Investigation of bioactivity and variations in alkaloid and hydrocarbon profiles of *Solenopsis invicta* Buren minim workers in relation to their age

by

Meng Chen

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 5, 2017

Keywords: *Solenopsis invicta* Buren, minims,
bioactivity, alkaloid, hydrocarbon

Approved by

Xing Ping Hu, Major Advisor, Professor of Entomology and Plant Pathology
Jian Chen, Research Entomologist, USDA-ARS
Kathy Flanders, Professor, Auburn University

Abstract

Red imported fire ant, *Solenopsis invicta* Buren, is a significant invasive pest introduced into the United States in the early 1930s. Venom alkaloids and hydrocarbons play important roles in the social life of the red imported fire ant. The minim is a unique caste in the fire ant colony development. However, the information on venom alkaloids and hydrocarbons of minims is very limited. In this study, alkaloid and hydrocarbon profiles of minims were examined from eclosion up to eight days after eclosion. Unlike the normal workers, the venom alkaloid component of the minim is dominated by a single piperidine alkaloid, 2-methyl-6-tridecenylpiperidine ($C_{13:1}$). The hydrocarbon profile of minims is very similar to that of normal workers, which was dominated by 5 hydrocarbons (heptacosane, 13-methylheptacosane, 13,15-dimethylheptacosane, 3-methylheptacosane, and 3,9-dimethylheptacosane). Heptacosane was the most dominant within five days after eclosion. Squalene, a triterpene, was also detected in minims. Both hydrocarbons and squalene were detected at one day after eclosion. Alkaloids were detected at one day after eclosion in ten out of the thirteen colonies. Alakloids, cuticular hydrocarbons and squalene varied quantitatively among different colonies and different ages of the minims from the same colony. This study also investigated the biological activity of heptadecane and squalene on fire ant workers. A behavioral bioassay demonstrated heptadecane was attractive to workers, while squalene was attractive at low concentrations (<=0.5%) but repellent at higher concentrations (>=2.5%).

Acknowledgments

I would like to thank my supervisor, Dr. Xing Ping Hu, for her expert guidance and persistent help during my two years' research at Auburn University. Without Dr. Hu's wisdom, patience and encouragement, this thesis would not have been possible. I am also indebted to my committee members, Dr. Jian Chen and Dr. Kathy Flanders for their sincere help and support during my research and defense.

I am thankful for all the support from Yuexun Tian for her advice during my research. I especially would like to give thanks to Liu Yang, who has shared with me countless experience.

Finally, I have immense gratitude for my family's love and understanding, for my Auburn friends' support, and for the encouragement from my friends in China.

Table of Contents

List of Figures

CHAPTER 1

GENERAL INTRODUCTION

Biology of *Solenopsis invicta* Buren

Taxonomy

Solenopsis invicta Buren belongs to the family Formicidae which contains more than 12,000 described species and 21 subfamilies (Bolton et al. 2006). The Formicidae is nested within the superorder Holometabola and resides within the order Hymenoptera. In 1916, Santschi described and named *Solenopsis saevissima wagneri* from Argentina, and later from Paraguay and Bolivia (Shattuck et al. 1999). Wilson examined the *Solenopsis saevissima* species complex and placed *wagneri* as junior synonym of *S. saevissima saevissima* in 1952 (Wilson 1952). In 1972, Buren recognized two distinct species in southern USA, *Solenopsis richteri* Forel and an undescribed species for which he proposed the name *S. invicta* (Buren 1972). In 1991, Trager examined the *S. geminata* species group, which included *S. invicta*, *S. saevissima* and related species, and concluded that *S. wagneri* was conspecific with *S. invicta*, and not *S. saevissima* as previously believed (Trager 1991). In 1995, Bolton recognized *S. wagneri* as an available name and treated *S. invicta* as a junior synonym of *S. wagneri* (Shattuck et al. 1999). Because the use of the name *S. wagneri* would cause considerable confusion and disrupt the non-taxonomic scientific literature concerning this species, Shattuck proposed that the use of *S. invicta* should be maintained because of its extensive use in the scientific literature (Shattuck et al. 1999). In 2002, James Pitts subjected a large number of characters from all life stages and castes to phylogenetic analysis designed to reveal the probable evolutionary relationships among the species (Pitts

2002). The mtDNA sequences of the inquiline social parasite *S. daguerrei* clade appears to be distantly related to sequences from the several host species. This corresponds to the James' discovery (Shoemaker et al. 2006). *S. invicta* queens and males' sculpture is not reduced and metapleuron not fused to propodeum if their size is greater than 5.0 mm in length. Major worker's pronotum is higher and posterior face of postpetiole is broader than high. Its median frontal streak and queen's frontal streak are present. Male's head is usually completely granulate and could see shagreened (Pitts 2002). These morphological features could also be used for identification of *S. invicta*.

Geographical Distribution

Solenopsis invicta, commonly known as the red imported fire ant*,* is native to the tropical areas of Central and South America, where it has an expansive geographical range from southeastern Peru to central Argentina and the south of Brazil (Vinson and Sorensen 1986, Pitts 2002).

Being one of the world's worst invasive alien species, *Solenopsis invicta,* is now having a wide distribution around the world (Lowe et al. 2000). *S. invicta* has been reported in southern China, Malaysia and Singapore (Chen et al. 2005, TianCi and Shi 2006). In Oceania, it has been detected in New Zealand and Australia (Drees and Gold 2003). Infestations also occur in a number of Caribbean island countries, including Puerto Rico, Antigua and Barbuda, British Virgin Islands and Trinidad and Tobago (Davis et al. 2001, Wetterer and Davis 2010).

In the USA, *S. invicta* is believed to have been introduced from northern Argentina or southern Brazil to the US port of Mobile, Alabama, between 1930 and 1945 (Hölldobler and Wilson 1990). It was inadvertently introduced as stowaways in cargo shipped from its native

South American range (Taber 2000). Two different species of fire ants were imported, both from South America. *S. richteri* Forel is referred to as the black imported fire ant and *S. invicta* is referred to as the red imported fire ant (Vinson 1997). However, *S. invicta* spread much more rapidly and more aggressively than *S. richteri.* In USA, *S. invicta* have gradually spread north and west despite intense efforts to stop or eliminate them. As of 2016, they were found in most of the southern states: Alabama, Arkansas, Arizona, California, Delaware, Florida, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, New Mexico, Oklahoma, South Carolina, Tennessee, Texas and Virginia. Morrison predicted that *S. invicta* could o become established over almost half of terrestrial land masses with the models of future range expansion based primarily on historical temperature and precipitation data (Morrison et al. 2004).

Monogyne Colony Cycle

Fire ants are social insects and live in colonies. They have two socially distinct forms. Colonies of the monogyne form have a single fertile queen, while the polygyne form contains multiple fertile queens per colony (Glancey et al 1973). Their life cycle occurs on two levels: the lives of individual ants and the life of the colony. A 1975 study found the founding of colonies is similar with most nonparasitic Myrniicine ants and begins when new queens alight on the ground after their nuptial flight (Lofgren et al. 1975). The female adult sexuals are reddish-brown whereas the males are shiny and black with a smaller head, Adult sexuals are not ready to fly and mate right after emerging from the pupae. The females, especially, must undergo maturation for about half per month (Keller and Ross 1993, Tschinkel 1993). During the period, the females almost triple their body weight while the males gain little weight. A large part of the queen's weight gain is probably storage protein and sequestered fat that makes up the metabolic store

from which females rear the first brood during the claustral period (Toom et al. 1976, Wheeler and Martinez 1995).

Although nuptial flights have been noted in every month of the year (Morrill 1974), peak activity occurs from late May through August subsequent to the periods of the highest production of sexual brood (Bass and Hays 1979). Substantial rain, especially when it follows a prolonged dry spell triggers the nuptial flights. Usually, the mating flight takes place on the day after the rain during the late morning or early afternoon (Markin and Dillier 1971, Obin and Vander Meer 1994). Alates fly hundreds of feet up from the ground and mate in the air. Males fly 40% faster than females, which makes obvious sense for males to catch females (Vogt et al. 2000). After mating, males die soon. Newly mated queens either descend directly to the ground or fly some distance before descending. Studies show it is easier for queens to survive by flying downwind (Rhoades and Davis 1967).

Once females land, they break off their wings and burrow into the soil to a depth of about 7-20 cm, sealing the entrance with soil to establish new colony (Vinson 1997). Many newly-mated fire ant queens are eaten by predators such as spiders, lizards, dragonflies, other ants, and ground beetles and birds. If a queen is attacked by the local established ant colonies before she sheds her wings, she occasionally takes flight again (Nickerson et al. 1975).

A monogyne colony is founded by single mated female sealed within the small chamber, a phenomenon known as claustral colony foundation (Vinson 1997). Within 24 to 72 hours, the queen starts to lay eggs and usually has a clump of 15 to 20 by the third day, typically 30 to 70 by one week. Queens frequently lick and move these eggs while keeping them in a clump (Markin et al. 1972, O'Neal and Markin 1973). Half of the eggs the queen lays do not form embryos and it was believed that they might be laid as food for the first-stage larvae (Glancey et

al. 1973, Voss et al. 1986). These eggs are common among ants and are called trophic eggs (Voss and Blum 1987). Fourth instar larvae may be a digestive and metabolic caste that processes protein for egg production by the queen (Tschinkel 1988). Queens will not eat before the minim workers eclose. As a queen loses half of her body weight as the claustral period wears on. The first batch of workers are uniformly tiny and are termed minims or nanitics. They are the smallest workers in the whole life cycle (Calabi and Porter 1989, Tschinkel 2006). The minims burrow out of the chamber and begin foraging for food to feed the queen and new larvae. The minims also begin construction of the mound (Vinson 1997).

Minim workers are an unique caste produced by the independently founding ant queens. They are regarded as a trade-off between size and number (Calabi and Porter 1989). Because a queen begins laying eggs with fixed reserves. She can only produce small ones. Minims are also influenced by the fixed larvae growth rate. The emergence of the minim workers indicates the start of the incipient phase which is one of the most confusing phases of the colony life cycle because of the dynamic behaviors in the colony (Rissing and Pollock 1991). The incipient colony stage is second only to the claustral stage in vulnerability (Hood and Tschinkel 1990). In this period, the nests might be shifted, the brood is transported like cargo, worker and queen quantities rise and fall. When is over, queens have fought to the death or been executed by workers until only one is left. (Tschinkel 1992). If they come into other incipient nests, they engage in reciprocal brood-stealing contests (Bartz and Hölldobler 1982). By about six months the colony has reached several thousand workers, which include a few large workers (major workers), many medium sized workers (median workers), and a majority of small workers (minor workers). These three types of workers are all sterile females and serve to perform tasks necessary to maintain the colony. In the process of rearrangement called “ brood raiding”, minor

workers are attacked less vigorously by minims when they are frosted as pupae in an incipient colony compared to a mature colony (Balas and Adams 1996). In the lab, there is only one important factor affects the outcome of raids--- the number of workers.In the fields, brood-raiding was limited in the first few weeks after the emergence of workers and it's negatively correlated to humidity (Tschinkel 1992).

The queen controls the colony by producing different eggs and releasing influencing pheromones (Vinson 1997). Temperature plays a vital role in the development rate from egg to adult ant. Growth in established colonies only occurred between 24 and 36℃, with maximal growth around 32℃. Colony growth ceased below 24℃ even though 17℃ was the theoretical minimum for brood development (Porter 1988). Eggs hatch into legless larvae in 7-10 days (Vinson 1997). The larvae will molt four times over half a month and the pupal stage of workers last 10-17 days (Jemal and Hugh-Jones 1993).When the larvae reach the fourth instar, they are able to digest solid foods and regurgitate amino acids and soluble proteins to feed the queen for egg production (Tschinkel 1993). A worker's function is partly determined by its age, size and the needs of the colony. As the colony matures, it begins to produce winged sexuals after at least containing 23,000 to 33,000 workers (Vargo 1988). Queens are able to control the sex ratio via fertilization of eggs and workers via the development of rearing or killing brood (Bourke and Franks 1995). Unfertilized eggs develop into males. Fertilized eggs can develop into workers, potential queens or female gynes. Some females won't be reproductive because the ovaries do not develop (Vinson 1997). Nutrition and juvenile hormone are also involved in determining whether a fertilized egg will develop into a worker or female gyne which could be a potential or actual queen (Passera et al. 2001).

Polygyny

Polygynous populations were first reported in the southeastern USA in the early 1970s (Hung et al. 1974, Glancey et al. 1975). Queens from polygyne colonies prefer to dig their founding chambers in infested soil avoiding soil from other polygyne *S. invicta* colonies (Kaspari and Vargo 1994). If there are many newly mated females in an area, they may aggregate and co-found colonies (Vinson 1997). However, if an incipient colony contains more than one queen, and the advantages of competing outweigh the advantages of cooperating, the workers that are produced begin to execute these queens until only one remains (Bernasconi and Keller 1996, 1999). A number of studies have shown that it might be related to the individual queen's fecundity, number of dominant workers and the queen's fighting ability (Adams and Tschinkel 1995, Adams and Balas 1999). These multiple queen or polygyne colonies consist of several mounds containing numerous queens with workers moving freely from one mound to another (Vinson 1997). The alate females from polygyne colonies are unlikely to establish independent colonies due to suppression of alate production, suppression of egg laying and inhibition of embryonation (Tschinkel 2006). For some polygyne colonies, some females, which could only survive in polygyne populations, could produce diploid males and these males are sterile (Crozier 1971). The reduction on longevity is common in polygyne ant species, the life span of a polygyne queen is at most about half the 7 year life expectancy of a monogyne queen (Vargo and Porter 1989). Many critical differences between the monogyne and polygyne social forms are associated with *Gp-9* genotype.

Ecology

The most prominent feature of *S. invicta* is that it is mainly a creature of disturbed habitats (Williams and Whelan 1991). It was rare in undisturbed ecosystems but was common and often dominant in disturbed areas in Florida (King 2004). *S. invicta* displaces some native ants and suppresses others (Tschinkel 2006) during and shortly after the initial phase of the invasion (Morrison 2002). *S. invicta* is also a predator of other insect pests such as boll weevils and borers infesting sugarcane and cotton (Sterling 1978). In 1972, researcher also found that reducing *S. invicta* population led to a 69% increasing pest damage (Reagan et al. 1972), and a two-five times increase in horn fly and stable fly populations (Summerlin et al. 1977, Summerlin and Kunz 1978). However, it is not quite convincing to encourage the beneficial role of *S. invicta* in agriculture (Tedders et al. 1990, Kaplan and Eubanks 2002). *S. invicta* attacks vertebrates including Loggerhead turtle and birds nestling (Sikes and Arnold 1986, Moulis 1997). Unlike many other insect pests that are either an urban, agricultural, or medical problem, *S. invicta* is a problem in all of these areas (Vinson 1997). Aside from its damage to electrical equipment, lawns, ornamentals, and gardens along with pesticide overuse, *S. invicta* is infamous for its stings which provoke a painful burning sensation with a white pustule formed in 24 hours. The sting is the main reason for insect venom allergy in the southeastern United States (Hoffman 1993). Hives and swollen blood vessels have become the most common problems for allergy suffers. It is reported that fatal reactions are becoming more common especially in Florida and Texas (Adams 1986).

Venom Alkaloids

Ants developed the stinger from a waspish ancestor while evolving. It is essentially a modified ovipositor and defines the aculeate Hymenoptera (Hermann and Blum 1981). Once the

social Hymenoptera evolved, the worker's egg-conduit function was no longer needed and the ovipositor evolved into hypodermic syringes for injecting venoms produced by accessory glands (Schmidt 1990). Some ants have lost the sting but fire ants are not among them. Obviously, their name is derived from their stings and effect of venom (Callahan et al. 1959). Small workers (*S. invicta*) deliver 75% more venom during each stinging bout compared to large workers, despite the fact workers have 75 % more weight and more venom. Also small workers' sting behavior is more frequent according to laboratory experiments (Haight 2002, Haight and Tschinkel 2003).

Unlike wasp or other ant venom in which the effective ingredients are usually biologically active protein, fire ant venom is made of alkaloids with only 0.1% protein. Study showed only venom of *Solenopsis* and *Monomorium* contains alkaloids (Blum 1988). The venom of *S. invicta* was identified as 6-methyl piperidine alkaloids with a variety of 2-substituted alkyl or alkenyl side chains (Brand et al. 1972). It contains trans-2-methyl-6-n-undecyl (*trans* C_{11}), *trans*-2-methyl-6-*n*-tridecyl (or tridecenyl) piperidine (*trans* C_{13} and *trans* $C_{13:1}$) together with *trans*-2-methyl-6-*n*-pentadecyl (or pentadecenyl) piperidine (*trans* C_{15} and trans $C_{15:1}$) (Brand 1978). Studies show unsaturated C_{13} and C_{15} alkaloids decline greatly in their dominance over saturated ones as workers age and body size increases. Some alkaloids are positively allometric with body size, and some are negatively allometric (Deslippe and Guo 2000).

Minim workers (*S. invicta*) have about 94% $C_{13:1}$ alkaloids in their venom. Compared to the normal workers, minims reportedly lack the other three major alkaloids (C_{13}, C_{15}, $C_{15:1}$). The levels of $C_{13:1}$ alkaloids in minims were comparable to young minor workers (Vander Meer 1986); however, it was believed that Vander Meer had probably compared older minors to young minims (Deslippe and Guo 2000).

Venom alkaloid profiles can be used in taxonomy of fire ants . *Solenopsis invicta*'s venom consists of five major piperidine alkaloids and they are all in the *trans* configuration with only traces of *cis*. *S. richteri* only has C_{11} ,C_{13},$C_{13:1}$ in *trans* configuration. *S. geminata*, S. *aurea* and S. *xyloni* mainly have *cis*- and *trans*-C_{11} with traces of *cis*-C_{13} and *cis*-$C_{13:1}$ (Brand et al. 1972). Combined with morphological characters, these differences are large enough to be useful as taxonomic features for S*olenopsis* species (MacConnell et al. 1976, Trager 1991). Alkaloid profiles have been used to determine hybridization between *S. invicta* and *S. richteri* (Vander Meer and Lofgren 1988, 1990).

