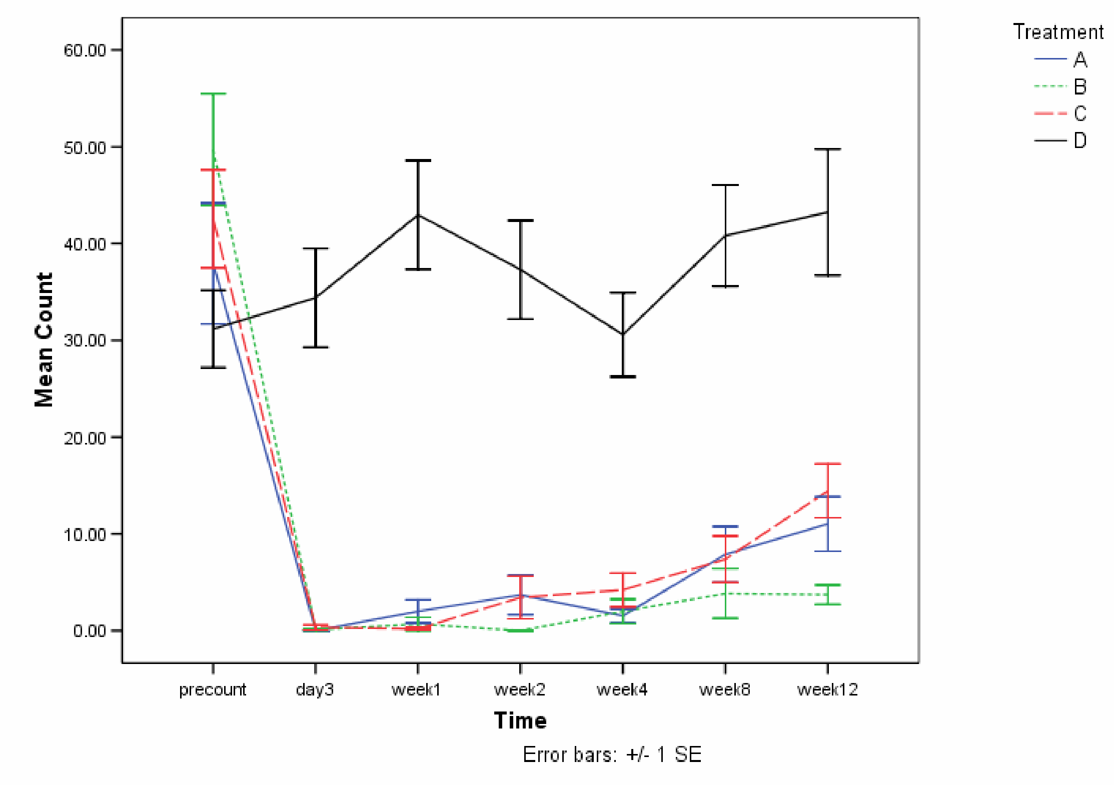

Figure 8.2. Mean number of *P*. sp. nr. *pubens* per vial by treatment over time.

Discussion

The statistical difference among food resource means does not signify either food-resource as a more accurate indicator of *P*. sp. nr. *pubens* activity. The statistical difference of food resource means suggest food resource attraction shifts in *P*. sp. nr. *pubens* over time. These data demonstrate a significant shift from the 12 wk protein-based food means and all other previous post-treatment protein counts. This suggests a significant increase in protein attraction during the 12 wk count. Also, 12 wk carbohydrate-based food results decreased significantly from the previous post-treatment carbohydrate counts. These results may suggest a transition in the attraction of food resources by *P*. sp. nr. *pubens*, reflecting a change from a more balanced selection of both protein and carbohydrates (found in pre-treatment counts). This *P*. sp. nr. *pubens* food resource attraction transition between 8 and 12 wk means, favors an increased protein-majority. These findings may also suggest, rather than using just one food resource, that use of both food resources is a more accurate indication of *P*. sp. nr. *pubens* activity during the months of July through October. These data may indicate a need for a temporally dynamic protein:carbohydrate bait mixture for control of *P*. sp. nr. *pubens* and to best indicate activity. However, more field and laboratory data will need to be gathered year-round and throughout multiple years to corroborate these results of *P*. sp. nr. *pubens* activity.

Treatments A (Phantom SC and current label Termidor SC), B (Phantom SC and expanded label Termidor SC), and C (Phantom SC, current label Termidor SC, and ACAB) means were significantly different from treatment D (no AI) over all time (Table

8.4). This indicates all AI treatments were efficacious when comparing the no AI treatment D controls. There were no statistical differences among AI treatments over all times. All treatments were statistically equal in effectiveness. Although the differences are not significant, these data appear to indicate treatment B as the treatment of choice (Fig. 8.1).

The statistical analysis using Tukey's HSD may be too stringent regarding mean separation of treatments. Some residents still regarded individual treatments as a failure, despite a lack of overall statistical mean separation among AI treatments (Table 8.4). There may be a few explanations for this. Although *P*. sp. nr. *pubens* were present at or proximal to many of the bait stations, they did not necessarily enter into the baited vials. Perhaps; 1) the ants may have been repelled by the treatments and alternately gathered food sources in non-treated areas; 2) food in treated areas may have become an unneeded resource; or 3) a likelier cause is that AI treatments caused adverse effects regarding their physiology and/or behavior(s) associated with food attraction.

Separating individual post-treatment counts from other post-treatment counts enabled a more precise view of treatment effects over time. This ensures examination of possible time-specific failure (or success) of treatments. By separating individual post-treatment counts from all other post-treatment counts over time, an expectation of identical statistical conclusions should occur between pre- and post-treatment counts, assuming no differences between treatments. This data isolation enables a view of treatments whose pre-treatment means may have influenced the overall statistical conclusions of the experiment. When regarding statistical analysis of treatments by isolating pre-treatment and 12 wk post-treatment data, statistical difference was found between treatment A and treatment D only (Table 8.5). The statistical similarity found between most treatments may indicate a failure of some treatments at 12 wk post-treatment. The near inversion of means resulting from pre-treatment to 12 wk post-treatment counts could suggest a trend of failure for treatments A and C. More data may be needed to corroborate this conclusion.

There were significant differences among mean numbers of *P*. sp. nr. *pubens* over time (Table 8.6). These data indicate significant differences among pre-treatment counts and all post-treatment counts. This indicates efficacy of treatments in causing a decrease in overall population of the treated houses. These data also show a gradual increase of mean *P*. sp. nr. *pubens* during post-treatment counts, which may indicate reduced effectiveness of treatments near the end of the experiment.

Percent reductions of *P*. sp. nr. *pubens* populations after treatment demonstrates a reduction from all AI treatments and an increase in control populations. The only treatment to offer consistent population control over the length of the study was treatment B. Control populations increased over time indicating that July may not be the month of highest *P*. sp. nr. *pubens* populations.

Logically, the farther and longer an individual ant travels through treated areas, the greater the amount of insecticide they will encounter. Dependent upon availability of an AI, insecticide dose-responses have positive correlations. This is not necessarily a desired outcome with social insects where contact, grooming, and trophallaxis can cause death from collective sublethal doses. This delayed toxicity is a positive effect with social insects. There may be a decreased likelihood of delayed toxicity when a greater surface area is treated. Exposure with treatment B (the highest probability for fipronil contact of the treatments) may have resulted in a decreased likelihood to display the desired delayed toxicity (Table 8.4). The greater surface area treated with Termidor SC surrounding the structures may have caused a more rapid morbidity or death to the ants because of increased exposure when compared to other treatments. This may have caused the un-hypothesized statistical similarity between treatments B and A. Despite no significant differences among AI treatments, these data demonstrate trends that indicate treatment B as the treatment of choice (Fig. 8.2). More evaluations should be evaluated to determine if lower (< 0.06%) fipronil concentrations are more effective when using the expanded usage Termidor SC (1 m up, 3 m out).

