

7-11-2022

SOIL MOISTURE MEDIATED BEHAVIORAL AND PHYSIOLOGICAL ALTERATIONS OF COPTOTERMES FORMOSANUS AND RETICULITERMES FLAVIPES (BLATTODEA: RHINOTERMITIDAE)

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**SOIL MOISTURE MEDIATED BEHAVIORAL AND
PHYSIOLOGICAL ALTERATIONS OF *COPTOTERMES*
FORMOSANUS AND *RETICULITERMES FLAVIPES*
(BLATTODEA: RHINOTERMITIDAE)**

A Thesis

Submitted to the Graduate Faculty
of the Louisiana State University
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Steven James Richardson
B.S., Texas Agricultural and Mechanical College, College Station, Texas, 2019
August 2022

“Nothing in the world is worth having or worth doing unless it means effort, pain, difficulty... I have never in my life envied a human being who led an easy life. I have envied a great many people who led difficult lives and led them well.”

(Theodore Roosevelt)

To my parents, Michael and Kristine, for their
time spent guiding, encouraging, and chastising
me to be better, for giving me advice and trusting
my judgment, and to everything that I am,
I dedicate my work.

Acknowledgments

For the last two years, I have had the opportunity to work and interact with many people in the department and across campus who have given me support and encouragement, and who have helped build my professional growth.

Firstly, I would like to thank Dr. Qian Sun for giving me the opportunity to pursue a master's degree at Louisiana State University Agricultural and Mechanical College and for her continual direction and guidance through this journey. I'm also extremely grateful to my graduate committee members, Dr. Claudia Husseneder and Dr. Rodrigo Diaz for their resources and constructive advice on my thesis and projects.

I would also like to thank my lab mates, Joseph McCarthy, Paula Castillo, and Arjun Khadka, for showing me how to operate equipment, run software, and perform field collections. And thank you Justice, for helping me out with setting up experiments. I enjoyed the time spent with all of you and I'm looking forward to seeing you all progress.

Lastly, I would like to thank my family, Mike and Kristine, for being there for me when I needed you to be. And for giving me the opportunity to pursue a higher level of education. And thank you Sarah, for being my sister and going on adventures with me around our neighborhood forests when we were younger.

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Abbreviations

RH	relative humidity
CHC	cuticular hydrocarbon
<i>n</i> -alkane	linear alkane
mb-alkane	methyl-branched alkane
ng/mg	nanogram per microgram
GLMM	generalized linear mixed model
ANOVA	analysis of variance
Lsmeans	least squared means test
Tukey's	Tukey's honest significance test
Dunn's	Dunn's multiple comparison post hoc test
SE	standard error
FAS	fatty acid synthesis
CoA	coenzyme A

Abstract

Substrate moisture is a critical environmental factor for the survival and behavior of subterranean termites (Blattodea: Rhinotermitidae). The invasive Formosan subterranean termite (*Coptotermes formosanus*) and the native eastern subterranean termite (*Reticulitermes flavipes*) co-occur in the southeastern United States, but *C. formosanus* is predominant in subtropical and *R. flavipes* is more common in temperate regions. The difference in their geographic distributions is largely attributed to their different behavioral and physiological ecology. While ample research has been done on foraging behavior of the two species, there was no empirical evidence for the effects of constant soil moisture on their foraging activity and colony survival. In addition, subterranean termites rely on soil moisture to maintain water balance, and an important physiological mechanism of desiccation resistance in insects is the prevention of transpirational water loss via cuticular hydrocarbons (CHCs). CHCs exhibit plastic response to environmental variations in many insects. Previous research on termite CHCs has analyzed their seasonal and geographical variations as well as the role in chemical communication, however, studies examining CHC plasticity and its correlation with desiccation resistance are lacking. In this study, we investigated the effects of soil moisture on the behavior and physiology of subterranean termites. We hypothesized that foraging activity and survivorship of both *C. formosanus* and *R. flavipes* alter in response to different substrate moisture regimes, and the effects differ between the two species. To test this, the tunnel area, survivorship, and food consumption were documented for 28 days under six sand moisture conditions (0%, 1%, 5%, 15%, 25%, 30%) in both *C. formosanus* and *R. flavipes*. We found that, in both species, no tunneling or feeding activity was performed at 0% substrate moisture, while no significant difference was found on tunnel area, food consumption, or survivorship among 5%, 15%, 25%, or 30%. Survivorships of termites at 0% and 1% substrate

moisture were significantly lower than that at 5%, 15%, and 25% for *C. formosanus*. For *R. flavipes*, survivorships at 0% and 1% substrate moisture were significantly lower than that at 5%, 15%, and 30%. In both species, a minimum of 1% substrate moisture is needed for foraging and 5% is required for colony survival. To examine the physiological effects, we further hypothesized that desiccation resistance and CHC profiles of *C. formosanus* change in response to soil moisture conditions. Upon acclimation to soil moisture conditions of 5%, 15%, and 25%, we found that *C. formosanus* workers expressed notable changes in CHC profiles in both linear and methyl-branched alkanes, where 12 of the 20 cuticular hydrocarbons detected expressed a significant increase in quantity at 5% soil moisture when compared to 25% soil moisture. Lastly, desiccation resistance of workers was significantly higher upon acclimation to 5% sand moisture (20.36 ± 0.79 hours, survival time under desiccation stress) when compared to 15% (17.47 ± 0.63 hours) and 25% (15.38 ± 0.65 hours). Our results demonstrate the effects of substrate moisture on the behavior and physiology of subterranean termites and suggest that CHC plasticity may be important for the climate adaptation of subterranean termites.