Young and old workers are known to produce less venom than intermediate-aged workers. The differences in venom composition also correspond to the size- and age-based functional role of workers (Deslippe and Guo 2000). *Solenopsis invicta* venom synthesis is restricted to early life, and injected venom dose tends to be modulated (Haight and Tschinkel 2003). A *S. invicta* study in Taiwan shows the difference in the proportions of unsaturated alkaloids in venom among workers. The ratios of C_{13} :$C_{13:1}$ and C_{15} :$C_{15:1}$ alkaloids might be a good indicator for distinguishing monogyne from polygyne forms (Lai et al. 2008). Sexually mature and non-productive queens from polygyne colonies have different proportion of *cis*-piperidine alkaloids depending on their *Gp-9* genotype (Eliyahu et al. 2011). There are also new alkaloids that might indicate new biosynthetic pathways and antifungal activities (Chen and Fadamiro 2009, Dai et al. 2011). Studies show efficient piperidines and piperideines in *S. invicta* venom alkaloids inhibit the soil borne plant pathogen, *Pythium.ultimum,* and green peach aphids, *Myzus persicae* (Li et al. 2012, Rashid et al. 2013).

S. invicta **Hydrocarbons**

Insect cuticular hydrocarbons usually contain complex mixtures of normal and methyl branched components. In *S. invicta* workers, saturated hydrocarbons ranging from 23 to 30 total carbons make up 65%-75% the cuticular lipids (Lok et al. 1975). Around 60% of hydrocarbons were found in the newly mated queen's post-pharyngeal gland (PPG) and crop with a lower proportion (Vinson et al. 1980). The hydrocarbons in the PPG increase rapidly between day 10 and 15 (post-mating time) and then decrease to the similar level of day 0 after 15 days (Vander Meer et al. 1982). A mixture of 3,9- and 3,11-dimethyltricosanes. 3,7,11-Trimethylalkanes were confirmed to be present in *S. invicta* (Nelson et al. 1980).. Four major hydrocarbons, 13-methylheptacosane; 13,15-dimethylheptacosane; 3-methylheptacosane; and 3,9-dimethylheptacosane in the PPG of mated queens were identified and confirmed in 1981 (Thompson et al. 1981). In addition, heptacosane was found to be a major hydrocarbon in PPG of virgin queens. The major hydrocarbons of the PPG are also reported as the major cuticular hydrocarbons of *S. invicta* (Nelson et al. 1980, Thompson et al. 1981).

Insect cuticular hydrocarbons have many functions such as territory marking, alarm pheromones, defensive secretions and sex pheromones (Howard and Blomquist 1982). Similar to venom alkaloids, cuticular hydrocarbons could also be used as a chemotaxonomic tool for insect groups and caste recognition in termites (Howard et al. 1978, Carlson 1980). The distinct cuticular hydrocarbon patterns can be evidence for hybridization between *S. invicta* and *S. richteri* (Vander Meer et al. 1985). No qualitative differences between nonmelanized and normal workers were found in cuticular hydrocarbons (Williams et al. 1987). Trail pheromone of *S. invicta* was characterized as a sesquiterpene hydrocarbon in 1976 (Barlin et al. 1976). In 1988, the main majority of hydrocarbons were the known saturated normal, methyl, and dimethyl branched compounds specific to *S. invicta* (Vander Meer et al. 1988). In 1989, the cuticular

hydrocarbons were suggested to be used as a model for heritable nestmate recognition because of the patterns vary in different colonies and change within colonies over time (Vander Meer et al. 1989). In 1999, the first direct behavioral evidence that isolated hydrocarbons influence nestmate recognition was provided on *Chrysops. niger* (Lahav et al. 1999). In Lahav's study, it was hypothesized that PPG might function as storage for cuticular hydrocarbons, which would explain the composition equivalence in several ant species. The study also showed the same hydrocarbon mixture is present in the PPG, metapleural-gland and cuticle of *S. invicta*, but it is much more concentrated in the PPG (Cabrera et al. 2004). Along with cuticular hydrocarbons, phosphoric acid, glycerol, and lactic acid were discovered being deposited by *S. invicta* in the moistened silica gel nest material (Obin and Vander Meer 1985, Chen 2007b). In ants, different species tend to use specific cuticular hydrocarbon structural types indicating it may be generated in part by switching particular biosynthetic pathways on or off (Van Wilgenburg et al. 2011). On the other hand, it is believed that cuticular hydrocarbons might not be the only compounds involved in discrimination of alien conspecifics from nestmates (Lavine et al. 2011). Cuticular hydrocarbons was also discovered related to regulating social organization via signaling queen *Gp-9* genotype in *S. invicta* (Eliyahu et al. 2011).

Squalene

Squalene, like many other terpene compound, is fat-soluble. It is widespread in nature, and commonly found in olive oil, shark oil, wheat germ, and rice bran (Reddy and Couvreur 2009). Squalene may play a significant role in lowering cancer rates (Owen et al. 2004) and can potentially protect the skin from oxidation and ultraviolet radiation. 12% of overall bodily

squalene content was secreted from the skin. It exhibits antiradioactive and antioxidative properties (Newmark 1997, Hashim et al. 2005).

In 1972, squalene was found in the third instar larva of *Sarcophaga bullata* Parker, but no significant sterol biosynthesis from squalene in *S. bullutu* third instar larvae could be demonstrated (Goodfellow and Liu 1972). One year later, a study suggested that the squalene was possibly originally coming from the diet (Goodfellow et al. 1973). Squalene was found in the defense secretion of ticks in 1993. It was speculated that either ticks extract squalene from the blood ingested by a preceding developmental stage or they synthesize this molecule (Yoder et al. 1993b). *Ixodes* ticks might be vulnerable to predation by ants because they lack the allomonal defensive secretion whose principle componet is squalene. However, other metastriate ticks could be protected against ants via the allomonal defensive secretion produced by the large wax glands (Yoder et al. 1993a). A study reported that adults, especially female *Sarcoptes scabiei* (L.) were attracted more than immatures to most compounds including squalene (Arlian and Vyszenski-Moher 1995). In 1999, a bioassay study showed squalene contributes more to the tick's ability to locate hosts at greater distances than aggregation-attachment pheromones, indicating the potential of squalene for being used in attractant baits (Yoder et al. 1999). Squalene was detected as one of the major constituents on the body surface of *Ixodes persulcatus* Schulze and the percentage was higher in males than females (Tkachev et al. 2000). In 2002, it was reported that squalene might be involved in host location of a parasitoid, *Pholetesor bicolor*on Nees, in its the third trophic level (Dutton et al. 2002). Detected in the extracts of all three larval types, squalene was identified as one of the main component of cuticular compounds in first instar diploid drone larvae in the honey bee (*Apis mellifera* Linnaeus) (Santomauro et al. 2004). Studies also showed that squalene is an exceedingly attractive semiochemical that

provides nearly species and sex-specific attraction of male *Ceralocyna nigricornis* Gounelle (Jones et al. 2011).

Heptadecane

Heptadecane is an unbranched alkane hydrocarbon with the chemical formula $C_{17}H_{36}$. In 1967, heptadecane was detected in Dufour's gland constituents of the bull ant, *Myrmecia gulosa* Fabricius. (Cavill and Williams 1967). Heptadecane was also found in the gasters of *Novomessor cockerelli* Andre (Hymenoptera: Formicidae) among the 8 main detected hydrocarbons (Vick et al. 1969). In 1972, n-heptadecane was found in the Dufour gland in *Myrmica rubra*, (Morgan and Wadhams 1972) and many other ant species including *Myrmica. scabrinodis*, *Formica. nigricans*, *F. rufa*, *and F. polyctena* (Bergström and Löfqvist 1973, Morgan et al. 1979), but not widely found in the gaster gland. It was detected on foraging trails in the field as a component of the territorial odor (Salzemann et al. 1992). In a study of mechanism of the hydrocarbon biosynthesis from aldehyde in insects, heptadecane was the hydrocarbon into which the aldehyde was converted. Carbon dioxide was the other product and an approximate 1/1 ratio of CO_2/heptadecane was formed (Mpuru et al. 1996). Pentadecane and heptadecane are often the most abundant alkanes in myrmicines, and undecane or tridecane the most abundant alkanes in formicines (Morgan 2008).

Objectives

Minims are the first tiny-sized workers produced by the independently founding ant queens. They are regarded as a product of trade-off size and number (Calabi and Porter 1989). Being a special caste in the colony life cycle, very little is known about its alkaloids and hydrocarbons. In

1986, Bosworth and Vander Meer detected no predominate peaks (C_{13},C_{15},$C_{15:1}$) in gas chromatographic analysis of minims, but did not specify minim ages that ere studied (Vander Meer 1986, Deslippe and Guo 2000). Hydrocarbons have been studied as taxonomy and chemical communication among insects including ants (Howard and Blomquist 1982). Several hydrocarbons are reported affecting insect behaviors (Yoder et al. 1993a). This study is to:

1) Identify and quantify main alkaloids and hydrocarbons in minim workers of *S. invicta*.

2) Investigate the alkaloid and hydrocarbon profiles of the minim workers in relation to their age.

3) Assess the bioactivity of two hydrocarbons on normal workers of *S. invicta*.

CHAPTER 2

Venom alkaloids and hydrocarbons in *Solenopsis invicta* Buren minim workers in relation to their age.

Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is an invasive species that was introduced from South America into the United States through the port of Mobile, Alabama in the early 1930s (Vinson and Sorensen 1986). Being one of the World's Worst Invasive Alien Species (Lowe et al. 2000), it rapidly widespread in the southern United States and has been introduced to other countries and regions of the world, including the Caribbean, Australia, New Zealand, Thailand, China (Ascunce et al. 2011). Studies have show that the invasion of *S. invicta* has a major impact on local ecosystems (Porter and Savignano 1990, Kaspari 2000). Fire ants also have detrimental influences on crops, machinery, livestock and public health (Davidson and Stone 1989, Vinson 1997, Taber 2000).

Minim workers, also known as dwarf workers and nanitic workers, are the first tiny-sized workers produced by the independently founding ant queens. They are regarded as a trade-off between size and number (Calabi and Porter 1989). The emergence of the minim workers indicates the start of the incipient phase (Rissing and Pollock 1991). When the claustral period comes to an end, the minim workers open the nest and explore around (Tschinkel 1992).

Minim workers (*S. invicta*) have about 94% $C_{13:1}$ alkaloids in their venom, which is very different to normal workers (Vander Meer 1986). Compared to normal *S. invicta* workers,

minims were found to lack of three major piperidine alkaloids found in normal workers (C_{13},C_{15},$C_{15:1}$). The levels of $C_{13:1}$ alkaloids in venom of minims were reported comparable to young minor workers (Vander Meer 1986, Deslippe and Guo 2000).

In 1980, it was confirmed that the previously identified 10,12-dimethyltricosane was a mixture of 3,9- and 3,11-dimethyltricosanes, and 3,7,11-trimethylalkanes were identified in *S. invicta* (Nelson et al. 1980). The next year, Z,E and E, E-a-farnesene were isolated and identified from terpenoid trail pheromone which is produced in the Dufour's gland of *S. invicta* workers (Vander Meer et al. 1981). Five major hydrocarbons, heptacosane; 13-methylheptacosane; 13,15-dimethylheptacosane; 3-methylheptacosane; and 3,9-dimethylheptacosane in the PPG of mated queens of *S. invicta* have been identified and confirmed (Thompson et al. 1981).

The objectives of this study were to: 1) identify and quantify main alkaloids and hydrocarbons in minim workers of *S. invicta* and 2) investigate the alkaloid and hydrocarbon profiles of the minim workers in relation to age. This study would provide a basis for the selection of hydrocarbons from minims for the bioactivity exploration.

Materials and Methods

Insect samples

Newly mated *Solenopsis invicta* queens that had shed their wings were collected from E.W. Shell Fisheries Center in Auburn AL (32°39'N, 85°29'W) during April to June in 2016. The queens were collected immediately after they fell on the ground and before they dug into the soil. Test tubes (15cm× 2cm) filled with distilled water to 1/3 and plugged with a cotton ball served as containers. The queens were placed individually in different tubes and each tube was plugged with a cotton ball to prevent queens from escaping. The glass tubes were held horizontally and

checked daily for egg-laying, larval development and minim worker emerging. After emergence of minims, three minims were collected daily from each test tube colony. The remaining minims in glass tubes were kept alive with daily changed hot-dog (1g per piece, 8g in total) and sugar-water (absorbed with sterile cotton, 10%). The collected minims were stored in 1.8-ml brown-colored ROBO Autosampler vials (VWR International, LLC, Radnor, PA, 19087) with 500ul hexane. The vials were sealed with the cap and sealed with paraffin film. Samples were stored in -80℃ freezer for further GS analysis.

Chemical identification and quantification

Single Point Internal Standard Methods was used for GS-MS profile (Lavine et al. 2011). (2R,6R) Solenopsin B solution was used as standard solution. The ratio of acetone and hexane was 1:1 and standard solution concentration is 95.9ug/ml. The first analysis was injecting 2ul sampling hexane. The second analysis was drying out the solvent of the sample by nitrogen flow and adding 50ul standard solution, followed by injecting 2ul mixed sample. All samples were tested by Gas chromatography-mass spectrometry (GC-MS) analyses on an Agilent 7890A GC coupled to a 5975C mass selective detector (G3170-90001,Agilent Technologies, Inc), carried through a DB-5MS capillary column (30 m × 0.25 mm ID, 0.25um film thickness) with helium as the carrier gas flowing at a rate of 1.2ml/min. The oven (Agilent 7890A GC) was programmed from 150 to 270℃ at 15℃/min with a 2 min pre-run hold and a 5 min post-run hold. Alkaloids were identified by analysis of their mass spectra produced by EI(electron-impact) (70 eV), as well as by comparison of characteristic peaks of the alkaloids (Jones et al. 1982).

For purification of alkaloids in the samples, simple column chromatograph on 0.5g of silica gel was conducted. Disposable pasteur pipet (Fisherbrand, Borosilicate glass, Cat. No. 13-678-

20A) was filled with 0.5g silica gel (Sigma-Aldrich, 236802-1 kg, High-purity grade) with glasswool(18423, Pyrex® fiber glass wool, Sigma-Aldrich) on the bottom. The sample was eluted followed by blank hexane through the mini silica gel. The extra hexane in the sample was evaporated after purification for further GS-MS analysis.

Chemical standard solutions were made by serial dilution method. Concentration was decreased by half for all the alkaloids, hydrocarbon and squalene. Concentration for $C_{13:1}$ alkaloids ranged from 4.8 ug/ml to 0.15 ug/ml in six standard solutions. Concentration for hydrocarbon ranged from 456 ug/ml to 1.8 ug/ml in nine standard solutions. Concentration for squalene ranges from 2.67ug/ml to 0.17 ug/ml in five standard solutions.

Data Analysis

Linear standard curve was calculated based on the standard alkaloid, hydrocarbon and squalene concentrations (Lavine et al. 2011).

Results

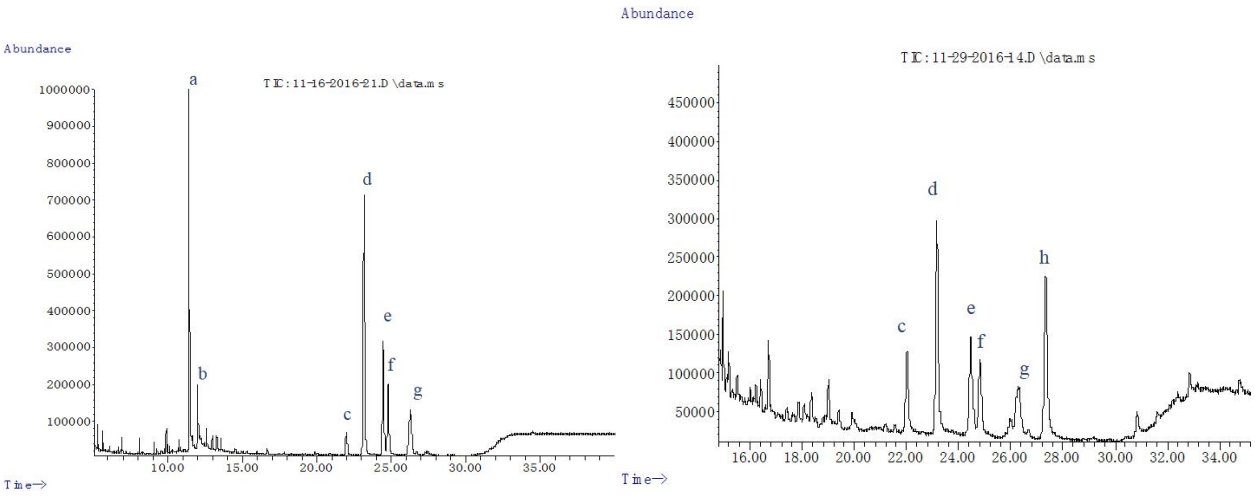
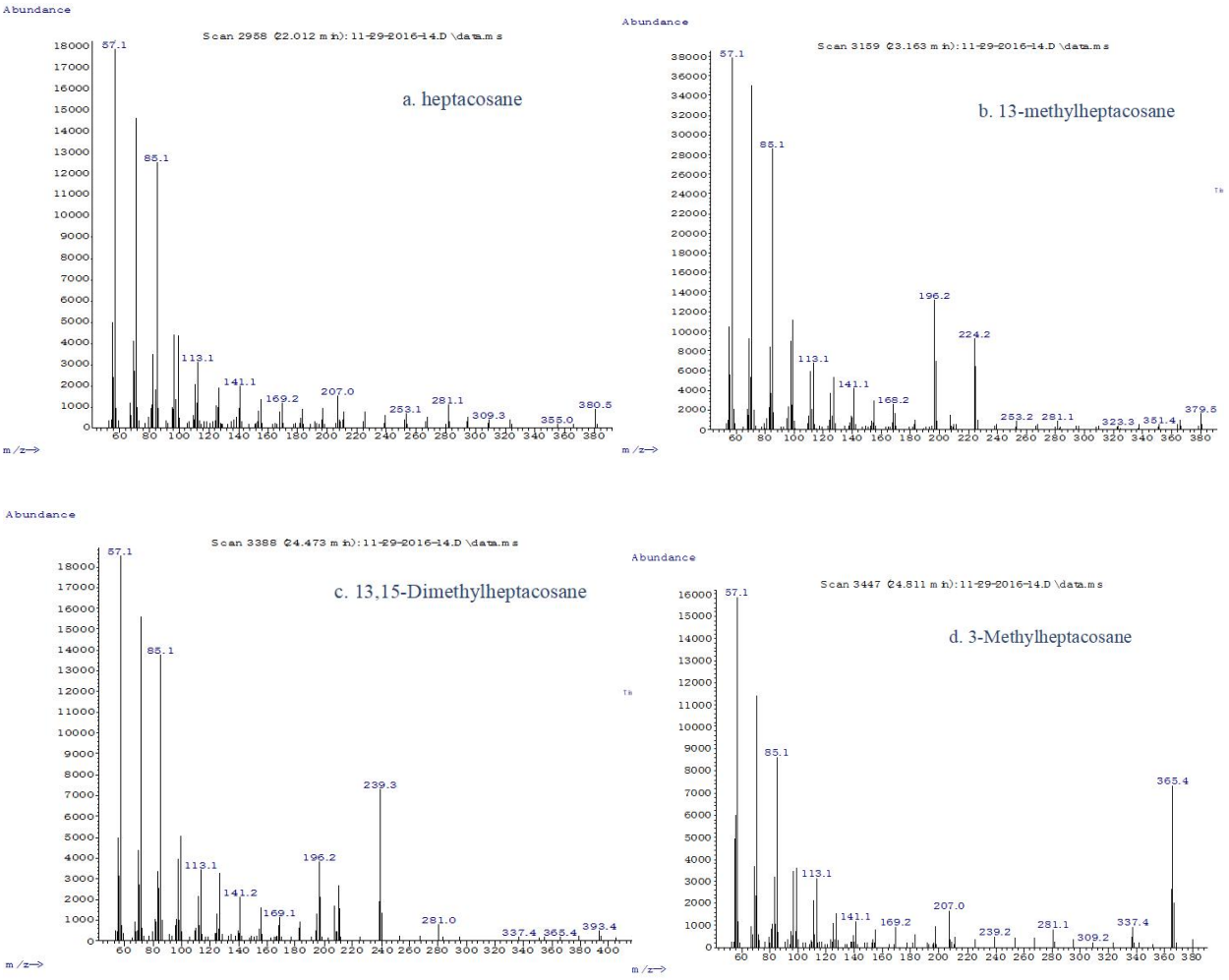