Further investigations of biological activity of Termidor SC, Phantom SC and ACAB should include post-treatment counts of 6-12 mo. Further investigation of seasonal food resource attraction is needed in both the laboratory and field for more accurate estimation of activity and efficacy of further experimental treatments. The lack of statistical differentiation of treatment C to other AI treatments does not support the label-use of ACAB as a peripheral treatment with Termidor SC and Phantom SC. Additional multiple control strategy experiments against *P.* sp. nr. *pubens* should evaluate the use of ACAB in much greater quantities. Further investigations should also include treatment with only Termidor SC or Phantom SC, respectively.

Exclusion of this pest may need to be integrated into the management strategy because of its associations with homopterans. It has been demonstrated that exclusion of

L. humile from homopteran sources may be an integral part of its population management (Shorey et al. 1996). Although exclusion techniques have been used successfully against *S. incvicta* (Pranschke et al. 2003) and *L. humile* (Rust et al. 1996, Klotz et al. 2003a), field reports from PCOs have indicated repeated and complete failures from using label rate repellents against *P*. sp. nr. *pubens*. These reports have indicated that under ideal conditions repellents have elicited ≤1 wk control of *P*. sp. nr. *pubens*. A more labor-intensive direct spray of all unapparent *P*. sp. nr. *pubens* nesting sites within the yard using Termidor SC should be investigated. This would greatly increase labor and costs associated with the treatment of *P*. sp. nr. *pubens* infested homes and structures; however, it would offer a more distinct zone of treatment. Sanitation was an issue with many residential homes and yards. Residents should be instructed to clean up yard debris, especially in structurally proximal areas.

In spatially acute control areas, such as a residence located in a neighborhood, control difficulties of *P*. sp. nr. *pubens* are compounded. Desired areas of control encounter population influx from surrounding, untreated areas. If a single or biannual visit regimen for pest control operators is not discovered, successful control of *P*. sp. nr. *pubens* will require multiple visits.

A comprehensive control program for *P*. sp. nr. *pubens* should include sanitation, vegetative maintenance, food and water resource prevention with repellants, supplementing with residual sprays and high quantity baiting. Satisfactory *P*. sp. nr. *pubens* control in neighborhoods may require that multiple residences or neighborhood-wide control programs be initiated. The successes and failures of this study demonstrate the difficulties associated with high density *P*. sp. nr. *pubens* infestations. The expanded label usage (1 m up, 3 m out) of Termidor SC demonstrate trends that support its use above the other presented treatments. Additional tactic(s) may need to be applied along side expanded Termidor SC in order to create longer term control of *P*. sp. nr. *pubens* and enhance consumer satisfaction.

CHAPTER IX

EFFECTIVENESS OF TRANSPORT 50 WP, TALSTAR G, AND TOP CHOICE FOR CONTROL OF *Paratrechina* SP. NR. *pubens* (HYMENOPTERA: FORMICIDAE)

Introduction

Given the myriad of current label insecticide products applied and deemed unacceptable for remedial control of *Paratrechina* sp. nr. *pubens* (Meyers et al. unpublished, Meyers and Gold unpublished c, d), other candidate active ingredients (AIs) are currently investigated. These unexamined products include Transport^{TM} 50 WP, Talstar G, and Top Choice^{TM}.

Acetamiprid, (E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine, is a neonicotinoid used as a contact and systemic insecticide (Thomson 2001). Acetamiprid blocks neuron activity causing paralysis (Yamada et al. 1999, Kiriyama et al. 2003). Acetamiprid and other neonicotinoids are very toxic to insects yet very low in mammalian toxicity (Kagabu 1997, Yamamoto et al. 1998). They are used in both agricultural and urban systems. *P*. sp. nr. *pubens* is a recently introduced pest ant in Texas that feeds on honeydew produced by homopteran insects. With the excellent systemic activity of acetamiprid (Takahashi et al. 1999, Wang et al. 2004), it may be a candidate to increase effectiveness of *P*. sp. nr. *pubens* control. This control can occur because of direct population reduction of homopterans and also from honeydew consumption by the ant. Negative effects on beneficial insects are always a concern when using systemic insecticide compounds. The use of acetamiprid in integrated control programs in agriculture systems with beneficial insects has caused significantly less predator mortality than other insecticides (Naranjo and Akey 2005).

Other potential control products may be needed to supplement a particular strategy for adequate control of numerically dense pests, such as *P*. sp. nr. *pubens*. Bifenthrin is a synthetic pyrethroid used primarily as a contact and stomach poison for arthropods (Thomson 2001). Bifenthrin, (2methyl[1,1'-bephenyl]-3-yl)methyl3-(2-chloro-3,3,3-triflouro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate, is a relatively stable compound in soil when applied at termiticidal rates (Baskaran et al. 1999). Bifenthrin has been formulated into a variety of insecticidal and repellant uses including; mosquito nets (Hougard et al. 2002, Chouaibou et al. 2006), exclusion of the red imported fire ant, *Solenopsis invicta* (Pranschke et al. 2003), direct contact and repellency of *S. invicta* (Oi and Williams 1996), and contact efficacy alone (Chen 2006). Successful control of a similar unicolonial ant, the Argentine ant, *Linepithema humile*, has been demonstrated using Talstar® PL granular (Klotz et al. 2007).

Termidor® has been proven to significantly decrease *P*. sp. nr. *pubens* populations for three mo using an expanded label technique (Meyers et al. unpublished). Fipronil is a broad spectrum insecticide utilized with great success. Fipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazol-3-carbonitrile, is a phenylpyrazole, a class of insecticides that act at the γ-aminobutyric acid (GABA)-gated chloride channel (Kidd and James 1991, Cole et al.

1993). Fipronil has been utilized against insects in a number of ways. Successful control has been demonstrated after fipronil soil treatments against a unicolonial tramp ant, the Argentine ant, *Linepithema humile* (Costa and Rust 1998). Significant reductions have been found when using bait integrated with fipronil against *L. humile* and the red imported fire ant, *Solenopsis invicta* (Klotz et al. 2003, Collins and Callcott 1998, respectively). The use of fipronil barrier treatments against *L. humile* have resulted in successful population control (Soeprono and Rust 2004). Spot treatment using fipronil against *L. humile* has also greatly reduced activity (Klotz et al. 2007). The use of Top Choice (fipronil) against *P.* sp. nr. *pubens* has not been investigated, however, some PCOs have claimed successful control relative to other commercial products.

A recent introduction of an exceptionally pestiferous ant, *Paratrechina* sp. nr. *pubens*, in Texas has challenged the effectiveness of typical pest ant management. This pest ant has been a considerable nuisance for residents and businesses due to their remarkable numbers. Since its introduction, *P.* sp. nr. *pubens* has become an immense pest due to its density and ability to cause electrical shortages in a variety of equipment (outlets, sewage lift pump stations, air conditioning units, computers, pool pumps, etc.). Residents of a Pearland, TX neighborhood have reported these annoyances in addition to household incursions by *P.* sp. nr. *pubens*. Reportedly, a number of compounds have killed *P.* sp. nr. *pubens*, however, none have reached customer or pest control operator satisfaction. A population management plan using a variety of tactics should be investigated. Area-wide management plans may need to be implemented to combat the population influx of *P.* sp. nr. *pubens* from non-treated areas.

The synergy of two insecticidal compounds may increase the effectiveness of a control product. In this study, the formulation of acetamiprid and bifenthrin into Transport 50 WP along with Talstar G was evaluated against *P*. sp. nr. *pubens*. There is currently no published research on the use of Transport 50 WP on pest ants. This study intended to evaluate the efficacy of treatments including the commercially available Transport 50 WP (abamectin and bifenthrin) with Talstar G (bifenthrin), and Termidor SC (fipronil) with Top Choice (fipronil) against *P*. sp. nr. *pubens*.

Materials and Methods

Center for Urban and Structural Entomology personnel located structures with active infestations of *Paratrechina* sp. nr. *pubens*. A total of 12 houses were treated in a Pearland, TX neighborhood. Two treatments were used, including Transport 50 WP with Talstar G and Termidor SC with Top Choice G. Six replications per treatment were used for a total of 12 structures. All commercial products were used in a manner consistent with current label instructions. Treatments were conducted mid July 2007 and post-treatment counts were continued until mid-August. Pre and post-treatment counts were done using baited vials (bait stations) at/or near the corners of the perimeter of each structure and/or near active trails. Bait stations included two vials containing hot dog or honey-water soaked cotton, respectively. Glass vials were placed in 2.54 cm diameter white PVC pipe to prevent overheating. Pre-treatment counts were conducted 1-2 d prior to treatment. Post-treatment counts were made on or about 2, 14, and 28 d after application.

Data were then analyzed using SPSS® software (SPSS 2005). Univariate ANOVA was conducted on these data to determine significant differences among treatments. Tukey's HSD was used for means separation of means over time. A paired t-test was conducted for separation of treatments over time. Survival ratios were analyzed by dividing mean counts of all respective post-treatment counts by pre-treatment. This transforms the data for statistical conclusions within the treatments, but not between treatments.

Results

Statistical analyses did not indicate significant differences between the two treatments (t= 3.53, df = 191, $P = 0.001$) (Table 9.1). ANOVA of *P*. sp. nr. *pubens* means per vial over time demonstrate significant differences between counts (treatment 1; $F = 50.74$, df = 3, 191, $P < 0.001$; treatment 2; $F = 26.23$, df = 3, 190, $P < 0.001$) (Table 9.2). Both treatments demonstrated effectiveness at 2 wk post-treatment, but were ineffective at 4 wk post-treatment. Survival ratios of mean number of *P*. sp. nr. *pubens* per vial for pre- and post-treatment counts did not demonstrate a statistical conclusion; however, showed slight differences in survival of the treatments (Table 9.3).

Table 9.1. Mean number of *P*. sp. nr. *pubens* per vial (±SE) by treatment over all time counts.

Treatment	Mean # of *P*. sp. nr. *pubens* per vial (± SE)[a]
Transport/Bifen granules	45.99 (4.54)
Termidor/Top Choice	29.17 (3.73)

[a] $t = 3.53$, df = 191, $P = 0.001$

Table 9.2. ANOVA of mean number of *P*. sp. nr. *pubens* per vial (±SE) over all time.

	Mean (± SE) # of *P*. sp. nr. *pubens* per vial[a]	
Time	Transport/Bifen granules[b]	Termidor/Top Choice[c]
0	98.90 (9.45) a	62.08 (9.14) a
2 d	0.0 (0.0) b	0.0 (0.0) b
2 wk	10.1 (3.20) b	3.15 (1.92) b
4 wk	74.96 (9.25) a	52.51 (8.65) a

[a] Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).

[b] $F = 50.74$, df = 3, 191, $P < 0.001$

[c] $F = 26.23$, df = 3, 190, $P < 0.001$

Table 9.3. Survival ratios of mean number of *P*. sp. nr. *pubens* per vial for each post-treatment count against pre-treatment counts during Transport/Bifen granules and Termidor/Top Choice treatments.

	Survival ratios between time intervals		
Treatment	Time 2 d	Time 2 wk	Time 4 wk
Transport/Bifen granules	0.00	0.10	0.76
Termidor/Top Choice	0.00	0.05	0.83

Discussion

Neither treatment was determined to be successful at 4 wk post-treatment. Granular applications were activated, as sufficient rainfall (0.29 inches on July 12, 2007, Underground Weather) occurred in the evening after all treatments were completed. Previous field observations from both researchers and pest control operators have suggested that there have been temporally varying post-application population influxes despite treatments. The post-treatment count at 4 wk may have been during a population influx that may have eventually been controlled by the treatments. However, this is unlikely as several residents complained the population influx had been occurring for several days prior to the 4 wk count. Due to this observed occurrence, several residents determined the treatments a failure (from field interviews with present residents).

If the study had included control-treatment (no active ingredient) experimental units, the populations at control homes infested with *P*. sp. nr. *pubens* may have increased throughout the time of the study and given a more accurate estimation of treatment success or failure.

The 4 wk post-treatment *P*. sp. nr. *pubens* mean were not significantly different from the pre-treatment counts. These statistical conclusions may be misleading. The use of control replications may have indicated successful treatments despite the lack of statistical disparity in the current study. Control replication populations of *P*. sp. nr. *pubens* may have increased during the experiment, which could have indicated treatment success. The use of control replications would offer a more accurate representation of the reality of temporal population fluxes that are inherent throughout the length of the study. Previous studies have shown (Meyers et al., unpublished) that typical baited vial entrance by *P*. sp. nr. *pubens* is skewed when placed in treated areas. This is likely due to the negative physiologically and/or behavior reactions to AI treatments. However, in urban areas often times “control” homes are impossible due to homeowner concern of safety issues and non-experimentally designed consumer control efforts. These concerns may preclude scientific accuracy in evaluating effectiveness of AIs under “actual” conditions.

Combining other successes from behaviorally similar pest species, *L. humile* may assist population management practices regarding *P*. sp. nr. *pubens*. The use of spot treatments against *L. humile* demonstrated activity reductions (Klotz et al. 2007) and should be investigated against *P*. sp. nr. *pubens*. Although not demonstrated in this study successful population management has occurred against *L. humile* using bifenthrin granules (Klotz et al. 2007). This may indicate that bifenthrin either induces susceptibility in *L. humile*, has ineffective activity against *P.* sp. nr. *pubens*, or an unknown cause occurred creating reduced control of *P.* sp. nr. *pubens* throughout time.

Given the rapid kill demonstrated by bifenthrin (11 to 34 min) (Soeprono and Rust 2004), bifenthrin may act as a repellent as well as causing rapid recognition of mortality within treated areas. Delayed toxicity is a critical factor when using baits against *L. humile* (Rust et al. 2004). The success of Termidor against *P.* sp. nr. *pubens* (Meyers et al. unpublished) may be from the relatively delayed toxicity presented by fipronil (270 to 960 min.) against *L. humile* (Soeprono and Rust 2004b). It has been proposed that fipronil is transferred through trophollaxis (Hooper-Bui and Rust 2000) and through contact alone (Soeprono and Rust 2004a, b). Whether fipronil may or may not be ingested, this delayed toxicity can allow the product to act in similar function as a bait due to the delayed response to treated areas. To the contrary, nonrepellent insecticides (including fipronil) do not fit the liquid bait models (e.g. the Formosan subterranean termite, *Coptotermes formosanus*) (Su 2005). Some pest control operators have suggested that the use of both Termidor and Top Choice, both of which contain fipronil, against *P.* sp. nr. *pubens* is highly effective against *P.* sp. nr. *pubens* in the field compared with other treatment regimes. Therefore, it was surprising that there was complete failure of the treatment. This demonstrates the variability that likely occurs from one application to another and underscores the need for a more consistent delivery mechanism for remedial control efforts against this ant. This may also imply the need for control programs for *P.* sp. nr. *pubens* population management to begin earlier in the season, as there are extremely high numbers during the months of the current study.

The formulation of an insecticide that contains both a slow and quick-acting compound (acetamiprid and bifenthrin, respectively) may not be effective against *P*. sp. nr. *pubens*. The neighborhood presented an ideal environment for pesticide activation (rain) and avoidance of compound degradation (shade). As previously discussed, sufficient rainfall had occurred in order to activate the granular products (Talstar G and Top Choice). Most residences had high amounts of shade from large trees and considerable amounts of vegetation from landscaping and gardens. Evapotranspiration (from urban vegetation) can result in both reduced air temperature and increased humidity (Sailor 1998). These characters likely decreased temperature and increased humidity and moisture. This environment may not be advantageous for all insecticides. These environmental parameters may not be advantageous for environmentally non-persistent insecticides such as neonicotinoids. The results of the study may indicate that these characters are more advantageous for *P*. sp. nr. *pubens* than either treatment.

Results suggest suppression for 2 wk but ultimately no control of populations. A combination of AI tactics are most likely going to be the best chance at elimination on small scales, or when possible, coordinate neighborhood efforts. Considerable research will need to be done regarding the biological activity of many current insecticides and novel chemistries against *P*. sp. nr. *pubens* before ideal treatments and subsequent management plans are discovered. The relative unsuccessful treatments presented in the current study underscore the difficulties of finding effective treatments. Further studies should be employed that consider early season treatment of *P*. sp. nr. *pubens* when populations are smaller and more manageable. An integrated urban pest management plan for *P*. sp. nr. *pubens* is needed. This plan will need to consider a temporally comprehensive treatment plan that includes sanitation, vegetation maintenance and multiple insecticide tactics.