Fig. 1. Typical total ion chromatogram of alkaloids and hydrocarbons (including squalene) in the whole body extracts of *S. invicta* minims. Peak (a) $C_{13:1}$ alkaloid; peak (b) C_{13} alkaloid; peak (c)

heptacosane; peak (d) 13-methylheptacosane; Peak (e) 13,15-dimethylheptacosane; peak (f) 3-methylheptacosane; peak (g) 3,9-dimethylheptacosane; peak (h) squalene.

Two alkaloids , five hydrocarbons and squalene (a hydrocarbon and triterpene) were detected in all the 13 minim groups from different queens. The distribution and retention time is shown in the Figure 1.

The mass spectra identified the major five hydrocarbon is c) heptacosane; peak (d) 13-methylheptacosane; Peak (e) 13,15-dimethylheptacosane; peak (f) 3-methylheptacosane; peak (g) 3,9-dimethylheptacosane followed by peak (h) squalene (Fig 2) (Thompson et al. 1981).



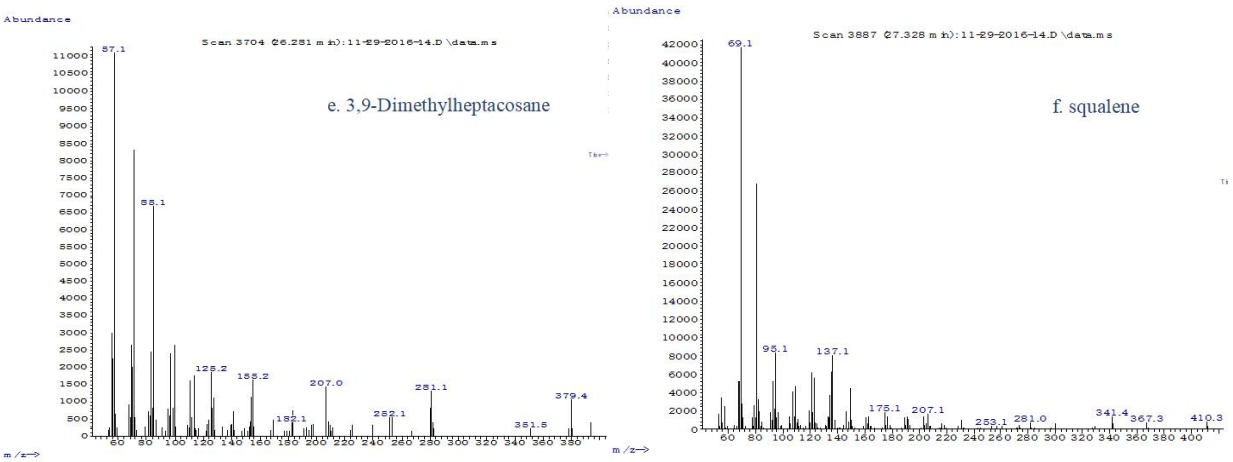
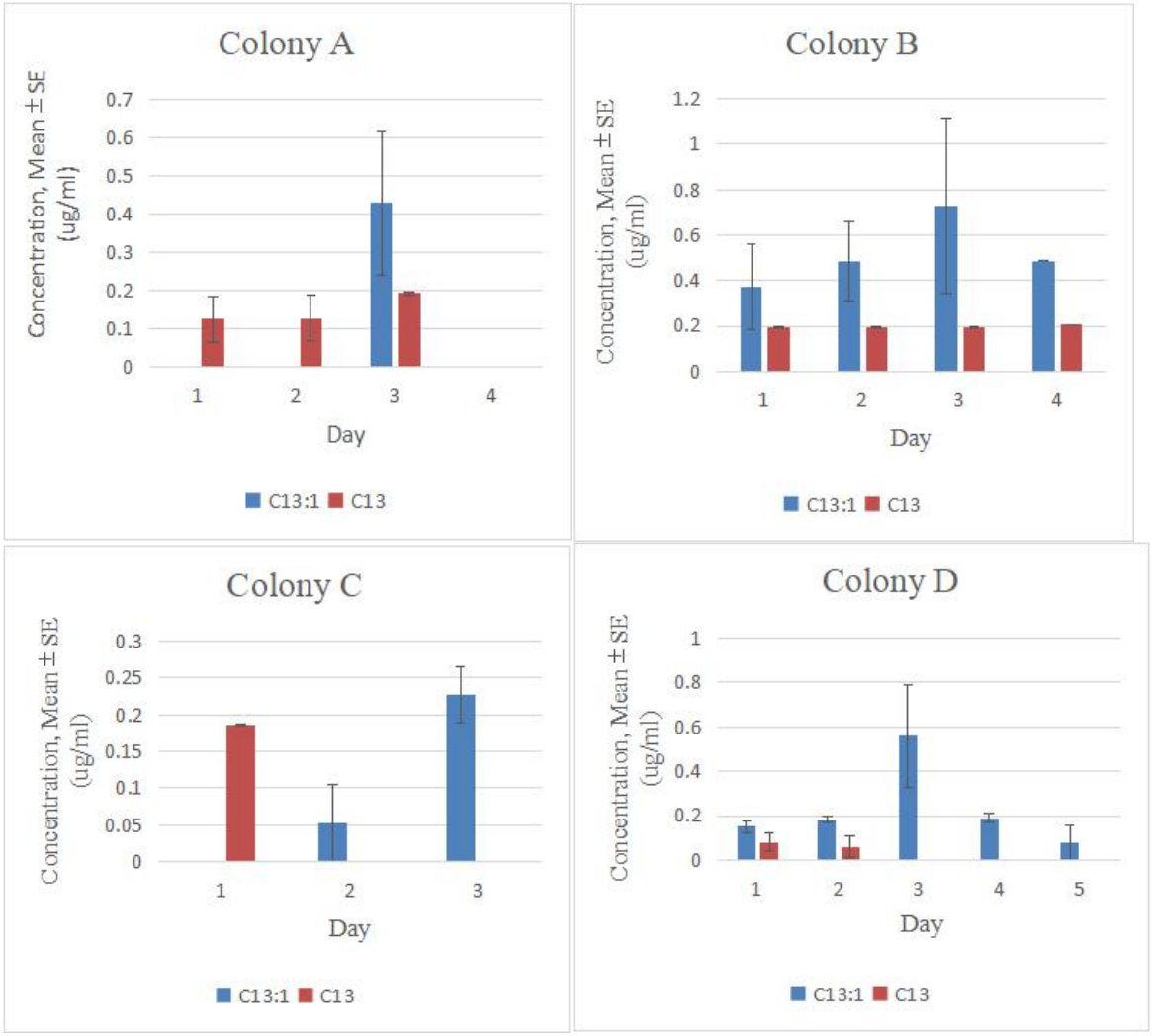
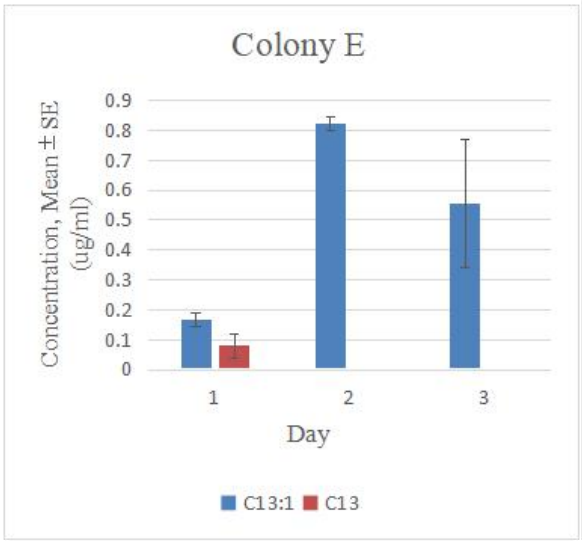
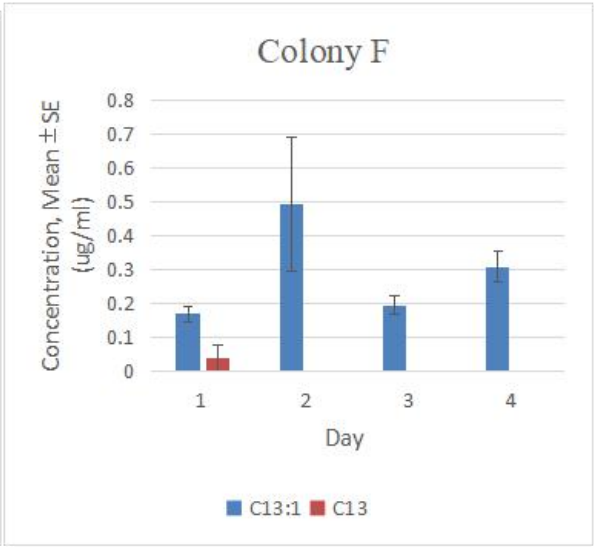
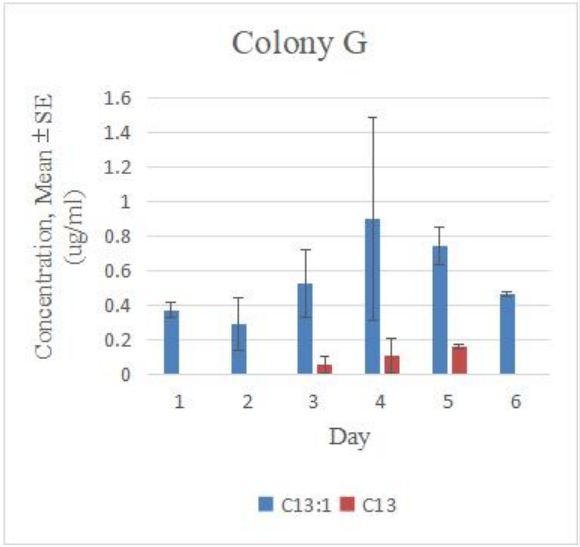


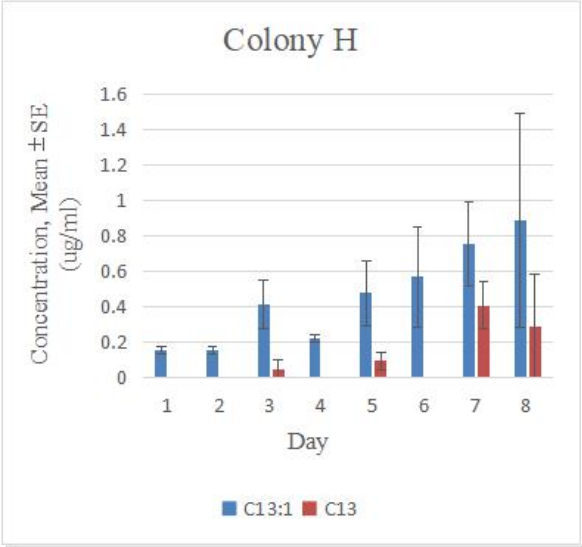
Fig 2. Mass spectra of major peaks in the whole body extracts of *S. invicta* minims. Peak assignment: a. Heptacosane, b.13-methylheptacosane, c.13,15-dimethylheptacosane, d. 3-methylheptacosane, e. 3,9-dimethylheptacosane and f. Squalene.

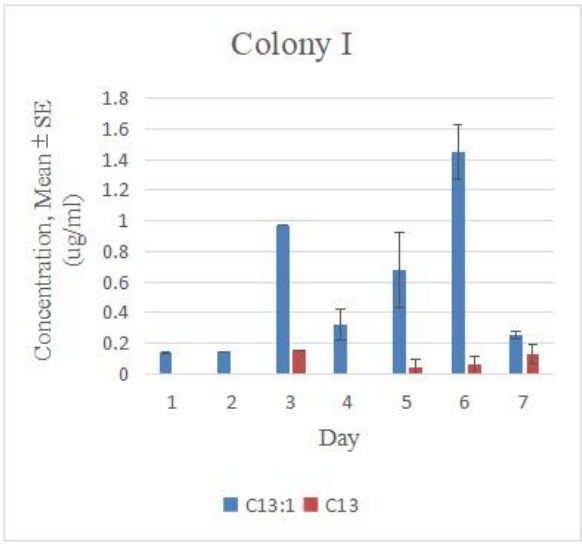


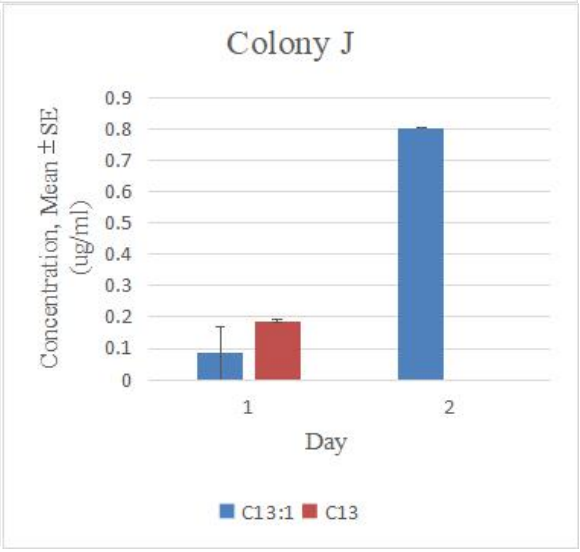

Colony E
Concentration, Mean ± SE (ug/ml)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
Day
C13:1
C13


Colony F
Concentration, Mean ± SE (ug/ml)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Day
C13:1
C13


Colony G
Concentration, Mean ± SE (ug/ml)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
Day
C13:1
C13


Colony H
Concentration, Mean ± SE (ug/ml)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
Day
C13:1
C13


Colony I
Concentration, Mean ± SE (ug/ml)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
Day
C13:1
C13


Colony J
Concentration, Mean ± SE (ug/ml)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
Day
C13:1
C13

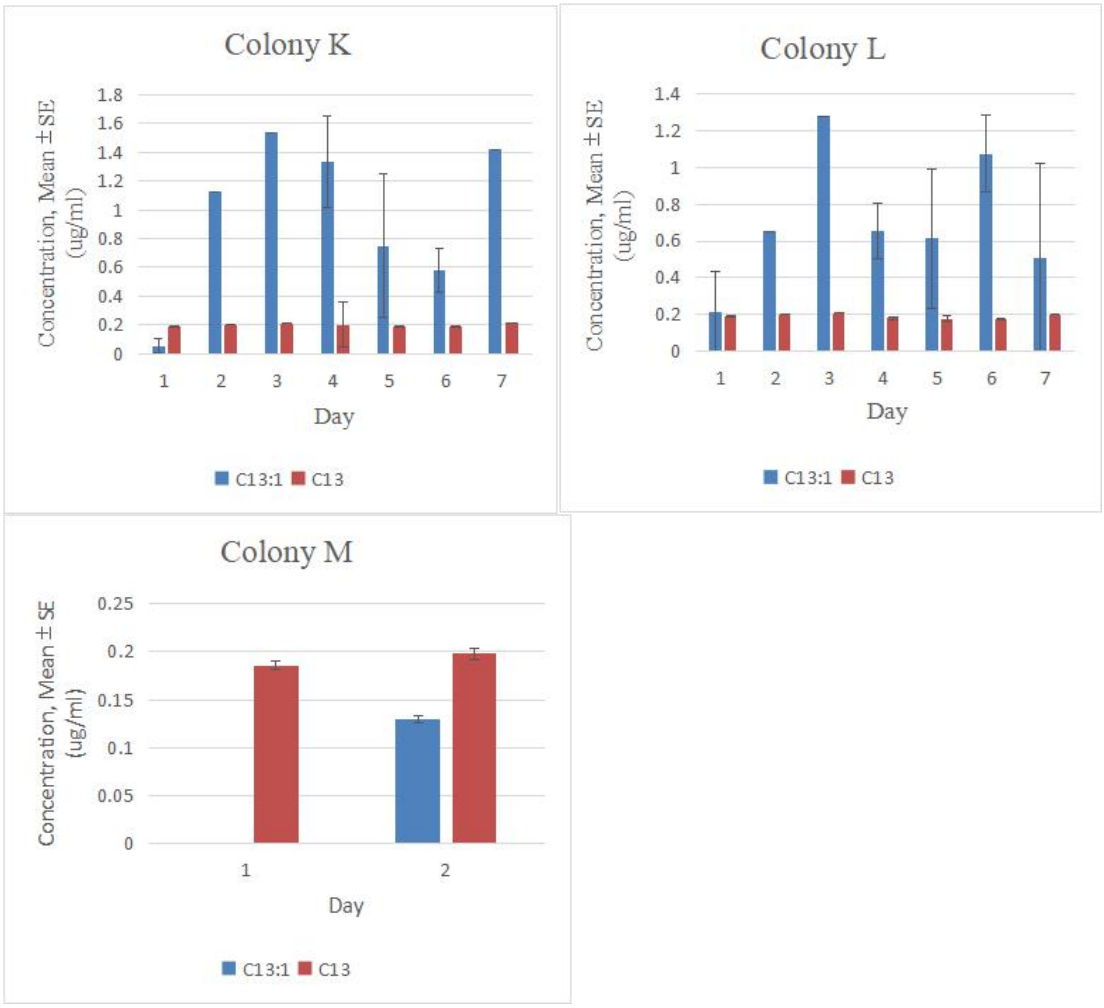


Fig. 3. 2-methyl-6-tridecenylpiperidine ($C_{13:1}$) and 2-methyl-6-tridecylpiperidine (C_{13}) alkaloid concentration (Mean ± SE) distribution in minims from 13 colonies in relation to their age.