CHAPTER X

CONCLUSIONS

Morphometric and phylogenetic analyses of *Paratrechina* sp. nr. *pubens* revealed inconclusive results regarding its identification. More comprehensive sampling and/or diagnostic evidence is needed to discover the taxonomic position of *P*. sp. nr. *pubens*. Therefore, the taxonomic identity of *P*. sp. nr. *pubens* populations in Texas will remain unchanged. The phylogenetic study of *P*. sp. nr. *pubens* and other *P*. spp. is the first significant endeavor describing the phylogenetic relationships among several *Paratrechina* species. The study found misidentifications of *Paratrechina* samples. The conclusions also may reflect paleogeographic events within the complex Fulva. The results may suggest that the three clades presented as one species, *P*. *pubens*. This procedure may also offer proof that an undescribed species may cause deleterious ecological and economical effects to Texas and St. Croix. More research regarding behavior, mating compatibility, phylogeny, or other analyses of these populations should be conducted before raising the *P*. sp. nr. *pubens* Texas populations to the status of an undescribed species.

As this dissertation was being concluded, preliminary conclusions from a concurrent *Paratrechina* spp. phylogenetic study, conducted in collaboration with the author, was received. These analyses cladistically identified the *P*. sp. nr. *pubens* populations investigated herein, as *P*. *pubens*. These findings corroborated the current conclusion presented herein, that a more comprehensive sampling of *P. pubens* and near *pubens* was needed to positively identify this pest species. These populations of *P.* sp. nr. *pubens* and *P. pubens* constitute a monophyletic invasive species that remains irrelevant to the overwhelming demand to more carefully evaluate this introduction event and subsequent spread. This species is a very important invasive pest and the future authoritative identification of these populations is imperative for possible remedial control scenarios.

Given its establishment in South Texas and the synanthropic behavior of *P.* sp. nr. *pubens*, continued introductions are unavoidable without local, state and/or federal expansion-preventing measures in place. Should this ant spread to further locations outside the current area of infestation, it may become a regionally exotic species with remarkably harmful consequences.

The relative success of the laboratory study on dinotefuran against *P.* sp. nr. *pubens* warrants further evaluations and initial field effectiveness investigations. These findings may assist pest control operators during their efforts to control the numerically superior pest.

The failure of the IGR, novaluron, in a laboratory study against *P.* sp. nr. *pubens* underscores the difficulties of maintaining relatively small, queenless colonies of *P.* sp. nr. *pubens* in the insectary. Although it is not known whether the lack of queens adversely affected the outcome of the study, it could be one of the contributing factors.

P. sp. nr. *pubens* are considerably attracted to the Advance Carpenter Ant Bait (ACAB) matrix in the laboratory and field. It is therefore recommended that ACAB with novaluron be tested against large laboratory colonies (with a full compliment of castes) or in the field. Field observations suggest an immense increase in numbers of *P*. sp. nr. *pubens* brood and worker members during early spring. During this period, foraging for food sources high in protein is needed for brood production. ACAB contains a crustacean lipid based attractant. Therefore, this product may be a viable option as part of a temporally dynamic control program against *P*. sp. nr. *pubens*.

The study of ACAB amended with dinotefuran against *P*. sp. nr. *pubens* represents the only field effectiveness evaluation against insects using ACAB with dinotefuran to date. This field experiment demonstrated initial population reduction of overwhelming numbers of *P*. sp. nr. *pubens*. The lack of consistent temporal *P*. sp. nr. *pubens* control indicates the need for larger concentrations and/or rates of ACAB with dinotefuran. Additional temporal applications of ACAB with dinotefuran against *P*. sp. nr. *pubens* should be evaluated. Also, an integration of an ACAB with dinotefuran treatment into a temporally comprehensive control strategy should be investigated. The likely application of ACAB with dinotefuran may be more effective within a comprehensive control plan for the numerically superior *P*. sp. nr. *pubens*.

The use of current and expanded label insecticide usage against *P*. sp. nr. *pubens* revealed a variety of conclusions. A temporally comprehensive control program for *P*. sp. nr. *pubens* should include sanitation, vegetative maintenance, food and water resource prevention with repellants, supplementing with residual sprays and high quantity baiting. Satisfactory *P*. sp. nr. *pubens* control in neighborhoods may require that multiple residence or neighborhood-wide control programs be initiated. The successes and failures of this study demonstrate the difficulties associated with high density *P*. sp. nr. *pubens* infestations. The expanded label usage (1 m up, 3 m out) of Termidor SC demonstrate trends that support its use above the other presented treatments. Additional tactic(s) may need to be applied along side expanded Termidor SC in order to create longer term control of *P*. sp. nr. *pubens*.

The formulation of an insecticide that contains both a slow and quick-acting compound (acetamiprid and bifenthrin, respectively) may not be efficacious against *P*. sp. nr. *pubens*. Results suggest suppression for 2 wk, but ultimately no control of populations through time. A combination of pesticide tactics are most likely going to be the best chance at elimination on small scales, or when possible coordinate neighborhood efforts. The Pearland, TX neighborhood represented an ideal environment for pesticide activation (rain) and avoidance of compound degradation (shade). This environment may not be advantageous for all insecticides. These environmental parameters may not be advantageous for environmentally non-persistent insecticides such as neonicotinoids. The results of the study may indicate that these characters are more advantageous for *P*. sp. nr. *pubens* than either treatment.

Considerable research will need to be done regarding the biological activity of many current insecticides and novel chemistries against *P*. sp. nr. *pubens* before ideal treatments and subsequent management plans are discovered. The relative unsuccessful treatments presented in the current study underscore the difficulties of finding efficacious treatments. Further studies should be employed that consider early season treatment of *P*. sp. nr. *pubens* when populations are smaller and more manageable. An integrated urban pest management plan for *P*. sp. nr. *pubens* is needed. This plan will need to consider a temporally comprehensive treatment plan that includes sanitation, vegetation maintenance and multiple insecticide tactics.

The biological and temporal caveats associated with successful invasive populations of social arthropods are quite complex. It is a rare scientific opportunity to follow the incipient biology of an unexpected, invasive, and dominant pest. Information and conclusions gained from these and future studies on *P*. sp. nr. *pubens* populations of Texas may assist research of other impending arthropod invaders, especially social insects.

REFERENCES CITED

Abbott, K.L. 2006. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Diversity Distributions. 12(1): 101-110.

Abbott, K.L. and P.T. Green. 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. Oikos. 116: 1238-1246.

Akçakaya, H.R. 2001. Linking population-level risk assessment with landscape and habitat models. Sci. Total Environment. 274: 283-291.

Allen, C.R., R.S. Lutz, and S. Demaris. 1995. Red imported fire ant impacts on northern bobwhite populations. Ecol. Appl. 5: 632-638.

Ameen, A., W. Kaakeh, and G. Bennett. 2000. Integration of chlorfenapyr into a management program for the German cockroach (Dictyoptera: Blattellidae). J. Agric. Urban Entomol. 17(3): 135-142.

Appel, A.G., M.J. Eva, and S.R. Sims. 2005. Toxicity of granular ant bait formulations against cockroaches (Dictyoptera: Blattellidae and Blattidae). Socio. 46(1): 65-72.

Arthur, F.H. 2008. Efficacy of chlorfenapyr against *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) adults exposed on concrete, vinyl tile, and plywood surfaces. J. Stored Products Res. 44(2): 145-151.

Austin, J.W., G.J. Glenn, A.L. Szalanski, J.A. McKern, and R.E. Gold. 2007. Confirmation of Asian cockroach *Blatella asahinai* (Blattodea: Blattelidae) introduction to Texas based on genetic, morphology, and behavior. Flor. Entomol. 90(3): 574-576.

Banks, W.A., B.M. Glancey, C.E. Stringer, D.P. Jouvenaz, C.S. Lofgren, and D.E. Weidhaas. 1973. Imported fire ants: Eradication trials with mirex bait. J. Econ. Entomol. 66: 785-789.