Both $C_{13:1}$ and C_{13} alkaloid were detected in minims of the 13 colonies. $C_{13:1}$ alkaloid was the dominant in the absolute amount of all ages. Out of the thirteen colonies, it was detected on day one after eclosion in ten colonies and on day two after eclosion in the other three colonies. C_{13} alkaloid was detected on the first three days after eclosion in colonies A, C, D, E, F, J, and M, but on or after the third day in colonies G, H, and I. (Fig 3).

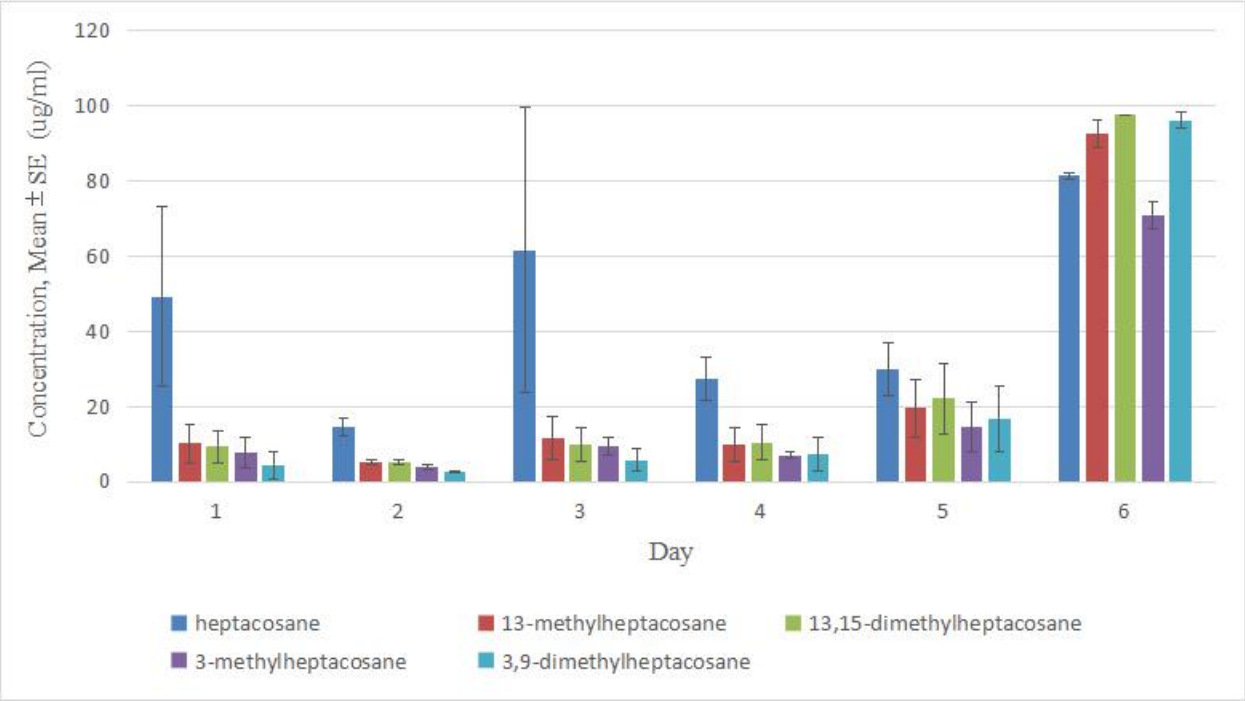


Fig. 4. Concentration of five major hydrocarbons (Mean±SE) in minims from colony G in relation to their age.

The minim colony G contained 3 minim samples in both Day 1 and Day 3 and 2 minim samples in Day 2, 4, 5 and 6. All major hydrocarbons were detected in 14 minims and there is only one minim showed no trace of 13-methylheptaeosane in the third day. Heptacosane was always the dominant hydrocarbons in the extracts except the sixth day which is most aged minims we could obtain. The amount of 13-methylheptacosane; 13,15-dimethylheptacosane; 3-methylheptacosane; and 3,9-dimethylheptacosane showed a significant increase in the sixth day (Fig4).

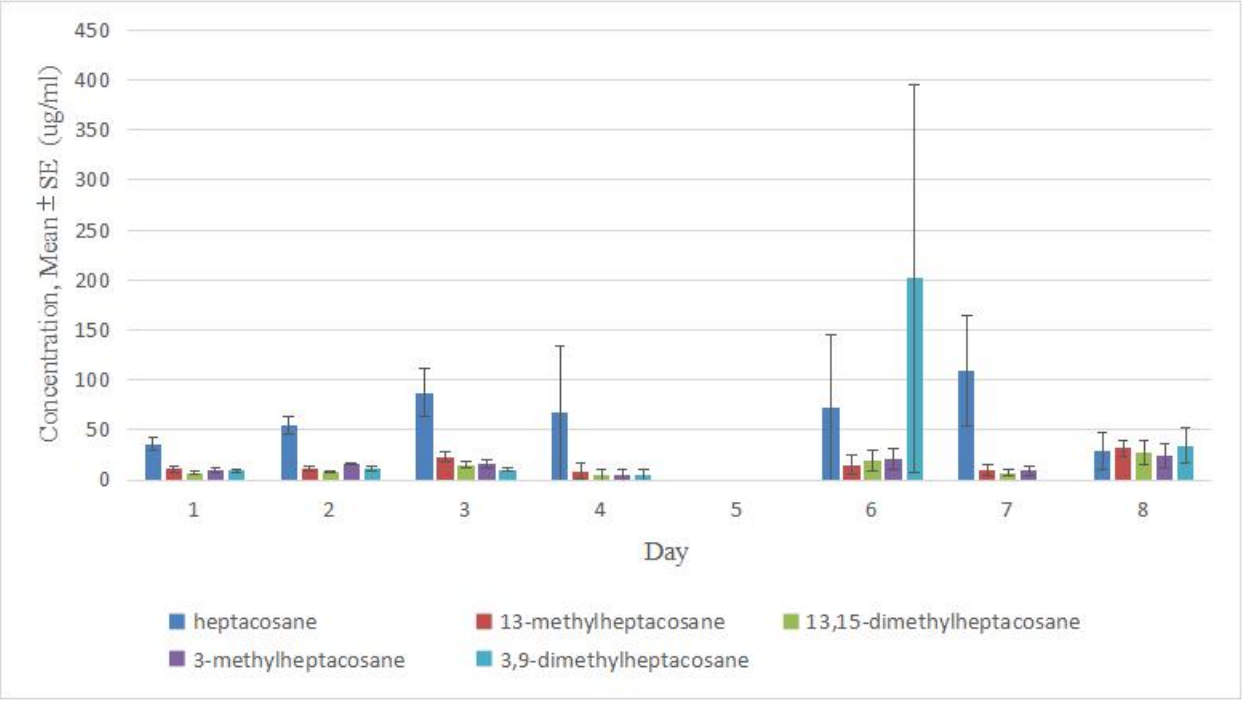


Fig. 5. Concentration of five major hydrocarbons (Mean ± SE) in minims from colony H in relation to their age

In colony H, heptacosane was still the major component in all the five hydrocarbons. We obtain 23 ant samples from eight days with 3 minims each for the first seven days and two 8-day-old minims. Interestingly, no hydrocarbons were detected in the 2 samples of the fourth day, 1 sample each for six and seventh day and all 3 in the fifth day (Fig 5).

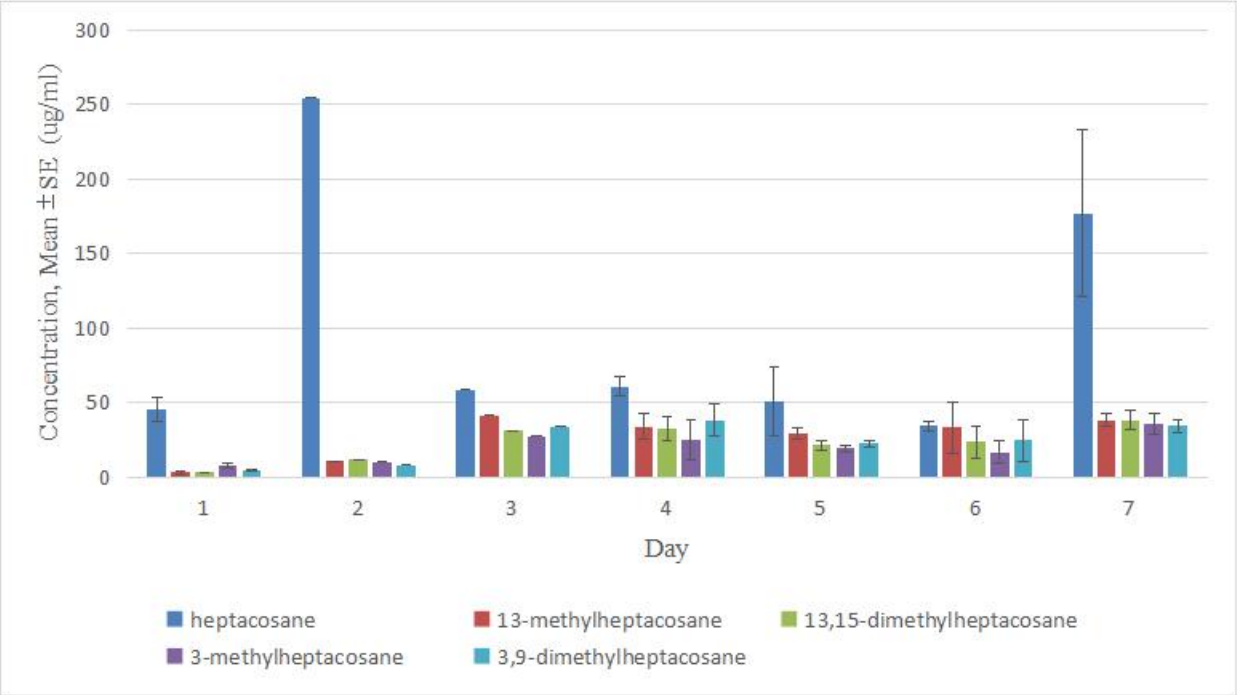


Fig. 6. Concentration of five major hydrocarbons (Mean±SE) in minims from colony I in relation to their age

In colony I, only one ant sample each was taken from second and third day and three minims each were taken in the next four days. Like the last two groups, heptacosane is still the main hydrocarbon among the top five. The other four hydrocarbons showed no significant difference in concentration while three extraordinarily high concentrations (254,199 and 261ug/ml) of heptacosane were detected in the second days and seventh day. Concentrations of 13-methylheptaeosane, 13,15-dimethylheptacosane, 3-methylheptacosane, and 3,9-dimethylheptacosane increased on third day and start to decrease on fifth day (Fig 6).

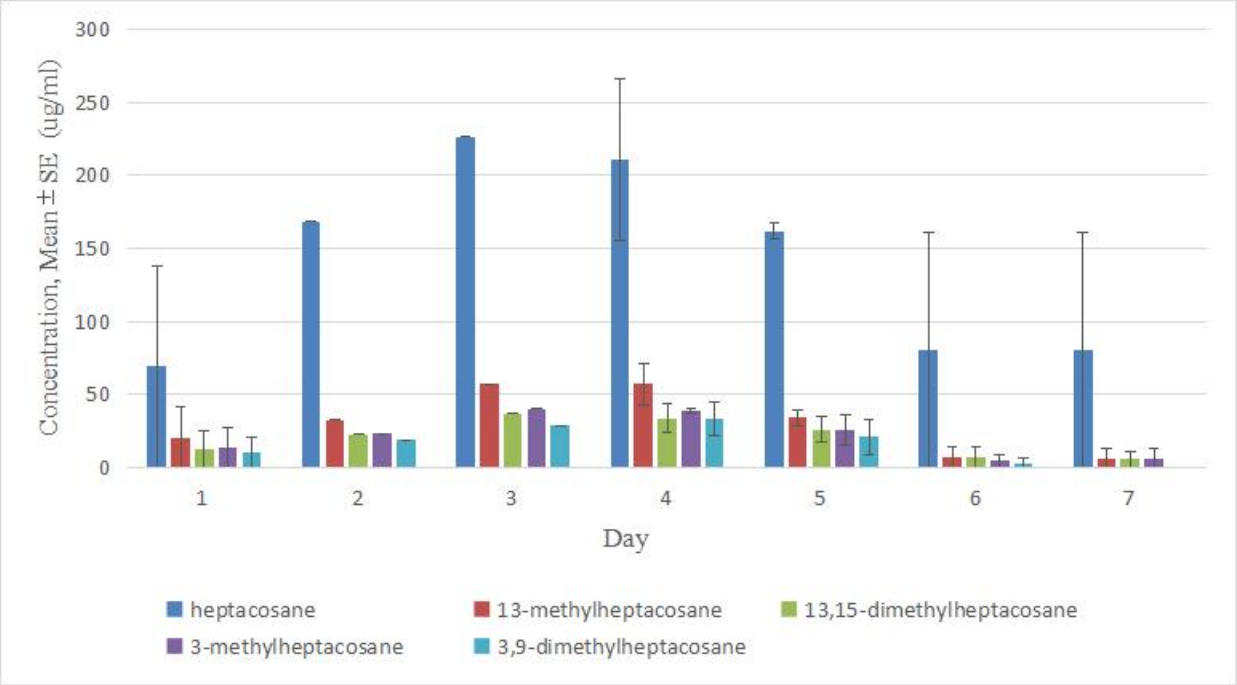


Fig. 7. Concentration of five major hydrocarbons (Mean±SE) in minims from colony L in relation to their age

Unlike other three sample group: heptacosane in colony L shows averagely significant higher concentration compared to other hydrocarbons in all different aged samples. Additionally, younger minims have higher concentrations of hydrocarbons especially in the 3-day-old and 4-day-old minims samples. In this group, we first discover the minims without trace of hydrocarbons in the first-day samples. Two samples were obtained from this group, the other sample shows a concentration of 138.4ug/ml heptacosane41.3ug/ml; 13-methylheptacosane; 25.1ug/ml 13,15-dimethylheptacosane; 27.8ug/ml 3-methylheptacosane; 20.7ug/ml 3,9-dimethylheptacosane and 2.4ug/ml squalene (Fig 7).

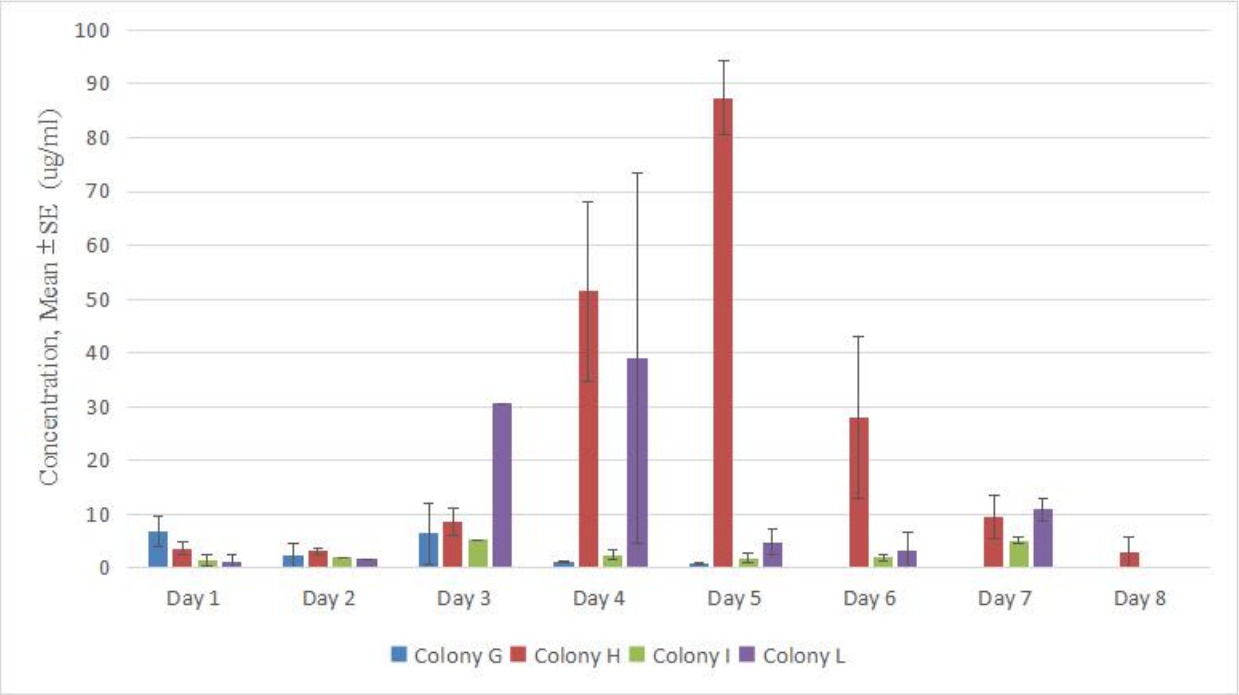


Fig. 8. Concentration (Mean ± SE) distribution of squalene in minims from colonies G, H, I and L in relation to their age.

Squalene was detected in all ant samples except for the ants from the sixth days in colony G. The concentration ranges from 0.55 to 96.18 ug/ml depending on different colony and age. In all four colonies, the concentration of squalene increase during Day 3 to Day 5 (Fig8).

Discussion

As previously noted, GC-MS is the method of choice for determining the molecular weight of every component in complex alkane mixtures such as those found in insect cuticle (Howard et al. 1980). The results of this study indicated that two main alkaloids, trans-2-methyl-6-n-tridecyl (or tridecenyl) piperidine (trans C_{13} and trans $C_{13:1}$), were found in minims. Venom alkaloid component of the minim is dominated by a single piperidine alkaloid, $C_{13:1}$. This agrees with previous study which showed $C_{13:1}$ alkaloids was the predominate component in venom (Vander Meer 1986). However, our study detected C_{13} alkaloid in minims of all 13 colonies. This is not

consistent with the minims lacking the other three predominate peaks (C_{13}, C_{15},$C_{15:1}$) in gas chromatographic analysis (Bosworth and Vander Meer 1984). Bosworth and Vander Meer used minims' weight and head widths to estimate the maturity of *S. invicta* colony (Bosworth and Vander Meer 1984). Our study avoided the inaccurate selection of different-aged minims. The results agree with the founding that $C_{13:1}$ amount in minims differentiated significantly from a positively correlation of the size-composition relationship for the percent of $C_{13:1}$ alkaloids (Vander Meer 1984). Young and old workers are known to produce less venom than intermediate-aged workers. The differences in venom composition also correspond to the size- and age-based functional role of workers (Deslippe and Guo 2000). This pattern is not consistent with your different aged minims. We speculate it might only be applicable between different castes. Unsaturated C_{13} and C_{15} alkaloids declined greatly in their dominance over saturated ones as worker age and body size increases (Deslippe and Guo 2000). The difference in the proportions of unsaturated alkaloids in venom and the ratios of C_{13} :$C_{13:1}$ and C_{15} :$C_{15:1}$ alkaloids might be a good indicator for distinguishing monogyne from polygyne form (Lai et al. 2008). We speculate both $C_{13:1}$ and C_{13} are functional venom alkaloids in minims. And the different ratio might indicate different biology features of the colony. Considering the limitation of current information, further study is need.

Five major hydrocarbons (heptacosane,13-methylheptacosane,13,15-Dimethylheptacosane, 3-Methylheptacosane, 3,9-Dimethylheptacosane) were found in minims. This study is the first to identify squalene in *S. invicta* minims. This five major hydrocarbon is also the five major ones in PPG and cuticular hydrocarbons of *S. invicta* (Nelson et al. 1980, Thompson et al. 1981). However, Our study showed heptacosane was the most dominant in five major hydrocarbons

within five days after eclosion. 13-Methylheptacosane remained the predominant hydrocarbon of PPG of queens 2-8 weeks after mating. Heptacosane is a major hydrocarbon in PPG of virgin queens (Thompson et al. 1981). Minim workers are an unique caste produced by the independently founding ant queens (Calabi and Porter 1989). This result shows the consistence of predominate hydrocarbon between virgin queens and minims. *Ixodes* ticks might be vulnerable to predation by ants because they lack the allomonal defensive secretion whose principle componet is squalene. However, other metastriate ticks could be protected against ants via the allomonal defensive secretion produced by the large wax glands (Yoder et al. 1993a). We speculated squlene that first time identified in *S. invicta* minims might have some functions in the colony. Additionally, the profiles of the three groups of chemicals varied quantitatively among different colonies and different ages of the minims from the same colony.

In conclusion, Venom alkaloid component of the minim is dominated by a single piperidine alkaloid, C13:1. Heptacosane was the most dominant in five major hydrocarbons within five days after eclosion. This study is the first to identify squalene in *S. invicta* minims. Our study fill the limited information of venom alkaloids and hydrocarbons of *S. invicta* minims.

CHAPTER 3

Bioactivity of Squalene and Heptadecane to Red Imported Fire Ants (Hymenoptera: Formicidae)

Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is an invasive species that was introduced from South America into the United States through the port of Mobile, Alabama in the early 1930s (Vinson and Sorensen 1986). Being one of the World's Worst Invasive Alien Species (Lowe et al. 2000), it rapidly widespread in the southern United States and has been introduced to other countries and regions of the world, including the Caribbean, Australia, New Zealand, Thailand, China (Ascunce et al. 2011). Studies have show that the invasion of *S. invicta* has a major impact on local ecosystems (Porter and Savignano 1990, Kaspari 2000). Fire ants also have detrimental influences on crops, machinery, livestock and public health (Davidson and Stone 1989, Vinson 1997, Taber 2000).

Squalene, like many other terpene compound, is fat-soluble. It is widespread in nature, and commonly found in olive oil, shark oil, wheat germ, and rice bran (Reddy and Couvreur 2009). Squalene was found in the defense secretion of ticks in 1993. It was speculated that either ticks extract squalene from the blood ingested by a preceding developmental stage or they synthesize this molecule (Yoder et al. 1993b). *Ixodes* ticks might be vulnerable to predation by ants because they lack the allomonal defensive secretion whose principle componet is squalene. However, other metastriate ticks could be protected against ants via the allomonal defensive secretion produced by the large wax glands (Yoder et al. 1993a). In 1999, a bioassay study showed

squalene contributes more to the tick's ability to locate hosts at greater distances than aggregation-attachment pheromones, indicating the potential of squalene for being used in attractant baits (Yoder et al. 1999). In 2002, it was reported that squalene might be involved in host location of a parasitoid, *Pholetesor bicolor*on Nees, in its the third trophic level (Dutton et al. 2002). Studies also showed that squalene is an exceedingly attractive semiochemical that provides nearly species and sex-specific attraction of male *Ceralocyna nigricornis* Gounelle (Jones et al. 2011).

Heptadecane is an alkane hydrocarbon with the chemical formula $C_{17}H_{36}$, the unbranched isomer is normal or n-heptadecane (Ingold 1953). N-heptadecane was found in the gasters of *Novomessor cockerelli* Andre (Hymenoptera: Formicidae) among the 8 main detected hydrocarbons (Vick et al. 1969). In 1972, n-heptadecane was examined in the Dufour gland in *Myrmica rubra*, but no volatile materials were found in the sting gland (Morgan and Wadhams 1972). Heptadecane has been found in Dufour's gland of workers of many ant species including *Myrmica. scabrinodis*, *Formica. nigricans*, *F. rufa*, *and F. polyctena* (Bergström and Löfqvist 1973, Morgan et al. 1979), but not widely found in the gaster gland. It was detected on foraging trails in the field as a component of the territorial odor (Salzemann et al. 1992). Pentadecane and heptadecane are often the most abundant alkanes in Myrmicines, and undecane or tridecane the most abundant alkanes in formicines (Morgan 2008). Heptadecane and pentadecane were produced by significantly more queens than other hydrocarbons and whose quantities were also significantly higher at lift off than the others (Richards et al. 2015). Heptadecane plays a vital role in regulating the behavior of swarming workers in honey bee (*Apis mellifera*).

In the last chapter, we first identified squalene in *S. invicta* minims. Combining the previous research information, our objective of this study was to assess the bioactivity of squalene and heptadecane on normal workers of *S. invicta*.

Materials and Methods

Source of normal workers

Solenopsis. invicta colonies were collected from Town Creek Park in Auburn, Alabama (32°35'N, 85°28'W). The workers were separated from the soil by the water drip method (Chen 2007a). Workers were placed into a plastic tray (wall was covered with baby powder to prevent ants from escaping) (40 by 25 by 12 cm) and held at 20–24°C and 45–55% RH, exposed to a photoperiod of 12:12 (L:D) h, and supplied with water, a 5% sugar solution, and dead crickets (*Acheta domesticus* Linnaeus, Armstrong Crickets*)* ad libitum. Ants were used for the laboratory experiments within 48 h of collection. Ants were transferred by brushes and index cards during experiment and no anesthesia was used to facilitate handling.

Chemicals

Squalene (S3626, ≥98%) and heptadecane (128503, 99%) were obtained from Sigma and Aldrich (3050 Spruce Street, St. Louis, MO 63103). The test components were prepared in five concentrations 5%, 2.5%, 1% ,0.5% and 0.1% with hexane by gradient dilution method.

Choice Test

Bioactivity of the test materials was determined using 10cm diameter× 1.5cm height glass petri dishes (Z666246 SIGMA). The inside of upper vertical wall of each petri dish was coated

with baby powder to prevent the ants from escaping. Half of the bottom surface inside the Petri dish was uniformly sprayed with 200ul of the suqlaene of heptadcane solution test component using a pipette(Z709263 SIGMA) and the other half of the bottom surface was uniformly sprayed with the same amount of hexane as the control. The Petri dishes were placed under an exhaust hood for 3 minutes before the bioassay. Groups of 10 fire ant workers were introduced into the center of each petri dish using index cards and a centrifuge tube(15 mL, 17x120, mmT1818 SIGMA). The ants were able to move freely to make selections between the treated and control surfaces. Each petri dish of ants was uncovered and exposed to the air. Each concentration was tested with five replicates (from same colony) and each Petri dish was used only once. The petri dishes for the control study were only sprayed with hexane. The numbers of ants on each side of each Petri dish were counted at different post-treatment periods. The studies were conducted under ambient temperature and relative humidity, averaging 22±2°C and 50 ± 5% RH The Pertri dishes were exposed to constant light (150–200 lux, INS Digital Lux Meter, Markson Scientific, Phoenix, AZ). Responding behaviors and responding behavioral changes of ants towards the treatment and control were recorded at different post-treatment times. Repellency was defined as the mean percentage of ants present on the treated side of the Petri dish(If more ants on treated side means attractance). Five replicates dishes containing 10 ants each were used for each treatment in a completely randomized design.

Data Analysis

Repellency (percentage of ants in the treated side of the Petri dish) was analyzed using usingTukey's honestly significant difference (HSD) test in SPSS (17.0). A significance level of P=0.05 was used for all statistical tests.

Results

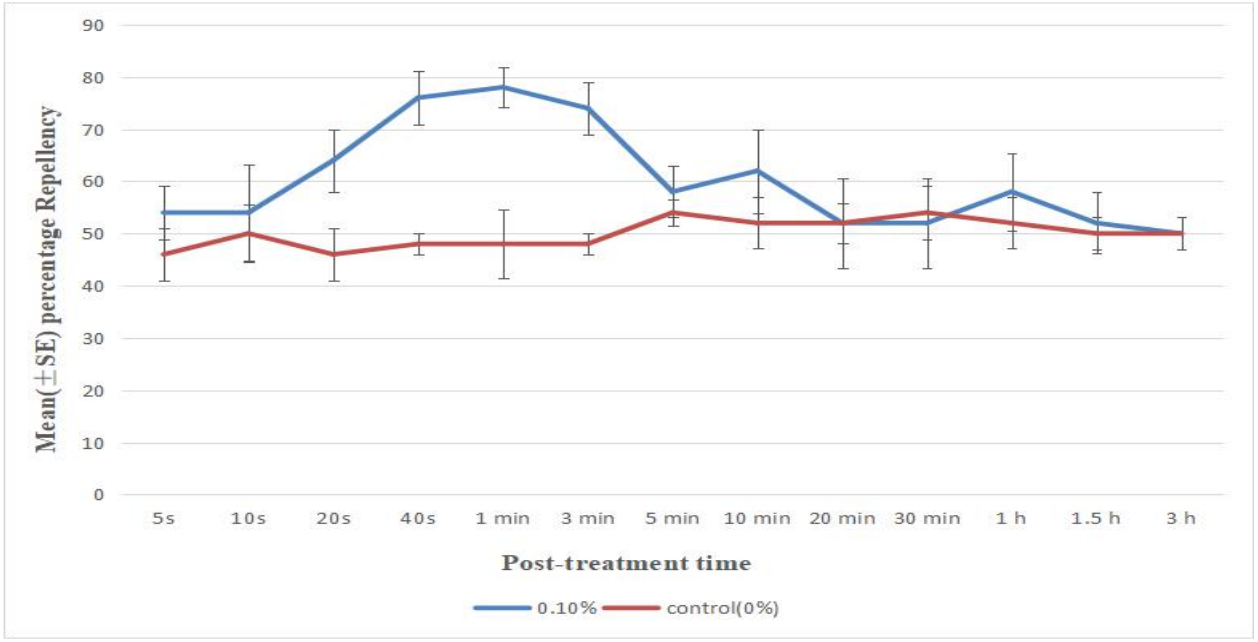


Fig. 9. Mean(±SE) percentage attractancy of 0.1% squalene to RIFA workers in relation to time.

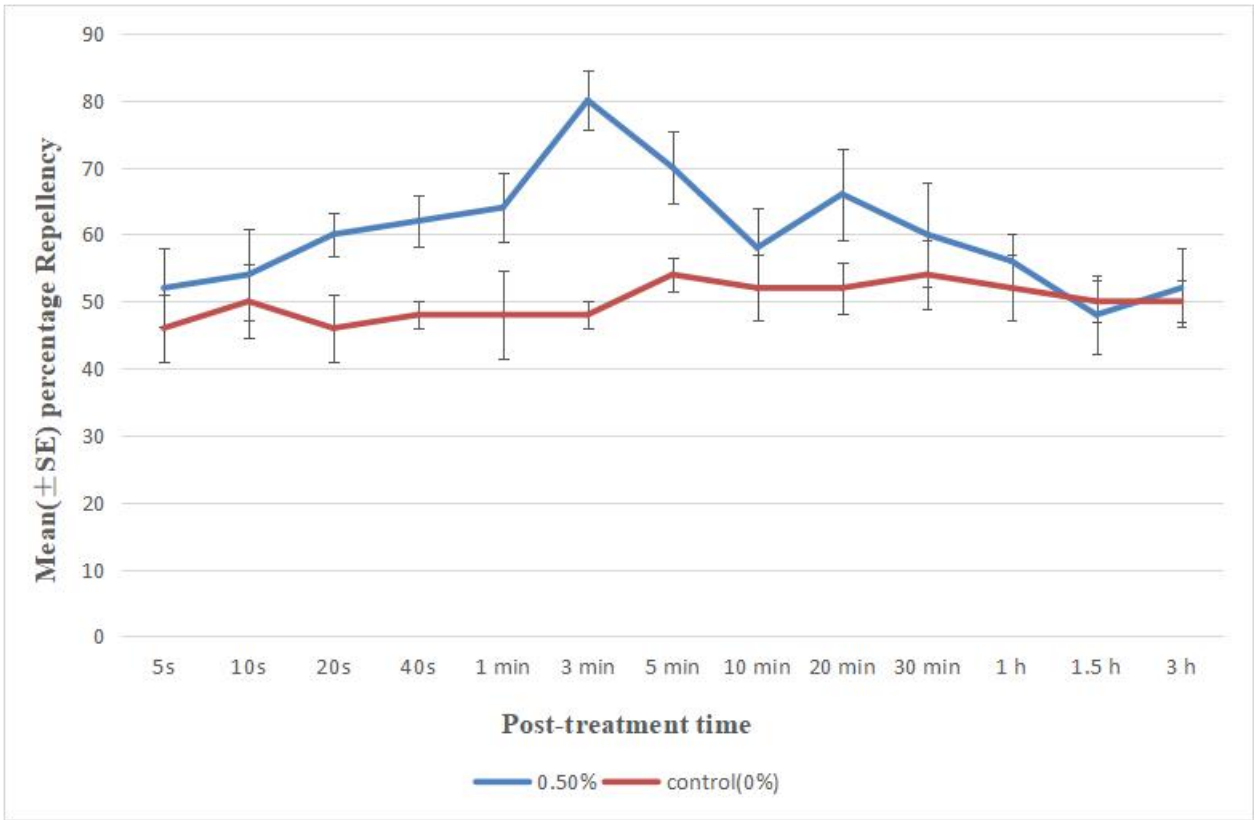


Fig. 10. Mean(±SE) percentage attractancy of 0.5% squalene to RIFA workers in relation to time.