Banks, W.A., L.R. Miles, and D.P. Harlan. 1983. The effects of insect growth regulators and their potential as control agents for imported fire ants (Hymenoptera: Formicidae). Florida Entomologist. 66(1): 172-181.

Baskaran, S., R.S. Kookana, and R. Naidu. 1999. Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. Pesticide Sci. 55(12): 1222-1228.

Bolton, B., G. Alpert, P.S. Ward, and P. Naskrecki. 2006. Bolton's Catalogue of Ants of the World: 1758-2005. Harvard University Press, Cambridge, MA.

Bond, W.J. and P. Slingsby. 1984. Collapse of an ant-plant mutualism: the argentine ant (*Iridomyrmex humilis*) and myrmecochorous Proteaceae. Ecology 65: 1031-1037.

Buczkowski, G., M.E. Scharf, C.R. Ratliff, and G.W. Bennett. 2005. Efficacy of simulated barrier treatments against laboratory colonies of pharaoh ant. J. Econ. Entomol. 98(2): 485-492.

Buys, B. 1987. Competition for nectar between Argentine ants (*Iridomyrmex humilis*) and honeybees (*Apis mellifera*) on black ironbark (*Eucalyptus sideroxylon*). South African J. Zoo. 22: 173-174.

Byers, J.E., S. Reichard, J.M. Randall, I. M. Parker, C.S. Smith, W.M. Lonsdale, I.A.E. Atkinson, T.R. Seastedt, M. Williamson, E. Chronesky, and D. Hayes. 2002. Directing research to reduce the impacts of nonindigenous species. Cons. Bio. 16: 630-640.

Cabrera, A.R., R.A. Cloyd, and E.R. Zaborski. 2005. Lethal and sub-lethal effects of novaluron (Pedestal®) on the soil-dwelling predatory mite, *Stratiolaelaps scimitus* (Womersley) (Acari: Mesostigmata: Laelapidae), under laboratory conditions. J. Entomological Sci. 40(1): 47-53.

Chapman, R.F. and F.G. Bourke. 2001. The influence of sociality on the conservation biology of social insects. Ecology Letters. 4: 650-662.

Chaton, P.F., P. Ravanel, M. Tissut, and J.C. Meyran. 2002. Toxicity and bioaccumulation of fipronil in the nontarget arthropodan fauna associated with subalpine mosquito breeding sites. Ecotoxicology Environmental Safety. 52(1): 8-12.

Chen, J. 2006. Digging behavior of *Solenopsis invicta* workers when exposed to contact insecticides. J. Econ. Entomol. 99(3): 634-640.

Chouaibou, M., F. Simard, F. Chandre, J. Etang, F. Darriet, and J.-M. Hougard. 2006. Efficacy of bifenthrin-impregnated bednets against *Anopheles funestus* and pyrethroid-resistant *Anopheles gambiae* in North Cameroon. Malaria J. 5:77.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657-1659.

Cole, F.R., A.C. Medeiros, L.L. Loope, and W.W. Zuehlke. 1992. Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. Ecology. 73: 1313-1322.

Cole, L.M., R.A. Nicholson, and J.E. Casida. 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pesticide Biochemistry Physiology. 46(1): 47-54.

Collins, H.L. and A.M.A. Callcott. 1998. Fipronil: an ultra-low-dose bait toxicant for control of red imported fire ants (Hymenoptera: Formicidae). Florida Entomol. 81(3): 407-415.

Connelly, P. 2001. Environmental fate of fipronil. Dept. Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA.

Corbel, V., S. Duchon, M. Zaim, and J.-M. Hougard. 2004. Dinotefuran: a potential neonicotinoid insecticide against resistant mosquitoes. J. Med. Entomol. 41(4): 712-717.

Costa, H.S. and M.K. Rust. 1998. Mortality and foraging rates of Argentine ants (Hymenoptera: Formicidae) colonies exposed to potted plants treated with fipronil. J. Agric. Urban Entomol. 16(1): 37-48.

Creighton, W.S. 1950. Ants of North America. Bull. Mus. Comp. Zool. 104: 402-410.

Davis, A.M. and T.F. Glick. 1978. Urban ecosystems and island biogeography. Environmental Cons. 5: 299-304.

De Kock, A.E. 1990. Interactions between the introduced Argentine ant, *Iridomyrmex humilis* Mayr, and two indigenous fynbos ant species. J. Entomol. Soc. Southern Africa. 53: 107-108.

Doherty, W.M., N.P. Stewart, R.M. Cobb, and P.J. Keiran. 1994. In-vitro comparison of the larvicidal activity of moxidectin and abamectin against *Onthophagus gazella* (F.) (Coleoptera: Scarabaeidae) and *Haematobia irritans exigua* De Meijere (Diptera: Muscidae). Austral. J. Entomol. 33(1): 71-74.

Dorward, K. 1956. Spread and new finds of some insects in the United States in 1956. Agr. Chem. 11: 57.

Drees, B.M. 1994. Red imported fire ant predation on nestlings of colonial waterbirds. Southwestern Entomol. 19: 355-359.

Drummond, A. J., and A. Rambaut. 2003. BEAST v1.0 http://evolve.zoo.ox.ac.uk/beast/.

Elbert, A., R. Nauen, and W. Leicht. 1998. Imidacloprid, a novel chloronicotynyl insecticide: biological activity and agricultural importance, pp. 50-73. *In* I. Ishaaya and D. Degheele [eds.], Insecticides with novel modes of action: mechanism and application. Springer, Berlin, Germany.

(EPA) Environmental Protection Agency. 2004. Dinotefuran. Pesticide Fact Sheet. Washington, D.C.

Erickson, J.M. 1971. The displacement of native ant species by the introduced Argentine ant *Iridomyrmex humilis* Mayr. Psyche. 78: 257-266.

Fellowes, J.R. 1999. Exotic ants in Asia: is the mainland at risk? The case of Hong Kong. Aliens. 9: 5-6.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Fluker, S.S. and J.W. Beardsley. 1970. Sympatric associations of three ants: *Iridomyrmex humilis*, *Pheidole megacephala* and *Anoplolepis longipes* in Hawaii. Ann. Ent. Soc. Amer. 63: 1290-1296.

Folgarait, P.J. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodiversity Cons. 7: 1221-1244.

Forel, A. 1893. Formicides de l'Antille St. Vincent, recoltees par Mons. H.H. Smith. Trans. Ent. Soc. London.

Freitag, A., K. Dorn, and D. Cherix. 2000. First occurrence of the crazy ant *Paratrechina longicornis* (Latreille) (Hym. Formicidae: Formicinae) in Switzerland. Bull. de la Societe Entomologique Suisse. 73: 301-303.

Giliomee, J.H. 1986. Seed dispersal by ants in the cape flora threatened by *Iridomyrmex humilis* (Hymenoptera: Formicidae). Entomologia Generalis. 11: 217-219.

Gilpin, M. 1990. Ecological prediction. Sci. 248: 88-89.

Guglielmone, A.A., M.M. Volpogni, O.S. Anziani, and S.G. Flores. 1999. Evaluation of injectable abamectin to control natural infestations of *Haematobia irritans* (Diptera: Muscidae) in cattle. J. Med. Entomol. 36(3): 325-328.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95-98.

Hallman, G.J. and C.P. Schwalbe. 2002. Invasive arthropods in agriculture: problems and solutions. Science Publishers, Inc., Enfield, NH.

Harris, R.J. 2002. Potential impact of the Argentine ant (*Linepithema humile*) in New Zealand and options for its control. Sci. Cons. 196. 36 pp.

Hedges, S.A. 1998. Field guide for the management of structure-infesting ants. G.I.E. Media Inc., Cleveland, OH.

Heintschel, B. P., J. W. Austin and R. E. Gold. 2006. Soldier labral morphology and genetic comparison of *Reticulitermes* (Isoptera: Rhinotermitidae) from Texas. Sociobiology. 48(1): 63-84.

Hölldobler, B. and E.O. Wilson. 1977. The number of queens: an important trait in ant evolution. Die Naturwissenschaften. 64: 8-15.

_____. 1990. The ants. Belknap Press of Harvard Univ. Press, Cambridge.

Holway, D.A. 1998a. Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. Oecologia. 116: 252-258.