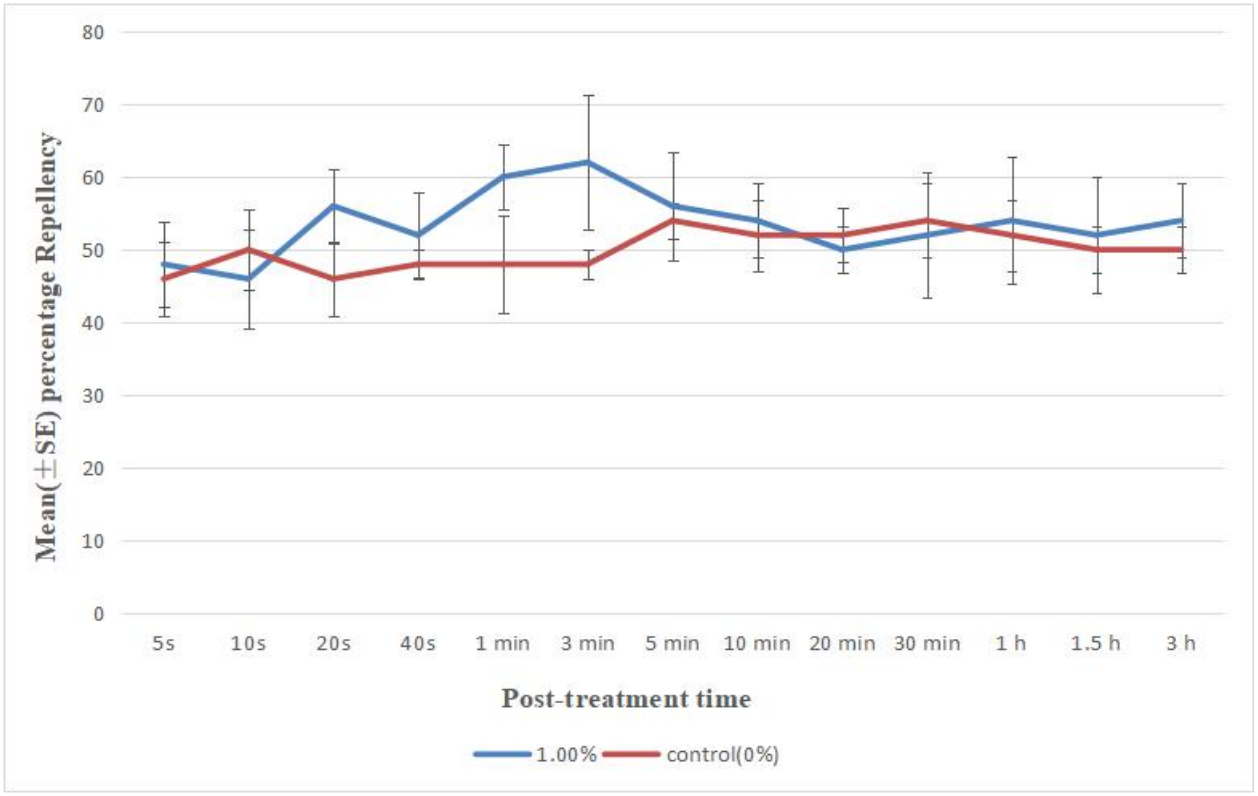


Fig. 11. Mean(±SE) percentage repellency of 1% squalene to RIFA workers in relation to time.

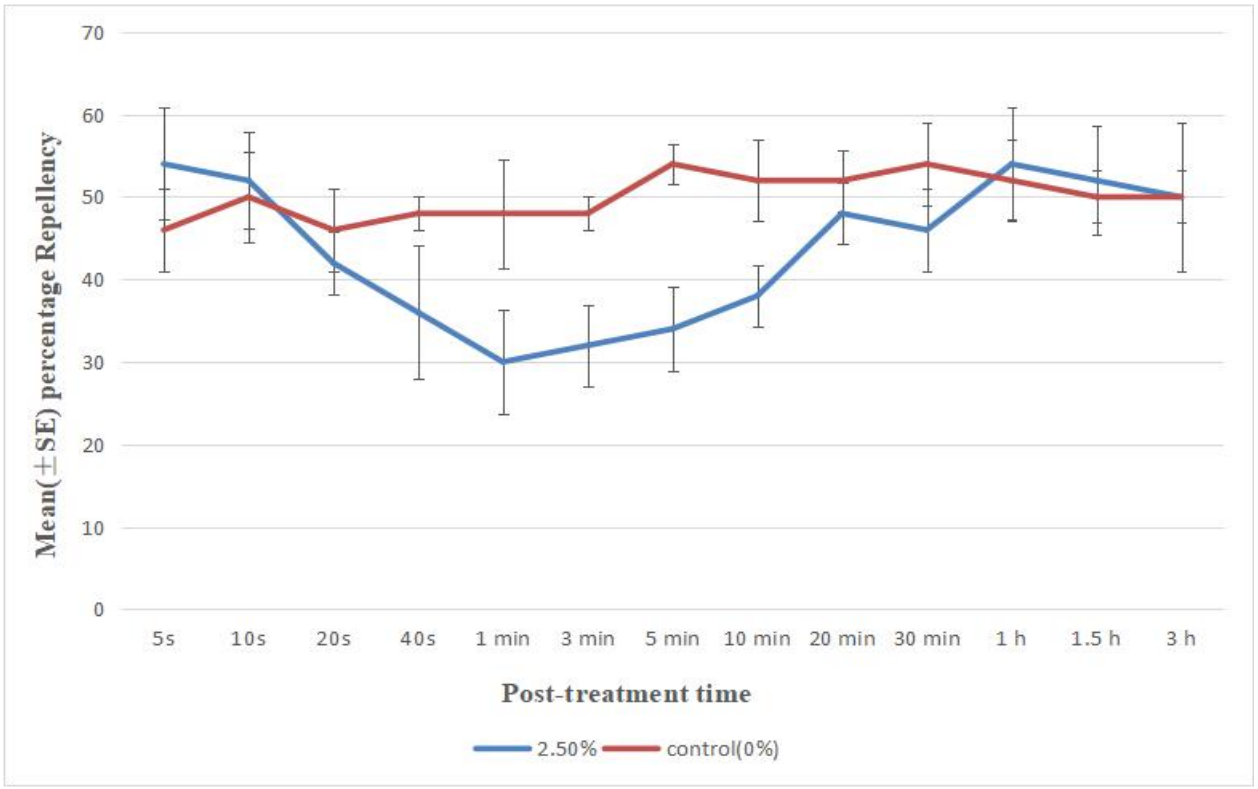


Fig. 12. Mean(±SE) percentage repellency of 2.5% squalene to RIFA workers in relation to time

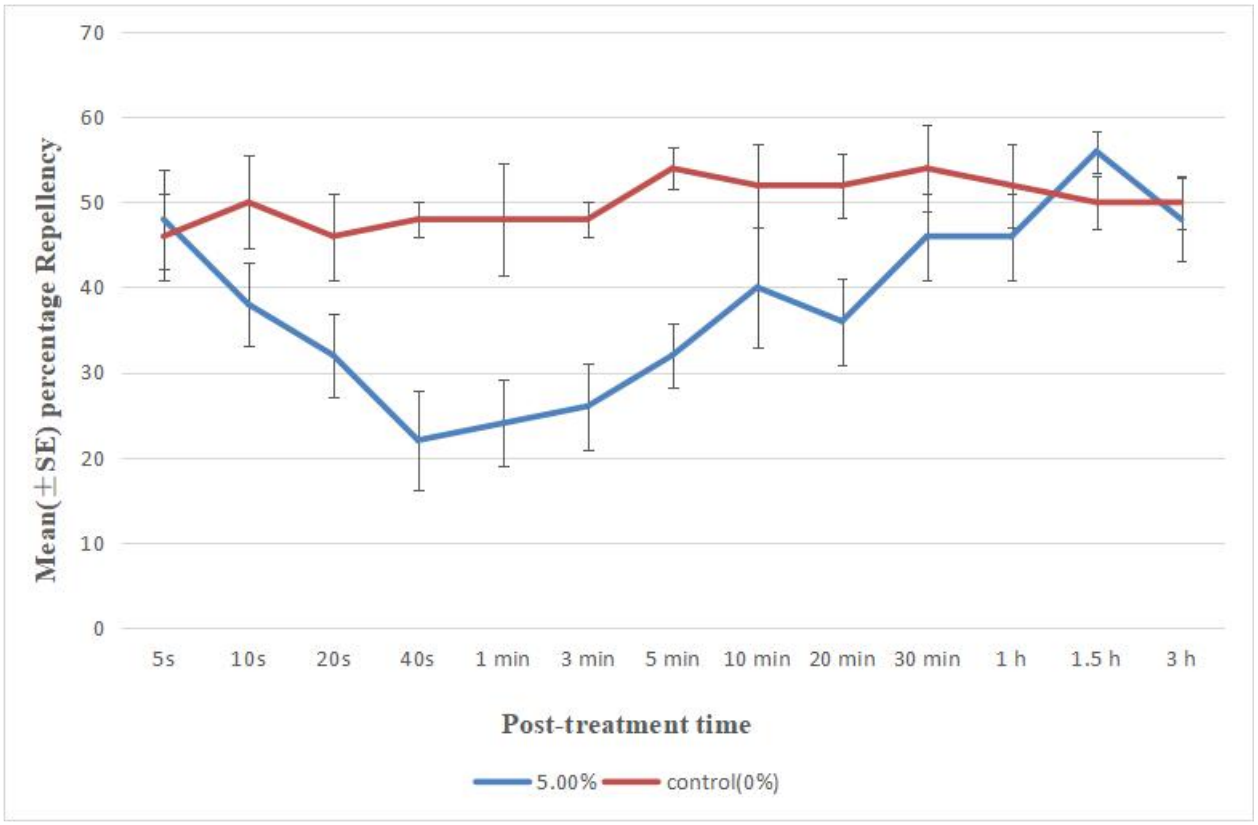


Fig. 13. Mean(±SE) percentage repellency of 5% squalene to RIFA workers in relation to time

0.1% squalene concentration showed attractance at 40s, 1 minute and 3 minute(P<0.05); 0.5% squalene concentration showed significant attractance at 3 minutes (P<0.05); 1% squalene concentration wasn't bioactive during recorded time(P>0.05); 2.5% squalene concentration showed repellency at 3 minute and 5 minutes(P<0.05); 5% squalene concentration showed repellency at 40s, 1 minute and 3 minute(P<0.05).

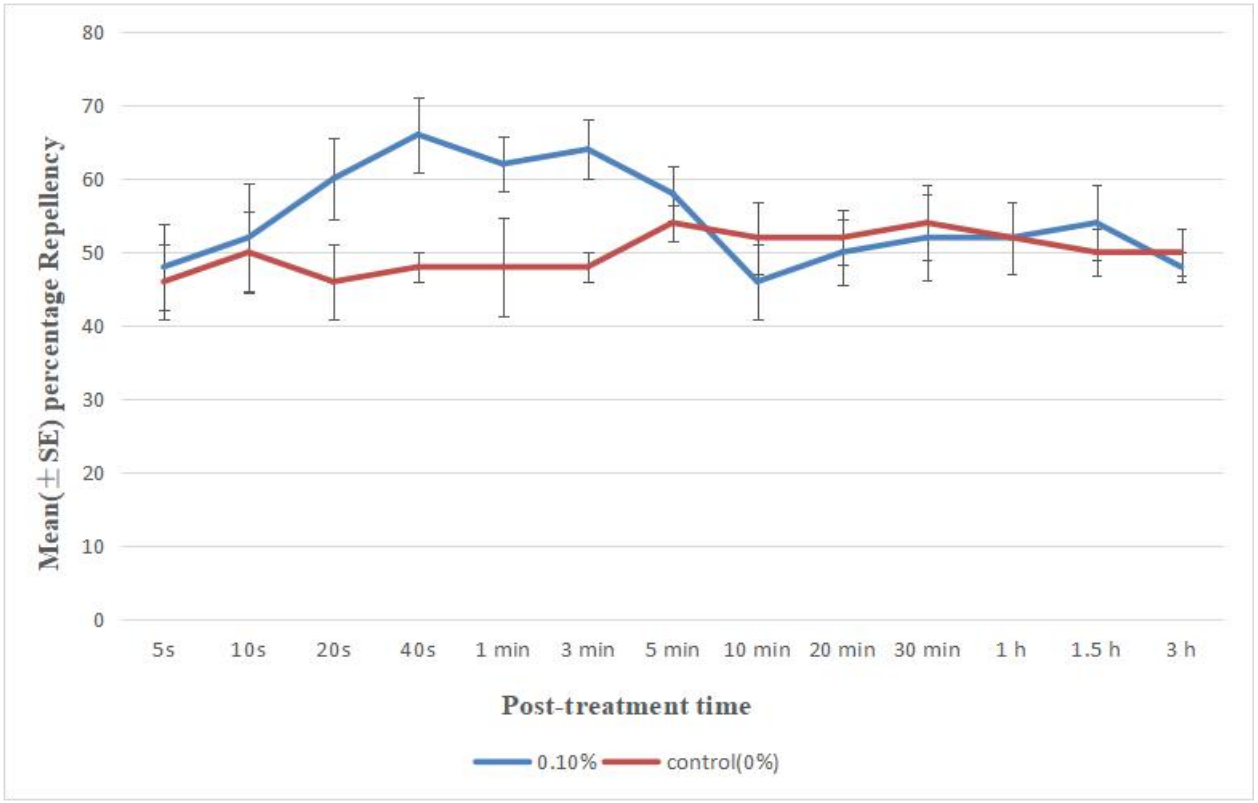


Fig.14. Mean (±SE) percentage attractancy of 0.1% heptadecane to RIFA workers in relation to time

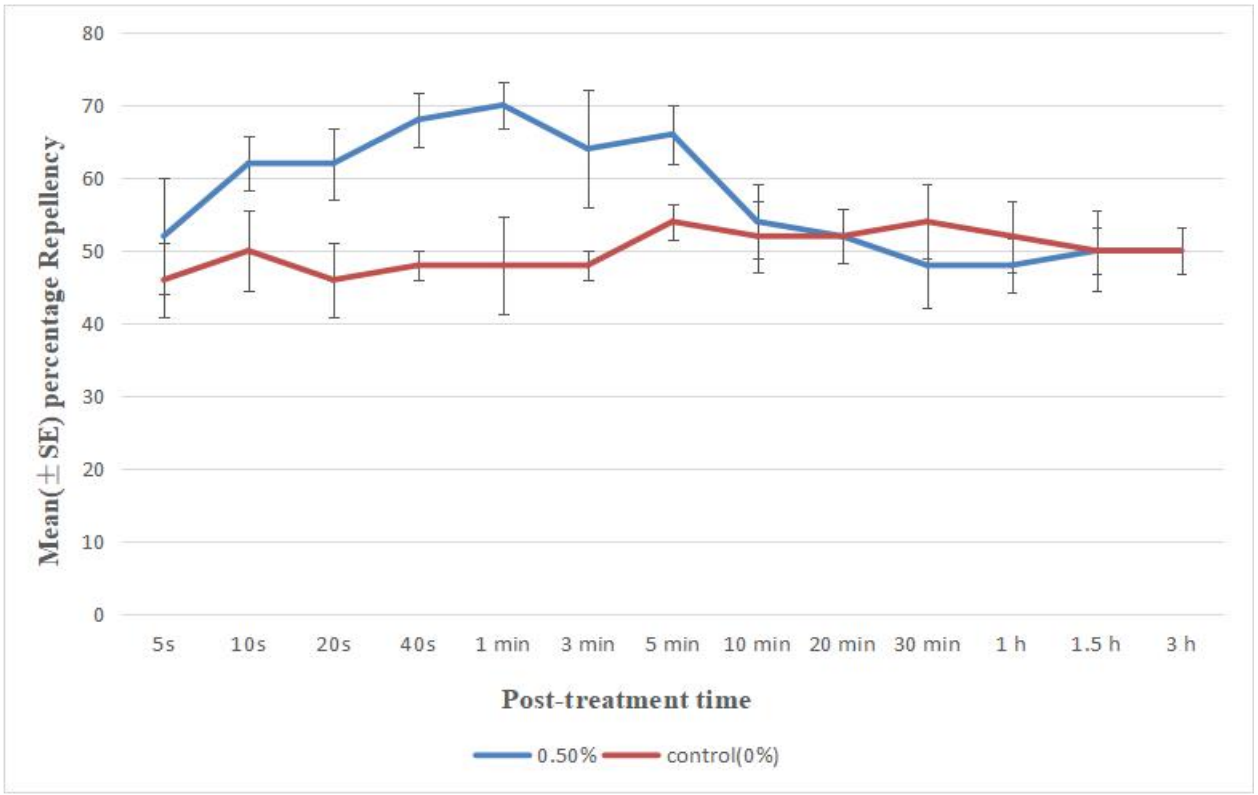


Fig. 15. Mean(±SE) percentage attractancy of 0.5% heptadecane to RIFA workers in relation to time

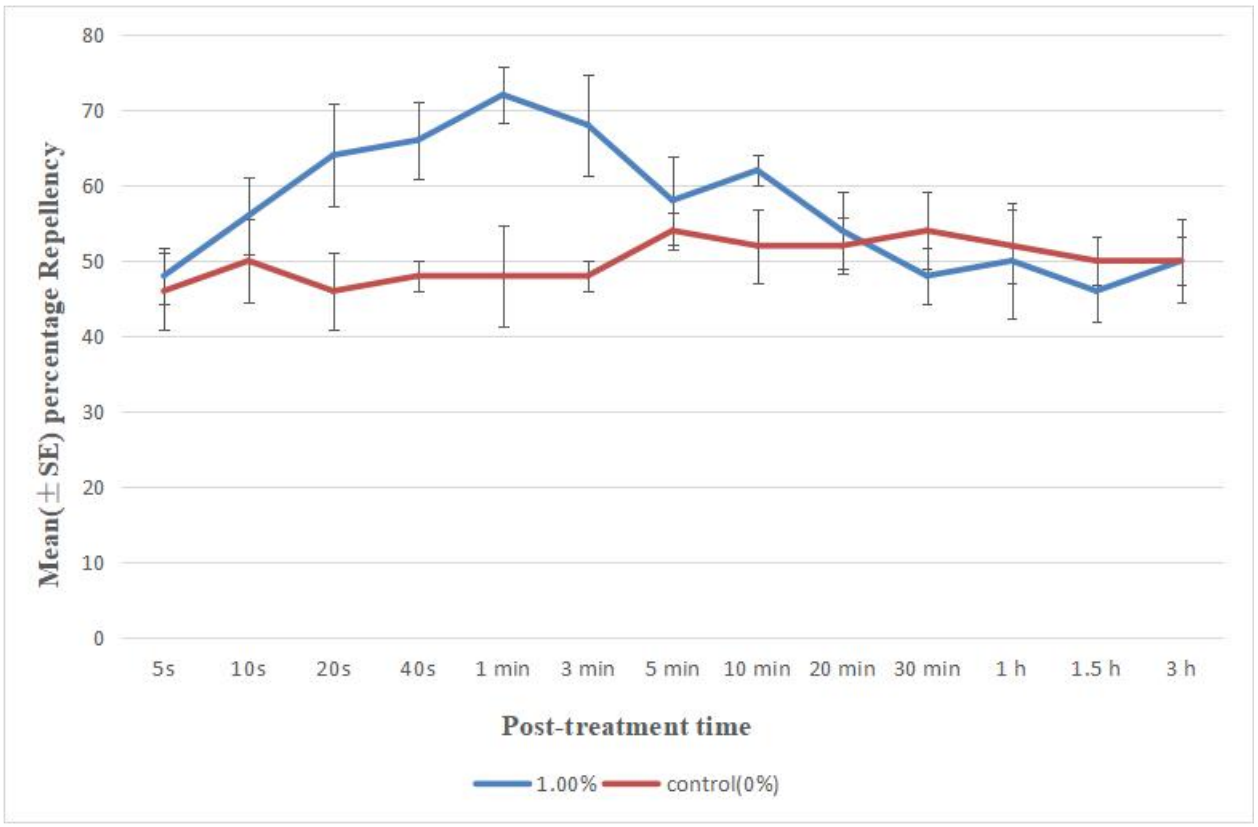


Fig. 16. Mean(±SE) percentage attractancy of 1% heptadecane to RIFA workers in relation to time

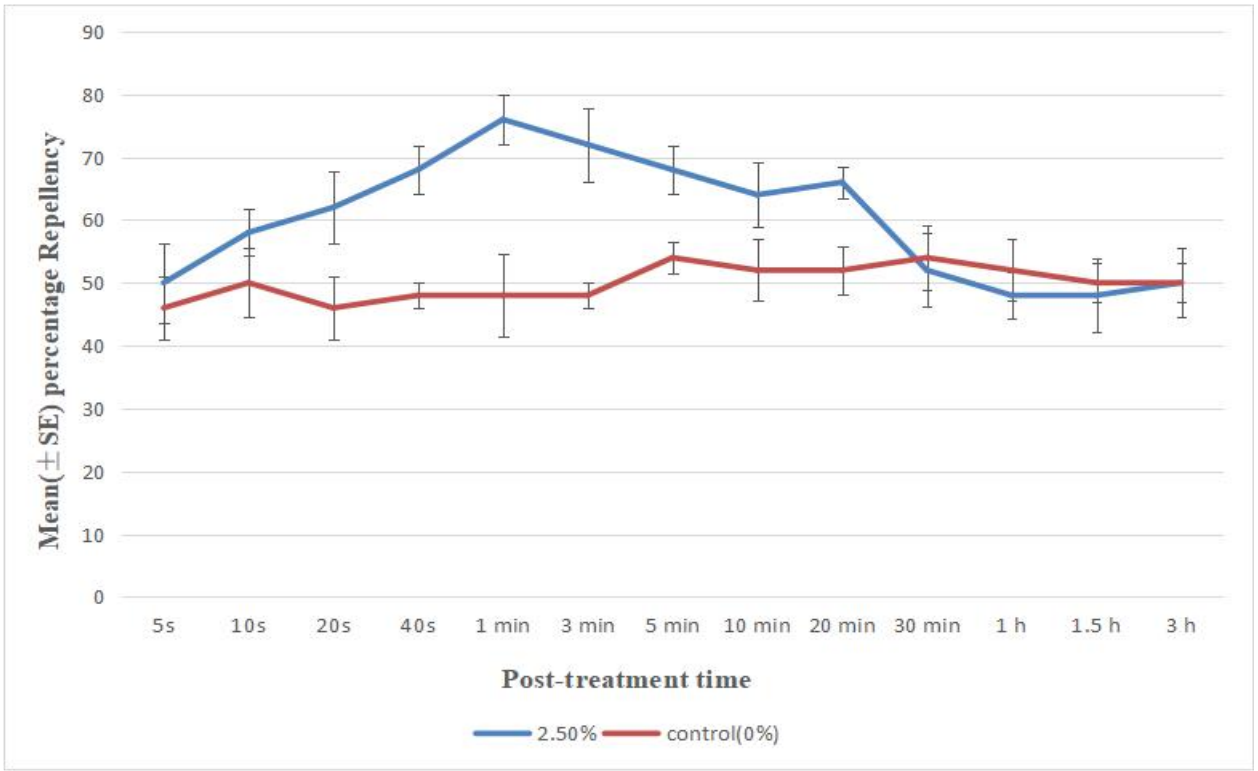


Fig. 17. Mean(±SE) percentage attractancy of 2.5% heptadecane to RIFA workers in relation to time

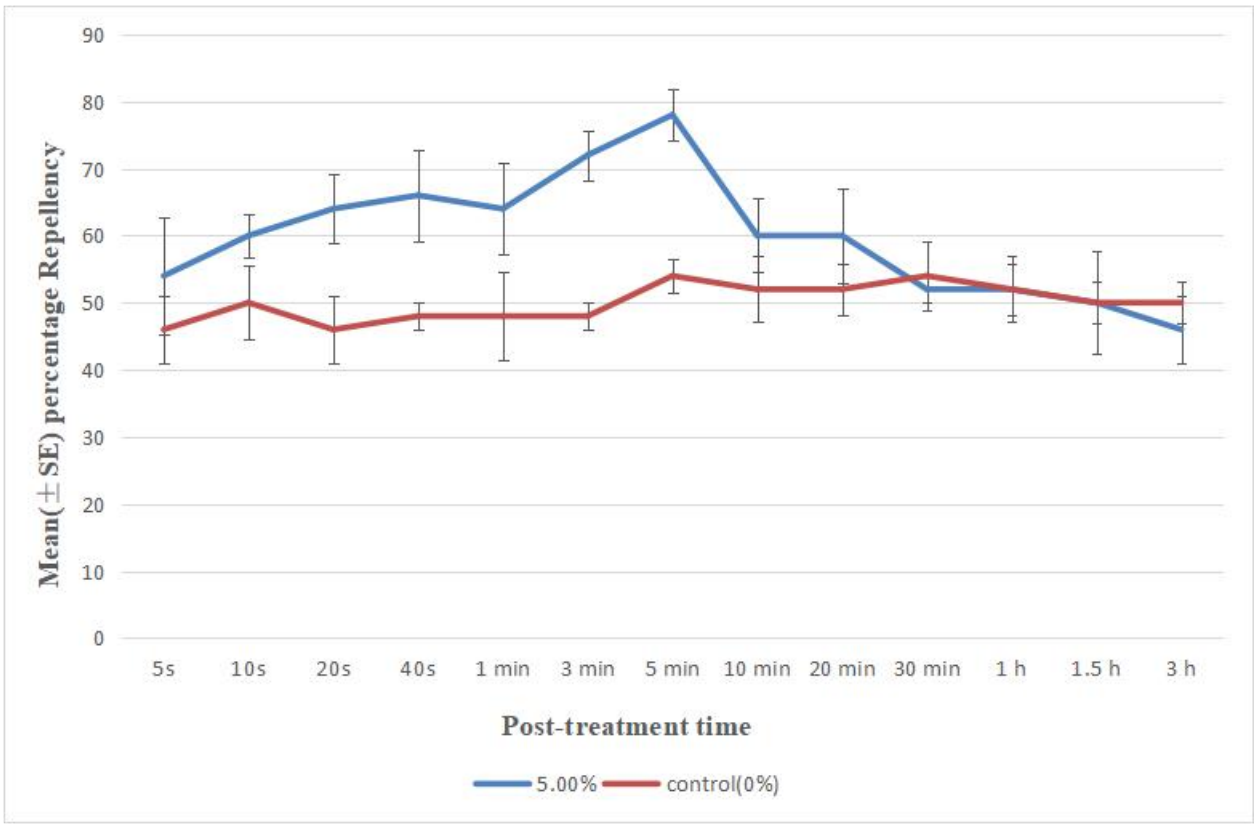


Fig. 18. Mean(±SE) percentage attractancy of 5% heptadecane to RIFA workers in relation to time

0.1% heptadecane concentration wasn't bioactive during during recorded time(P>0.05); 0.5% heptadecane concentration showed attractance 40s and 1 minute (P<0.05); 1% heptadecane concentration showed attractance 1 minute and 3 minute (P<0.05); 2.5% heptadecane concentration showed attractance during 40s, 1 minute and 3 minute (P<0.05); 5% heptadecane concentration showed attractance at 3 minute and 5 minute (P<0.05).

Discussion

Our study showed both squalene and heptdecane are biactive to normal workers of *S. invicta*. Squalene was attractive at low concentrations but repellent at higher concentrations. Heptadecane was attractive. Hydrocarbons have many functions such as territory marking, alarm pheromones, defensive secretions and sex pheromones in insects. (Howard and Blomquist 1982).

In Our study, we allow workers moving freely between control and treated side of the Petri dish. Results showed 0.1% squalene concentration showed attractance at 40s, 1 minute and 3 minute(P<0.05); 0.5% squalene concentration showed significant attractance at 3 minutes (P<0.05); 2.5% squalene concentration showed repellency at 3 minute and 5 minutes(P<0.05); 5% squalene concentration showed repellency at 40s, 1 minute and 3 minute(P<0.05). We speculate the different response of *S. invicta* workers and different concentration might be related. A study reported that adults, especially female *Sarcoptes scabiei* (L.) were attracted more than immatures to most compounds including squalene (Arlian and Vyszenski-Moher 1995). In 1999, a bioassay study showed squalene contributes more to the tick's ability to locate hosts at greater distances than aggregation-attachment pheromones, indicating the potential of squalene for being used in attractant baits (Yoder et al. 1999). Both squalene and heptadecane have been identified in semichemical extracts of insects in the family Formicidae. These provide the possibility of squalene function as a potential semiochemical in *Solenopsis invicta* (Hefetz et al. 2001).

Compared to squalene, heptadecane is more likely to be found in Dour's gland (such as *Lasius fuliginosus*) and trail pheromone from S*olenopsis invicta* (Akino and Yamaoka 1996). In our study, we found *S. invicta* was attractive to heptadecane in different concentration. Except for the 0.1% concentration, it showed attractance in all other concentration at different time. In 1967, heptadecane was detected in Dufour's gland constituents of the bull ant, *Myrmecia gulosa* Fabricius. (Cavill and Williams 1967). N-heptadecane was also found in the gasters of *Novomessor cockerelli* Andre (Hymenoptera: Formicidae) among the 8 main detected hydrocarbons (Vick et al. 1969). Heptadecane has been found in Dufour's gland of workers of many ant species including *Myrmica. scabrinodis*, *Formica. nigricans*, *F. rufa*, *and F. polyctena*

(Bergström and Löfqvist 1973, Morgan et al. 1979), but not widely found in the gaster gland. Heptadecane was detected on foraging trails in the field as a component of the territorial odor (Salzemann et al. 1992). These studies showed different functions of heptadecane and it is widely distribute in the Formicidae. Because both squalene and heptadecane are volites (Jirapong et al. 2010). This attribute and concentration might explain the different recorded bioactive time.

Our study shows two single hydrocarbons, squalene and heptadecane, are bioactive to *S invicta* normal workers. This might contribute to the further study of these two compounds' bioactivity to other castes of *S. invicta* as well as nestmate recognition (Lavine et al. 2011).

Reference

Adams, C. 1986. Agricultural and medical impact of the imported fire ants. Fire ants and leaf cutting ants: biology and management. Westview Press, Boulder, CO 435: 48-57.

Adams, E. S., and W. R. Tschinkel. 1995. Effects of foundress number on brood raids and queen survival in the fire ant *Solenopsis invicta*. Behavioral Ecology and Sociobiology 37: 233-242.

Adams, E. S., and M. T. Balas. 1999. Worker discrimination among queens in newly founded colonies of the fire ant *Solenopsis invicta*. Behavioral Ecology and Sociobiology 45: 330-338.

Akino, T., and R. Yamaoka. 1996. Purification of the trail pheromone of *Lasius fuliginosus* Latreille. Japanese Journal of Applied Entomology and Zoology 40: 233-238.

Arlian, L. G., and D. L. Vyszenski-Moher. 1995. Response of *Sarcoptes scabiei var. canis* (Acari: Sarcoptidae) to lipids of mammalian skin. Journal of Medical Entomology 32: 34-41.

Ascunce, M. S., C.-C. Yang, J. Oakey, L. Calcaterra, W.-J. Wu, C.-J. Shih, J. Goudet, K. G. Ross, and D. Shoemaker. 2011. Global invasion history of the fire ant *Solenopsis invicta*. Science 331: 1066-1068.

Balas, M. T., and E. S. Adams. 1996. Nestmate discrimination and competition in incipient colonies of fire ants. Animal Behaviour 51: 49-59.

Barlin, M. R., M. S. Blum, and M. M. Brand. 1976. Fire ant trail pheromones: Analysis of species specificity after gas chromatographic fractionation. Journal of Insect Physiology 22: 839-844.

Bartz, S. H., and B. Hölldobler. 1982. Colony founding in *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. Behavioral Ecology and Sociobiology 10: 137-147.

Bass, J., and S. Hays. 1979. Nuptial flights of the imported fire ant in South Carolina. Journal.

Bergström, G., and J. Löfqvist. 1973. Chemical congruence of the complex odoriferous secretions from Dufour's gland in three species of ants of the genus *Formica*. Journal of Insect Physiology 19: 877-907.

Bernasconi, G., and L. Keller. 1996. Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). Proceedings of the Royal Society of London B: Biological Sciences 263: 509-513.

Bernasconi, G., and L. Keller. 1999. Effect of queen phenotype and social environment on early queen mortality in incipient colonies of the fire ant, *Solenopsis invicta*. Animal Behaviour 57: 371-377.

Blum, M. S. 1988. Biocidal and deterrent activities of nitrogen heterocycles produced by venomous Myrmicine ants. ACS Publications.

Bolton, B., G. Alpert, P. Ward, and P. Naskrecki. 2006. Bolton's catalogue of ants of the world: 1758-2005, Harvard University Press Cambridge, MA.

Bosworth, J., and R. Vander Meer. Year. Published. Colony founding minims: A new *Solenopsis invicta* caste. In 1984 Imported Fire Ant Conference, Gainesville, Fla.(USA), 27-28 Mar 1984, 1984. Veterinary Medical Exp. Stn.

Bourke, A. F., and N. R. Franks. 1995. Social evolution in ants, Princeton University Press.

Brand, J., M. Blum, H. Fales, and J. MacConnell. 1972. Fire ant venoms: comparative analyses of alkaloidal components. Toxicon 10: 259-271.

Brand, J. M. 1978. Fire ant venom alkaloids: their contribution to chemosystematics and biochemical evolution. Biochemical Systematics and Ecology 6: 337-340.

Buren, W. F. 1972. Revisionary studies on the taxonomy of the imported fire ants. J. Ga. Entomol. Soc 7: 1-26.

Cabrera, A., D. Williams, J. V. Hernández, F. H. Caetano, and K. Jaffe. 2004. Metapleural-and Postpharyngeal-Gland Secretions from Workers of the Ants *Solenopsis invicta* and *S. geminata*. Chemistry & Biodiversity 1: 303-311.

Calabi, P., and S. D. Porter. 1989. Worker longevity in the fire ant *Solenopsis invicta*: ergonomic considerations of correlations between temperature, size and metabolic rates. Journal of Insect Physiology 35: 643-649.

Callahan, P. S., M. S. Blum, and J. R. Walker. 1959. Morphology and histology of the poison glands and sting of the imported fire ant (*Solenopsis saevissima* v. *richteri* Forel). Annals of the Entomological Society of America 52: 573-590.

Carlson, D. 1980. Identification of mosquitoes of *Anopheles gambiae* species complex A and B by analysis of cuticular components.

Cavill, G., and P. Williams. 1967. Constituents of Dufour's gland in *Myrmecia gulosa*. Journal of Insect Physiology 13: 1097-1103.

Chen, J. 2007a. Advancement on techniques for the separation and maintenance of the red imported fire ant colonies. Insect science 14: 1-4.

Chen, J. 2007b. Qualitative analysis of red imported fire ant nests constructed in silica gel. Journal of Chemical Ecology 33: 631-642.

Chen, L., and H. Y. Fadamiro. 2009. Re-investigation of venom chemistry of *Solenopsis* fire ants. II. Identification of novel alkaloids in *S. invicta*. Toxicon 53: 479-486.

Chen, Y.-F., H.-C. Chang, and C.-M. Chao. 2005. Fire ant, a new hazard to military camps in Taiwan. Journal of Medical Sciences 25: 161-165.

Crozier, R. H. 1971. Heterozygosity and sex determination in haplo-diploidy. The American Naturalist 105: 399-412.

Dai, L., M. R. Jacob, S. I. Khan, I. A. Khan, A. M. Clark, and X.-C. Li. 2011. Synthesis and antifungal activity of natural product-based 6-alkyl-2, 3, 4, 5-tetrahydropyridines. Journal of Natural Products 74: 2023-2026.

Davis Jr, L. R., R. K. Van der Meer, and S. D. Porter. 2001. Red imported fire ants expand their range across the West Indies. Florida Entomologist 84: 735.

Deslippe, R. J., and Y.-J. Guo. 2000. Venom alkaloids of fire ants in relation to worker size and age. Toxicon 38: 223-232.

Drees, B. M., and R. E. Gold. 2003. Development of Integrated Pest Management Programs for the Red Imported Fire Ant (Hymenoptera: Formicidae) 1. Journal of Entomological Science 38: 170-180.

Dutton, A., L. Mattiacci, R. Amadò, and S. Dorn. 2002. A novel function of the triterpene squalene in a tritrophic system. Journal of Chemical Ecology 28: 103-116.

Eliyahu, D., K. G. Ross, K. L. Haight, L. Keller, and J. Liebig. 2011. Venom alkaloid and cuticular hydrocarbon profiles are associated with social organization, queen fertility status, and queen genotype in the fire ant *Solenopsis invicta*. Journal of Chemical Ecology 37: 1242-1254.

Glancey, B. M., C. Stringer, and P. Bishop. 1973. Trophic egg production in the imported fire ant, *Solenopsis invicta*. Ga Entomol Soc J.

Glancey, B. M., C. Stringer, C. Craig, and P. Bishop. 1975. An extraordinary case of polygyny in the red imported fire ant. Annals of the Entomological Society of America 68: 922-922.

Goodfellow, R., and G. Liu. 1972. Squalene metabolism in the larva of the fly, *Sarcophaga bullata*. Journal of Insect Physiology 18: 95-103.

Goodfellow, R., H. Radtke, Y. Huang, and G. Liu. 1973. Studies on squalene biosynthesis in *Sarcophaga bullata* larvae. Insect Biochemistry 3: 61-65.

Haight, K. L. 2002. Fire ant venom economy: patterns of synthesis and use.