_____. 1998b. Factors governing rate of invasion: a natural experiment using Argentine ants. Oecologia. 115: 206-212.

_____. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology 80: 238-251.

Holway, D.A., L. Lach, A. Suarez, N. Tsutsui, and T.J. Case. 2002. The causes and consequences of ant invasions. Annu. Rev. Ecol. Syst. 33: 181-233.

Hooper-Bui, L.M. and M.K. Rust. 2000. Oral toxicity of abamectin, boric acid, fipronil, and hyrdramethylnon to laboratory colonies of Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 93(3): 858-864.

Hostettler, N.C., D.W. Hall, and R.H. Scheffrahn. 1995. Intracolony morphometric variation and labral shape in Florida *Reticulitermes* (Isoptera: Rhinotermitidae) soldiers: significance for identification. Florida Entomol. 78(1): 119-129.

Hougard, J.-M., S. Duchon, M. Zaim, and P. Guillet. 2002. Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. J. Med. Entomol. 39(3): 526-533.

Howarth, F.G. 1985. Impacts of alien land arthropods and mollusks on native plants and animals in Hawaii pp. 149-173. *In* Hawaii's terrestrial ecosystems: preservation and management C.P. Stone and J.M. Scott [eds.]. University of Hawaii press, Honolulu.

Human, K.G., and D.M. Gordon. 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. Oecologia 105: 405-412.

_____. 1997. Effects of Argentine ants on invertebrate biodiversity in Northern California. Cons. Bio. 11: 1242-1248.

Ibrahim, S.A., G. Henderson, and H. Fei. 2003. Toxicity, repellency, and horizontal transmission of fipronil in the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 96(2): 461-467.

Ishaaya, I., S. Kontsedalov, and A.R. Horowitz. 2003. Novaluron (Rimon), a novel IGR: potency and cross-resistance. Archives Insect Biochem. Physiology. 54: 157-164.

Jousson, O., J. Pawlowski, L. Zaninetti, A. Meinesz, and C.F. Boudouresque. 1998. Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. Marine Ecol. Progress Series. 172: 275-280.

Kabashima, J.N., L. Greenberg, M.K. Rust, and T.D. Paine. 2007. Aggressive interaction between *Solenopsis invicta* and *Linepithema humile* (Hymenoptera: Formicidae) under laboratory conditions. J. Econ. Entomol. 100(1): 148-154.

Kagabu, S. 1997. Chloronicotynyl insecticides: Discovery, application and future perspective. Rev. Toxicol. 1(7-8): 75-129.

Keane, R.M. and M.J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. TRENDS Ecol. & Evol. 17(4): 164-170.

Kidd, H. and D. James [eds.]. 1991. The agrochemicals handbook, 3rd Ed. Royal Society of Chemistry Information Services, Cambridge, UK.

Kiriyama, K. and K. Nishimura. 2002. Structural effects of dinotefuran and analogues in insecticidal and neural activities. Pest Manag. Sci. 58: 669-676.

Kiriyama, K., H. Nishiwaki, Y. Nakagawa, and K. Nishimura. 2003. Insecticidal activity and nicotinic acetylcholine receptor binding of dinotefuran and its analogues in the housefly, *Musca domestica*. Pest Manag. Sci. 59: 1093-1100.

Klotz, J.H., M.K. Rust, H.S. Costa, D.A. Reierson, and K. Kido. 2003a. Strategies for controlling Argentine ants (Hymenoptera: Formicidae) with sprays and baits. J. Agric. Urban Entomol. 19(2): 85-94.

Klotz, J.H., M.K. Rust, D. Gonzalez, L. Greenberg, H. Costa, P. Phillips, C. Gispert, D.A. Reierson, and K. Kido. 2003b. Directed sprays and liquid baits to manage ants in vineyards and citrus groves. J. Agric. Urban Entomol. 20(1): 31-40.

Klotz, J.H., M.K. Rust, L. Greenberg, H.C. Field, and K. Kupfer. 2007. An evaluation of several urban pest management strategies to control Argentine ants (Hymenoptera: Formicidae). Socio. 50(2) 391-398.

Kolar, C.S. and D.M. Lodge. 2001. Progress in invasion biology: predicting invaders. TRENDS Ecol. Evol. 16(4): 199-204.

Kopanic, R.J. and C. Schal. 1999. Coprophagy facilitates horizontal transmission of bait among cockroaches (Dictyoptera: Blattellidae). J. Environ. Entomol. 28(3): 431-438.

Kruchelnycky, P.D., L.L. Loope, and S.M. Joe. 2004. Limiting spread of a unicolonial invasive insect and characterization of seasonal patterns of range expansion. Biological Invasions. 6: 47-57.

Lasota, J.A. and R.A. Dybas. 1991. Avermectins, a novel class of compounds: implications for use in arthropod pest control. Annu. Rev. Entomol. 36: 91-117.

Leung, B., D.M. Lodge, D. Finnoff, J.F. Shogren, M.A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proc. R. Soc. Lond. 269: 2407-2413.

Lockley, T.C. 1995. Effect of imported fire ant predation on a population of the least tern – an endangered species. Southwest Entomol. 20: 517-519.

Lopez, R., D.W. Held, and D.A. Potter. 2000. Management of a mound-building ant, *Lasius neoniger* Emery, on golf putting greens and tees using delayed-action baits or fipronil. Crop Sci. 40: 511-517.

MacArthur, R.H. and E.O. Wilson. 1963. An equilibrium theory of insular zoogeography. Evolution. 17: 373-387.

_____. 1967. Theory of island biogeography. Princeton, NJ, Princeton University.

Macom, T.E. and S.D. Porter. 1996. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. Ann. Entomol. Soc. Am. 89: 535-543.

Mayr, G. 1862. Myrmecologische Studien. Verh. K-K. Zool.-Bot. Ges. Wien 12: 649-776.

McGlynn, T.P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. J. Biogeography. 26: 535-548.

McKinney, M.L. and J.L. Lockwood. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. TRENDS in Ecol. & Evol. 14(11): 450-453.

Meyers, J.M. and R.E. Gold. Unpublished a. Molecular phylogeography of an invasive *Paratrechina* sp. and other *Paratrechina* spp. from various countries.

_____. Unpublished b. Distribution and spread of an exotic ant, *Paratrechina* sp. nr. *pubens*, in Texas.

_____. Unpublished c. Morphometric assessment of *Paratrechina* sp. nr. *pubens* (Hymenoptera: Formicidae) populations in Texas.

_____. Unpublished d. Laboratory evaluation of dinotefuran in liquid ant bait against *Paratrechina* sp. nr. *pubens* (Hymenoptera: Formicidae).

Meyers, J.M., R. Davis, and R.E. Gold. Unpublished. Field efficacy of current and expanded label treatments against an invasive ant pest, *Paratrechina* sp. nr. *pubens* (Hymenoptera: Formicidae), of Texas.

Mitchell, C.E. and A.G. Power. 2003. Release of invasive plants from fungal and viral pathogens. Nature. 421: 625-627.

Miyagi, S., I. Komaki, and Y. Ozoe. 2006. Identification of a high-affinity binding site for dinotefuran in the nerve cord of the American cockroach. Pest Manag. Sci. 62: 293-298.

Moller, H. 1996. Lessons for invasion theory from social insects. Biological Cons. 78: 125-142.

Moller, H., C.R. Townsend, J.R. Ragg, and P. Bannister. 1993. Invasion and environmental impacts of New Organisms; can they be predicted? Research Report. Ministry of Environment, New Zealand. 11.3.2.

Mori, K., T. Okumoto, N. Kawahara, and Y. Ozoe. 2001. Interaction of dinotefuran and its analogues with nicotinic acetylcholine receptors of cockroach nerve cords. Pest Manag. Sci. 58: 190-196.

Morrison, L.W. 1998. The spatiotemporal dynamics of insular ant metapopulations. Ecol. 79(4): 1135-1146.

Naranjo, S.E. and D.H. Akey. 2005. Conservation of natural enemies in cotton: comparative selectivity of acetamiprid in the management of *Bemisia tabaci*. Pest Manag. Sci. 61(6): 555-566.