Haight, K. L., and W. R. Tschinkel. 2003. Patterns of venom synthesis and use in the fire ant, *Solenopsis invicta*. Toxicon 42: 673-682.

Hashim, Y. Z., M. Eng, C. I. Gill, H. McGlynn, and I. R. Rowland. 2005. Components of olive oil and chemoprevention of colorectal cancer. Nutrition reviews 63: 374-386.

Hefetz, A., V. Soroker, A. Dahbi, M. C. Malherbe, and D. Fresneau. 2001. The front basitarsal brush in *Pachycondyla apicalis* and its role in hydrocarbon circulation. Chemoecology 11: 17-24.

Hermann, H. R., and M. S. Blum. 1981. Defensive mechanisms in the social Hymenoptera. Social insects 2: 77-197.

Hoffman, D. R. 1993. Allergens in Hymenoptera venom XXV: The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. Journal of Allergy and Clinical Immunology 92: 707-716.

Hölldobler, B., and E. O. Wilson. 1990. The ants, Harvard University Press.

Hood, W. G., and W. R. Tschinkel. 1990. Desiccation resistance in arboreal and terrestrial ants. Physiological Entomology 15: 23-35.

Howard, R. W., and G. J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Annual Review of Entomology 27: 149-172.

Howard, R. W., C. McDaniel, and G. J. Blomquist. 1978. Cuticular hydrocarbons of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar)(Isoptera: Rhinotermitidae). Journal of Chemical Ecology 4: 233-245.

Howard, R. W., C. McDaniel, D. R. Nelson, and G. J. Blomquist. 1980. Chemical ionization mass spectrometry. Journal of Chemical Ecology 6: 609-623.

Hung, A. C., S. B. Vinson, and J. W. Summerlin. 1974. Male sterility in the red imported fire ant, *Solenopsis invicta*. Annals of the Entomological Society of America 67: 909-912.

Ingold, C. K. 1953. Structure and mechanism in organic chemistry, Cornell University Press; Ithaca; New York.

Jemal, A., and M. Hugh-Jones. 1993. A review of the red imported fire ant (*Solenopsis invicta* Buren) and its impacts on plant, animal, and human health. Preventive Veterinary Medicine 17: 19-32.

Jirapong, C., K. Inplub, and C. Wongs-Aree. Year. Published. Volatile compounds in four species of Thai waterlily (Nymphaea spp.), pp. 117-122. *In*, Asia Pacific Symposium on Postharvest Research, Education and Extension 943, 2010.

Jones, T. H., M. S. Blum, and H. M. Fales. 1982. Ant venom alkaloids from *Solenopsis* and *Monorium* species: recent developments. Tetrahedron 38: 1949-1958.

Jones, V. P., S. A. Steffan, N. G. Wiman, D. R. Horton, E. Miliczky, Q.-H. Zhang, and C. C. Baker. 2011. Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards. Biological Control 56: 98-105.

Kaplan, I., and M. D. Eubanks. 2002. Disruption of cotton aphid (Homoptera: Aphididae)—natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). Environmental Entomology 31: 1175-1183.

Kaspari, M. 2000. Do imported fire ants impact canopy arthropods? Evidence from simple arboreal pitfall traps. The Southwestern Naturalist: 118-122.

Kaspari, M., and E. Vargo. 1994. Nest site selection by fire ant queens. Insectes Sociaux 41: 331-333.

Keller, L., and K. G. Ross. 1993. Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta*. Behavioral Ecology and Sociobiology 33: 121-129.

King, J. R. 2004. Ant communities of Florida's upland ecosystems: ecology and sampling. University of Florida.

Lahav, S., V. Soroker, A. Hefetz, and R. K. Vander Meer. 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. Naturwissenschaften 86: 246-249.

Lai, L.-C., R.-N. Huang, and W.-J. Wu. 2008. Venom alkaloids of monogyne and polygyne forms of the red imported fire ant, *Solenopsis invicta*, in Taiwan. Insectes Sociaux 55: 443-449.

Lavine, B. K., N. Mirjankar, and R. K. Vander Meer. 2011. Analysis of chemical signals in red fire ants by gas chromatography and pattern recognition techniques. Talanta 83: 1308-1316.

Li, S., X. Jin, and J. Chen. 2012. Effects of piperidine and piperideine alkaloids from the venom of red imported fire ants, *Solenopsis invicta* Buren, on *Pythium ultimum* Trow growth in vitro and the application of piperideine alkaloids to control cucumber damping-off in the greenhouse. Pest Management Science 68: 1546-1552.

Lofgren, C., W. Banks, and B. Glancey. 1975. Biology and control of imported fire ants. Annual Review of Entomology 20: 1-30.

Lok, J. B., E. W. Cupp, and G. J. Blomquist. 1975. Cuticular lipids of the imported fire ants, *Solenopsis invicta* and *richteri*. Insect Biochemistry 5: 821-829.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database.

MacConnell, J., M. Blum, W. Buren, R. Williams, and H. Fales. 1976. Fire ant venoms: chemotaxonomic correlations with alkaloidal compositions. Toxicon 14: 69-78.

Markin, G. P., and J. H. Dillier. 1971. The seasonal life cycle of the imported fire ant, *Solenopsis saevissima* richteri, on the Gulf Coast of Mississippi. Annals of the Entomological Society of America 64: 562-565.

Markin, G. P., H. L. Collins, and J. H. Dillier. 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Annals of the Entomological Society of America 65: 1053-1058.

Morgan, E., and L. Wadhams. 1972. Chemical constituents of Dufour's gland in the ant, *Myrmica rubra*. Journal of Insect Physiology 18: 1125-1135.

Morgan, E., K. Parry, and R. Tyler. 1979. The chemical composition of the Dufour gland secretion of the ant *Myrmica scrabrinodis*. Insect Biochemistry 9: 117-121.

Morgan, E. D. 2008. Chemical sorcery for sociality: exocrine secretions of ants (Hymenoptera: Formicidae). Myrmecol News 11: 79-90.

Morrill, W. L. 1974. Production and flight of alate red imported fire ants. Environmental Entomology 3: 265-271.

Morrison, L. W. 2002. Long-term impacts of an arthropod-community invasion by the Imported Fire Ant, *Solenopsis invicta*. Ecology 83: 2337-2345.

Morrison, L. W., S. D. Porter, E. Daniels, and M. D. Korzukhin. 2004. Potential global range expansion of the invasive fire ant, *Solenopsis invicta*. Biological Invasions 6: 183-191.

Moulis, R. 1997. Predation by the Imported Fire Ant(*Solenopsis invicta*) on Loggerhead Sea Turtle(*Caretta caretta*) Nests on Wassaw National Wildlife Refuge, Georgia. Chelonian Conservation and Biology 2: 433-436.

Mpuru, S., J. R. Reed, R. C. Reitz, and G. J. Blomquist. 1996. Mechanism of hydrocarbon biosynthesis from aldehyde in selected insect species: Requirement for O_2 and NADPH

and carbonyl group released as CO_2. Insect Biochemistry and Molecular Biology 26: 203-208.

Nelson, D. R., C. L. Fatland, R. W. Howard, C. McDaniel, and G. J. Blomquist. 1980. Re-analysis of the cuticular methylalkanes of *Solenopsis invicta* and *S. richteri*. Insect Biochemistry 10: 409-418.

Newmark, H. L. 1997. Squalene, olive oil, and cancer risk: a review and hypothesis. Cancer Epidemiology and Prevention Biomarkers 6: 1101-1103.

Nickerson, J., W. Whitcomb, A. Bhatkar, and M. Naves. 1975. Predation on founding queens of *Solenopsis invicta* by workers of *Conomyrma insana*. Florida Entomologist: 75-82.

O'Neal, J., and G. Markin. 1973. Brood nutrition and parental relationships of the imported fire ant *Solenopsis invicta*. Journal of the Georgia Entomological Society.

Obin, M. S., and R. K. Vander Meer. 1985. Gaster flagging by fire ants (*Solenopsis spp*.): functional significance of venom dispersal behavior. Journal of Chemical Ecology 11: 1757-1768.

Obin, M. S., and R. K. Vander Meer. 1994. Alate semiochemicals release worker behavior during fire ant nuptial flights. Journal of Entomological Science 29: 143-151.

Owen, R., R. Haubner, G. Würtele, W. Hull, B. Spiegelhalder, and H. Bartsch. 2004. Olives and olive oil in cancer prevention. European Journal of Cancer Prevention 13: 319-326.

Passera, L., S. Aron, E. L. Vargo, and L. Keller. 2001. Queen control of sex ratio in fire ants. Science 293: 1308-1310.

Pitts Jr, J. P. 2002. A cladistic analysis of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). UGA.

Porter, S. D. 1988. Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. Journal of Insect Physiology 34: 1127-1133.

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

Rashid, T., J. Chen, and P. McLeod. 2013. Toxicity of newly isolated piperideine alkaloids from the red imported fire ant, *Solenopsis invicta* Buren, against the green peach aphid, *Myzus persicae* (Sulzer). Advances in Entomology 2013.

Reagan, T., G. Coburn, and S. Hensley. 1972. Effects of mirex on the arthropod fauna of a Louisiana sugarcane field. Environmental Entomology 1: 588-591.

Reddy, L. H., and P. Couvreur. 2009. Squalene: A natural triterpene for use in disease management and therapy. Advanced Drug Delivery Reviews 61: 1412-1426.

Rhoades, W., and D. Davis. 1967. Effects of meteorological factors on the biology and control of the imported fire ant. Journal of Economic Entomology 60: 554-558.

Richards, J., M. Carr-Markell, A. Hefetz, C. M. Grozinger, and H. R. Mattila. 2015. Queen-produced volatiles change dynamically during reproductive swarming and are associated with changes in honey bee (*Apis mellifera*) worker behavior. Apidologie 46: 679-690.

Rissing, S., and G. Pollock. 1991. An experimental analysis of pleometrotic advantage in the desert seed-harvester ant *Messor pergandei* (Hymenoptera; Formicidae). Insectes Sociaux 38: 205-211.

Salzemann, A., P. Nagnan, F. Tellier, and K. Jaffe. 1992. Leaf-cutting ant *Atta laevigata* (Formicidae: Attini) marks its territory with colony-specific Dufour gland secretion. Journal of Chemical Ecology 18: 183-196.

Santomauro, G., N. J. Oldham, W. Boland, and W. Engels. 2004. Cannibalism of diploid drone larvae in the honey bee (*Apis mellifera*) is released by odd pattern of cuticular substances. Journal of Apicultural Research 43: 69-74.

Schmidt, J. O. 1990. Hymenopteran venoms: striving toward the ultimate defense against vertebrates. Insect defenses: adaptive mechanisms and strategies of prey and predators: 387-419.

Shattuck, S., S. Porter, and D. Wojcik. 1999. *Solenopsis invicta* Buren, 1972 (Insecta, Hymenoptera): Proposed conservation of the specific name. The Bulletin.

Shoemaker, D. D., M. E. Ahrens, and K. G. Ross. 2006. Molecular phylogeny of fire ants of the *Solenopsis saevissima* species-group based on mtDNA sequences. Molecular Phylogenetics and Evolution 38: 200-215.

Sikes, P. J., and K. A. Arnold. 1986. Red imported fire ant (*Solenopsis invicta*) predation on cliff swallow (*Hirundo pyrrhonota*) nestlings in east central Texas. The Southwestern Naturalist (USA).

Sterling, W. L. 1978. Fortuitous biological suppression of the boll weevil by the red imported fire ant. Environmental Entomology 7: 564-568.

Summerlin, J., and S. Kunz. 1978. Predation of the red imported fire ant on stable flies. Southwestern Entomologist.

Summerlin, J., J. Olson, R. Blume, A. Aga, and D. Bay. 1977. Red imported fire ant: effects on *Onthophagus gazella* and the horn fly. Environmental Entomology 6: 440-442.

Taber, S. W. 2000. Fire ants, Texas A&M University Press.

Tedders, W., C. Reilly, B. Wood, R. Morrison, and C. Lofgren. 1990. Behavior of *Solenopsis invicta* (Hymenoptera: Formicidae) in pecan orchards. Environmental Entomology 19: 44-53.

Thompson, M. J., B. Glancey, W. E. Robbins, C. S. Lofgren, S. R. Dutky, J. Kochansky, R. K. Vandermeer, and A. R. Glover. 1981. Major hydrocarbons of the post-pharyngeal glands of mated queens of the red imported fire ant *Solenopsis invicta*. Lipids 16: 485-495.

TianCi, Y., and Z. Shi. 2006. Control of the red imported fire ant (*Solenopsis invicta*). Chinese Journal of Vector Biology and Control 17: 72-74.

Tkachev, A. V., A. K. Dobrotvorsky, A. I. Vjalkov, and S. V. Morozov. 2000. Chemical composition of lipophylic compounds from the body surface of unfed adult *Ixodes persulcatus* ticks (Acari: Ixodidae). Experimental and Applied Acarology 24: 145-158.

Toom, P. M., E. Cupp, C. P. Johnson, and I. Griffin. 1976. Utilization of body reserves for minim brood development by queens of the imported fire ant, *Solenopsis invicta*. Journal of insect Physiology 22: 217-220.

Trager, J. C. 1991. A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae: Myrmicinae). Journal of the New York Entomological Society: 141-198.

Tschinkel, W. R. 1988. Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. Physiological Entomology 13: 327-350.

Tschinkel, W. R. 1992. Brood raiding in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): laboratory and field observations. Annals of the Entomological Society of America 85: 638-646.

Tschinkel, W. R. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecological Monographs 63: 425-457.

Tschinkel, W. R. 2006. The fire ants, Harvard University Press.

Van Wilgenburg, E., M. Symonds, and M. Elgar. 2011. Evolution of cuticular hydrocarbon diversity in ants. Journal of Evolutionary Biology 24: 1188-1198.

Vander Meer, R. 1986. Chemical taxonomy as a tool for separating *Solenopsis spp*.

Vander Meer, R., and C. Lofgren. 1990. Chemotaxonomy applied to fire ant systematics in the United States and South America. Applied Myrmecology: A world perspective. Westview Press, Boulder, CO: 75-84.

Vander Meer, R., F. Williams, and C. Lofgren. 1981. Hydrocarbon components of the trail pheromone of the red imported fire ant, *Solenopsis invicta*. Tetrahedron Letters 22: 1651-1654.

Vander Meer, R., B. Glancey, and C. Lofgren. 1982. Biochemical changes in the crop, oesophagus and postpharyngeal gland of colony-founding red imported fire ant queens (*Solenopsis invicta*). Insect Biochemistry 12: 123-127.

Vander Meer, R. K., and C. S. Lofgren. 1988. Use of chemical characters in defining populations of fire ants, *Solenopsis saevissima* complex,(Hymenoptera: Formicidae). Florida Entomologist: 323-332.

Vander Meer, R. K., C. S. Lofgren, and F. M. Alvarez. 1985. Biochemical evidence for hybridization in fire ants. Florida Entomologist: 501-506.

Vander Meer, R. K., F. Alvarez, and C. S. Lofgren. 1988. Isolation of the trail recruitment pheromone of *Solenopsis invicta*. Journal of Chemical Ecology 14: 825-838.

Vander Meer, R. K., D. Saliwanchik, and B. Lavine. 1989. Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*. Journal of Chemical Ecology 15: 2115-2125.

Vargo, E. L. 1988. Effect of pleometrosis and colony size on the production of sexuals in monogyne colonies of the fire ant *Solenopsis invicta*. Advances in Myrmecology: 217-225.

Vargo, E. L., and S. D. Porter. 1989. Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 82: 307-313.

Vick, K., W. Drew, D. McGurk, E. Eisenbraun, and G. Waller. 1969. Identification of Hydrocarbons from *Novomessor cockerelli*. Annals of the Entomological Society of America 62: 723-725.

Vinson, S., S. Phillips, and H. Williams. 1980. The function of the post-pharyngeal glands of the red imported fire ant, *Solenopsis invicta* Buren. Journal of Insect Physiology 26: 645-650.

Vinson, S. B. 1997. Insect life: invasion of the red imported fire ant (Hymenoptera: Formicidae). American Entomologist 43: 23-39.

Vinson, S. B., and A. Sorensen. 1986. Imported fire ants: life history and impact.

Vogt, J. T., A. G. Appel, and M. S. West. 2000. Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren. Journal of Insect Physiology 46: 697-707.

Voss, S., and M. Blum. 1987. Trophic and embryonated egg production in founding colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology (USA).

Voss, S., J. Bryan, and C. Keith. Published. Early developmntal arrest in fire ant trophic eggs, pp. a244-a244. *In*, Journal of Cell Biology, 1986. Rockefeller Univ Press 1114 First Ave, 4th Fl, New York, NY 10021 USA.

Wetterer, J. K., and L. R. Davis Jr. 2010. *Solenopsis invicta* (Hymenoptera: Formicidae) in the Lesser Antilles. Florida Entomologist 93: 128-129.

Wheeler, D. E., and T. Martinez. 1995. Storage proteins in ants (Hymenoptera: Formicidae). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 112: 15-19.

Williams, D., and P. Whelan. 1991. Polygynous colonies of *Solenopsis geminata* (hymenoptera: Formicidae) in the Galapagos Islands. The Florida Entomologist 74: 368-371.

Williams, D. F., R. K. Vander Meer, and C. S. Lofgren. 1987. Workers of the Imported Fire Ant *Solenopsis invicta* Buren. Archives of Insect Biochemistry and Physiology 4: 251-259.

Wilson, E. O. 1952. The *Solenopsis saevissima* complex in South America (Hymenoptera: Formicidae). Memórias do Instituto Oswaldo Cruz 50: 49-68.

Yoder, J. A., R. J. Pollack, and A. Spielman. 1993a. An ant-diversionary secretion of ticks: First demonstration of an acarine allomone. Journal of Insect Physiology 39: 429-435.

Yoder, J. A., B. W. Stevens, and K. C. Crouch. 1999. Squalene: a naturally abundant mammalian skin secretion and long distance tick-attractant (Acari: Ixodidae). Journal of Medical Entomology 36: 526-529.

Yoder, J. A., R. J. Pollack, A. Spielman, D. E. Sonenshine, and D. E. Johnstons. 1993b. Secretion of squalene by ticks. Journal of Insect Physiology 39: 291-296.