Niemelä, J. 1999. Ecology and urban planning. Biodiversity Cons. 8: 119-131.

Oi, D.H. and D.F. Williams. 1996. Toxicity and repellency of potting soil treated with bifenthrin and tefluthrin to red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 89(6): 1526-1530.

Pasfield, G. 1968. Argentine ants. Aust. Natur. Hist. 16: 12-15.

Passera, L. 1994. Characteristics of tramp species. *In* D.F. Williams [ed.], Exotic ants: biology, impact and control of introduced species. Westview Press, Boulder, CO.

Pimental, D. 1995. Amounts of pesticides reaching target pests: environmental impacts and ethics. J. Agri. Environ. Ethics. 8(1): 17-29.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience. 50(1): 53-65.

Pimm, S.L. 1989. Theories of predicting success and impact of introduced species pp. 351-367. *In* Biological Invasions: a Global Perspective. Drake, J.A., et al. [eds.]. John Wiley & Sons, Chichester, England.

Porter, S.D. and D.A. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology. 71(6): 2095-2106.

Posada, D., and K. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Pranschke, A.M., L.M. Hooper-Bùi, and B. Moser. 2003. Efficacy of Bifenthrin treatment zones against red imported fire ant. J. Econ. Entomol. 96(1): 98-105.

Rae, D.J., D.M. Watson, W.G. Liang, B.L. Tan, M. Li, M.D. Huang, Y. Ding, J.J. Xiong, D.P. Du, and J. Tang. 1996. Comparison of petroleum spray oils, abamectin, cartap, and methomyl for control of citrus leafminer (Lepidoptera: Gracillariidae) in southern China. J. Econ. Entomol. 89(2): 493-500.

Ramakrishnan, P.S. and P.M. Vitousek. 1989. Ecosystem-level processes and the consequences of biological invasions. pp. 281-300. *In* Biological Invasions: a Global Perspective. J.A. Drake et al. [eds.] Wiley, New York.

Reichard, S.H. 1997. Prevention of invasive plant introductions on national and local levels. pp. 215-227. In Assessment and Management of Plant Invasions. Luken, J. and J. Thieret [eds.], Springer-Verlag, New York.

Rheindt, F.E. 2003. Mating Frequency and Caste Determination in *Pogonomyrmex badius* (Formicidae: Hymenoptera). Diploma Thesis, University of Würzburg (Germany), 77 pp.

Ricciardi, A. 2003. Predicting the impacts of an introduced species from its invasion history: an empirical approach applied to zebra mussel invasions. Freshwater Bio. 48: 972-981.

Ring, D.R., A.L. Morgan, and W.D. Woodson. 2001. The first two years of an area wide management programs for the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the French Quarter, New Orleans, Louisiana. Sociobiology. 37: 293-300.

Rust, M.K., K. Haagsma, and D.A. Reirson. 1996. Barrier sprays to control Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 89: 134-137.

Rust, M.K., D.A. Reierson, and J.H. Klotz. 2004. Delayed toxicity as a critical factor in the efficacy of aqueous baits for controlling Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97(3): 1017-1024.

Rust, M.K. and R.K. Saran. 2006. Toxicity, repellency, and transfer of chlorfenapyr against western subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 99(3): 864-872.

Sailor, D.J. 1998. Simulations of annual degree day impacts of urban vegetative augmentation. Atmospheric Environ. 32(1): 43-52.

Sanchez, A.C. 2005. Diversity, abundance, seasonality and interactions of ants (Hymenoptera: Formicidae) in pecans in Mumford, Robertson Co., Texas. M.S. Thesis, Texas A&M University.

Sarty, M., K.L. Abbott, and P.J. Lester. 2007. Community level impacts of an ant invader and food mediated coexistence. Insect Soc. 54: 166-173.

Shetlar, D.J. 2003. Control of the turfgrass ant, *Lasius neoniger*, in Ohio. Golf Course Management. 71 (2): 117-120.

Shorey, H.H., L.K. Gaston, R.G. Gerber, C.B. Sisk, and D.L. Wood. 1996. Formulating farnesol and other ant-repellent semiochemicals for exclusion of Argentine ants (Hymenoptera: Formicidae) from citrus trees. Environ. Entomol. 25:114-19.

Silverman, J. and R.J. Brightwell. 2008. The Argentine ant: challenges in managing an invasive unicolonial pest. Annu. Rev. Entomol. 53: 231-252.

Simberloff, D. 1981. Community effects of introduced species. pp. 53-80. *In* Biotic crises in ecological and evolutionary time. M.H. Nitecki [ed.]. Academic Press, New York.

_____. 1986. Introduced insects: a biogeographic and systematic perspective. *In* Mooney, H.A., and J.A. Drake [eds.], Ecology of biological invasions of North America and Hawaii. Springer-Verlag, New York.

_____. 1989. Which insect introductions succeed and which fail? *In* Drake, J.A., et al. [Eds.], Biological invasions: a global perspective. John Wiley & Sons, New York.

_____. 1992. Conservation of pristine habitats and unintended effects of biological control. pp. 103-117. *In* Selection criteria and ecological consequences of importing natural enemies. W.C. Kauffman and J.E. Nechols [eds.]. Entomological Society of America, Lanham, Maryland.

_____. 2003. How much information on population biology is needed to manage introduced species? Conservation Biology. 17(1): 83-92.

Simberloff, D. and P. Stiling. 1996. Risks of species introduced for biological control. Biological Cons. 78: 185-192.

Simberloff, D., I.M. Parker, and P.N. Windle. 2005. Introduced species policy, management, and future research needs. Front. Ecol. Environ. 3(1): 12-20.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu & P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Amer. 87: 651-701.

Smith, M.A., B.L. Fisher, and P.D.N. Hebert. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Phil. Trans. R. Soc. B. 360: 1825-1834.

Soeprono, A.M. and M.K. Rust. 2004a. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 1675-1681.

_____. 2004b. Effect of delayed toxicity of chemical barriers to control Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97(6) 2021-2028.

SPSS Inc. 2005. SPSS 14.0 for Windows. SPSS Science Marketing Dept., Chicago, IL.

Stanley, M.C. 2004. Review of the efficacy of baits used for ant control and eradication. Landcare Research Contract Report: LC0405/044. Aukland, New Zealand.

Steiner, F.M., B.C. Schlick-Steiner, and K. Moder. 2006. Morphology-based cyber identification engine to identify ants of the *Tetramorium caespitum*/*impurum* complex (Hymenoptera: Formicidae). Myrmecologische Nachrichten. 8: 175-180.

Stimac, J. L. and S. B. Alves. 1994. Ecology and biological control of fire ants, pp. 353-380. *In* D. Rosen, F. D. Bennet, and J. L. Capinera [eds.]. Pest management in the subtropics: biological control, a Florida perspective. Intercept Limited, Andover, UK.

Strehel, C.-P. and J. Gadau. 2004. Cladistic analysis of paleo-island populations of the Florida harvester ant (Hymenoptera: Formicidae) based upon divergence of mitochondrial DNA sequences. Flor. Entomol. 87(4): 576-581.

Suarez, A.V., J.Q. Richmond, and T.J. Case. 2000. Prey selection in horned lizards following the invasion of Argentine ants in southern California. Ecological Applications. 10: 711-725.

Su, N.-Y. 2005. Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. J. Econ. Entomol. 2143-2152.

Su, T., M.S. Mulla, and M. Zaim. 2003. Evaluations of novaluron, a new insect growth regulator (IGR), against *Culex* mosquitoes. J. Amer. Mosquito Control Assoc. 19(4): 408-418.

Swofford, D.L. 2001. PAUP*: phylogenetic analysis using parsimony (*and other methods), ver. 4.0b10. Sinauer, Sunderland, MA.

Szalanski, A.L., D.S. Sikes, R. Bischof, and M. Fritz. 2000. Population genetics and phylogenetics of the endangered American burying beetle, *Nicorphorus americanus* (Coleoptera: Silphidae). Ann. Entomol. Soc. Am. 93: 589-594.

Takahashi, H., J. Mitsui, M. Yano, T. Take, and T. Yamada. 1999. Efficacy of acetamiprid 2% granule against diamondback moth on cabbage by various application methods. Nippon Noyaku Gakkaishi. 24(1): 23-27.

Thomas, C.D., H. Moller, R.J. Toft, J.A.V. Tilley, and R. Harris. 1989. The impact of *Vespula* wasps on native insects and birds: second year research report. DSIR Ecol. Div. Rep., No. 25. Aukland, New Zealand.

Thomson, W.T. [ed.]. 2001. Agricultural chemicals, book 1, insecticides. Thomson Publications, Fresno, CA.

Trager, J.C. 1984a. A revision of the genus *Paratrechina* (Hymenoptera: Formicidae) of the continental United States. Sociobiology. 9(2): 51-162.

Trager, J.C. 1984b. A revision of the genus *Paratrechina* (Hymenoptera: Formicidae) of the continental United States. Ph.D. Dissertation, University of Florida.

Tsutsui, N.D., A.V. Suarez, D.A. Holway, and T.J. Case. 2000. Reduced genetic variation and the success of an invasive species. PNAS. 97(11): 5948-5953.

Tomizawa, M. and I. Yamamoto. 1993. Structure-activity relationships of nicotinoids and imidacloprid analogs. Nihon Noyaku Gakkaishi. J. Pestici. Sci. 18: 91-98.

(UCS) Union of Concerned Scientists. 2001. The science of invasive species: an information update by the Union of Concerned Scientists. Union of Concerned Scientists, Cambridge, MA, available at: www.ucsusa.org/assets/documents/invasive_species/UCS_SOIS_-_General_1.pdf pp. 12. Accessed: May 27, 2008.

(USDA APHIS) U.S. Department of Agriculture Animal and Plant Health Inspection Service. 2004. Weed-initiated pest risk assessment: guidelines for qualitative assessments. http:// www.aphis.usda.gov/ppq/weeds/weedsrisk99.html. Accessed: May 27, 2008.

Vander Meer, R.K., K. Jaffe, and A. Cedeno (eds.). 1990. Applied myrmecology: a world perspective. Westview Press, Boulder, CO.

Vega, S.Y. and M.K. Rust. 2003. Determining the foraging range and origin of resurgence after treatment of Argentine ant (Hymenoptera: Formicidae) in urban areas. J. Econ. Entomol. 96(3): 844-849.

Vinson, B.S., (ed.) 1986. Economic impact and control of social insects. Praeger, New York.

Visser, D., M.G. Wright, and J.H. Gliomee. 1996. The effect of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), on flower-visiting insects of *Protea nitida* Mill (Proteaceae). Afr. Entomol. 4: 285-287.

Wakita, T., K. Kinoshita, E. Yamada, N. Yasui, N. Kawahara, A. Naoi, M. Nakaya, K. Ebihara, H. Matsuno, and K. Kodaka. 2003. The discovery of dinotefuran: a novel neonicotinoid. Pest Manag. Sci. 59: 1016-1022.

Wang, X.-H. and P.J. Lester. 2004. A preliminary study of the usefulness of morphometric tools for splitting the *Monomorium antarcticum* (Smith) complex (Hymenoptera: Formicidae), New Zealand's most common native ants. New Zealand Entomol. 27: 103-108.

Wang, B., V.C. Mastro, R. Gao, and R.C. Reardon. 2004. Update on the efficacy of selected systemic insecticides for the control of the Asian longhorned beetle. Proc., XV USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species. GTR-NE-332, pp. 81-82.

Ward, P.S. 1987. Distribution of the introduced Argentine ant (*Iridomyrmex humilis*) in natural habitats of the lower Sacramento valley and its effects on the indigenous ant fauna. Hilgardia. 55: 1-16.

Warner, J. and R.H. Scheffrahn. 2004. Caribbean crazy ant, *Paratrechina pubens* Forel. UF/IFAS Featured Creatures. EENY – 284. Available at: http://creatures.ifas.ufl.edu/urban/ants/craibbean.crazy.ant.htm

Way, M.J., M.E. Cammell, and M.R. Paiva. 1992. Studies on egg predation by ants (Hymenoptera: Formicidae) especially on the eucalyptus borer *Phoracantha semipunctata* (Coleoptera: Cerambycidae) in Portugal. Bulletin Entomol. Res. 82: 425-432.

Wetterer, J.K. 2006. The vanished plague ants (Hymenoptera: Formicidae) of the 19th century Bermuda. Myrmec. Nachr. 8: 219-224.

Wetterer, J.K. 2007. *Paratrechina pubens* (Forel, 1893) (Hymenoptera: Formicidae), a candidate for the plague ant of 19th century Bermuda. Myrmec. Nachr. 10: 39-40.

Wetterer, J.K. and J.L.W. Keularts. Unpublished. Population explosion of the hairy crazy ant, *Paratrechina pubens* (Forel), on St. Croix, U.S. Virgin Islands (Hymenoptera: Formicidae).

Wetterer, J.K., S.E. Miller, D.E. Wheeler, C.A. Olson, D.A. Polhemus, M. Pitts, I.W. Aston, A.G. Himler, M.M. Yospin, K.R. Helms, E.L. Harken, J. Gallaher, C.E. Dunning, M. Nelson, J. Litsinger, A. Southern, and T.L. Burgess. 1999. Ecological dominance by *Paratrechina longicornis* (Hymenoptera: Formicidae), an invasive tramp ant, in Biosphere 2. Florida Entomol. 82(3): 381-388.

Whitcomb, W. H., A. Bhatkar and J. C. Nickelson. 1973. Predators of *Solenopsis invicta* queens prior to successful colony establishment. Environ. Entomol. 2: 1101-1103.

Wiedenmann, J., A. Baumstark, T.L. Pillen, A. Meinesz, and W. Vogel. 2001. DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Austrailian population. Marine Bio. 138: 229-234.

Williams, D.F. [ed.]. 1994. Exotic ants: biology, impact, and control of introduced species. Westview Press, Boulder, CO.

Williamson, M. 1989. Mathematical models of invasion. pp. 329-350. *In* Biological Invasions: a Global Perspective. J.A. Drake et al. [eds.]. John Wiley & Sons, New York.

Wilson, E.O. and R.W. Taylor. 1967. An estimate of the potential evolutionary increase in species density in the Polynesian ant fauna. Evol. 21(1): 1-10.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. Bioscience. 48(8): 607-615.

Wojcik, D.P. 1994. Impact of the red imported fire ant on native ant species in Florida. pp. 269-281. *In* Exotic ant biology, impact, and control of introduced species D.F. Williams [ed.]. Westview Press, Boulder, CO.

Wojcik, D.P., C.R. Allen, R.J. Brenner, E.A. Forys, D.P. Jouvenaz, and R.S. Lutz. 2001. Red imported fire ants: impact on biodiversity. Amer. Enomolgist. 47(1): 16-23.

Yamada, T., H. Takahashi, and R. Hatano. 1999. A novel insecticide, acetamiprid. pp. 149-176. *In* Nicotinoid insecticides and the nicotinic acetylcholine receptor. I. Yamamoto and J.E. Casida [eds.]. Springer, Tokyo.

Yamaguchi, T. 2005. Influence of urbanization on ant distribution in parks of Tokyo and Chiba City, Japan II: analysis of species. Entomological Sci. 8: 17-25.

Yamamoto, I., M. Tomizawa, T. Saito, T. Miyamoto, E.C. Walcott, and K. Sumikawa. 1998. Structural factors contributing to insecticidal and selective actions of neonicotinoids. Archives Insect Biochem. Physiol. 37(1): 24-32.

Zenner-Polania, I. 1990. Biological aspects of the "hormiga loca," *Paratrechina* (*Nylanderia*) *fulva* (Mayr), in Colombia. pp. 290-297. *In* Applied myrmecology: a world perspective. R.K. Vander Meer, K. Jaffe and A. Cedeno [eds.]. Westview Press, Boulder, CO.

Zenner-Polania, I. 1994. Impact of *Paratrechina fulva* on other ant species. pp. 121-132. *In* Exotic ants: biology, impact, and control of introduced species. D.F. Williams [ed.]. San Francisco, Westview Press.

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