Chapter 1. Introduction

1.1. Invasion History and Economic Impacts

The *Coptotermes* species (Blattodea: Rhinotermitidae) worldwide geographic distribution spans the majority of tropical and subtropical regions (Evans et al. 2019). The Formosan subterranean termite, *C. formosanus* (Shiraki) (Figure 1.1), poses high risks as a pest species against wood structures and agricultural products like forestry crops (Evans et al. 2013, Krishna et al. 2013). Native to southern continental China and Taiwan, in the 1700s, it was introduced to Japan (Evans et al. 2013). Prior to World War Two, *C. formosanus* was introduced to Marshall Island, Midway, Hawaii in 1907, then entered continental United States in the 1950s (Evans et al. 2013). The species is now highly pervasive across the southeastern United States (Scheffrahn and Crowe 2011, Chouvenc et al. 2015). Since *C. formosanus* was introduced to the continental United States over 70 years ago, its spread encompasses temperate, subtropical and tropical climates across the southeastern United States (Cao and Su 2016). It ranges as high north as Tennessee and has not been found farther west than southeast Texas, with a few isolated colonies in California (Beal 1967, Su and Tamashiro 1986, Howell 1987, Tseng et al. 2021). In contrast, the native termite species, the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is widely distributed across most temperate regions of North America and as high north as Ontario, Canada (Su and Scheffrahn 1990).

Globally, *C. formosanus* contributes a large portion of the \$40 billion termite damage and, in the US, causes \$1 billion in structural damage with \$100 million from Hawaii and \$300 million from

New Orleans, Louisiana (Pimentel et al. 2005, Rust and Su 2012, Tong et al. 2017). Economically, *C. formosanus* is of the most destructive and damaging pests in North America and the United States (Lax and Osbrink 2003). Economic damage incurred from *C. formosanus* can be attributed to several biological factors making *C. formosanus* more destructive than *R. flavipes*. Three primary factors include high reproductive potentials, large colony size, and broader ranging preferences for cellulose-based food sources (Raina 2004). Queens of *C. formosanus* from mature colonies can lay over 1000 eggs daily and have colony sizes ranging from 1-4 million individuals (Raina 2004). In combination, the species has cellulose-based food preferences encompassing structural wood in buildings to living trees and sugarcane. Daily, *C. formosanus* colonies consume over 400 grams of wood (Raina 2004). Lastly, *C. formosanus* workers forage over 100 meters from their nests central point, making locating and treating colonies difficult (Su et al. 2017). However, the colony remains centered around a central point and the colony does not move or spread to a different location underground (Shults et al. 2021).



Figure 1.1. *C. formosanus* soldier (A) and worker (B) photo taken by Steven Richardson.

1.2. Behavioral Adaptations and Water Availability

The primary way *C. formosanus* spreads to new areas is the same primary mode of invasion for most all invasive species, by human interference through transportation of agricultural products and other infested goods (Scheffrahn and Crowe 2011, Simberloff 2013). Most invasions from foreign species to different continents are unsuccessful because those species were unable to acclimate to biotic or abiotic conditions present in the locality they were introduced to (Simberloff 2013). Because of this, generalists with a wide range of physiological or behavioral adaptability, such as high reproduction rates for a rapid increase in population, wide thermal tolerances allowing for a broader range for invasion, or broad nutrient preferences causing more generalistic foraging behaviors, are the most successful when establishing (Raina 2004, Zerebecki and Sorte 2011, Simberloff 2013). And, in reference to abiotic factors, a crucial aspect to establishment in subterranean termites is water availability (Collins 1969). Both *C. formosanus* and *R. flavipes* express behavioral adaptations assisting water retention (Grube and Rudolph 1999, Gallagher and Jones 2010). In soil with low relative humidity (RH), *R. flavipes* workers eject water from salivary glands, increasing soil moisture, assisting tunnel formation, and maintaining favorable RH (Grube and Rudolph 1999).

Additionally, termites increase soil moisture levels and RH by using fecal matter as tunnel insulation for moisture (Grube et al. 1997). However, environmental augmentation is not always possible and requires seasonal changes in behavior to accommodate for extremes in water availability (Cornelius and Osbrink 2011, Gautam and Henderson 2011c). Low water availability or colder temperatures during winter months are correlated to a decrease in foraging activity and food consumption in *C. formosanus* (Cornelius and Osbrink 2011, Gautam and Henderson

2011c). In *R. flavipes*, foraging activity and tunneling behavior were positively correlated to water availability, suggesting reduced foraging and tunneling in dry seasons or areas with low soil moisture content but a subsequent increase in activity after rain or when tunneling through soil in close proximity to water sources (Houseman et al. 2001, Houseman and Gold 2003).

1.3. Cuticular Hydrocarbons and Water Balance

If behavioral mediation isn't sufficient for soil moisture augmentation, cuticular permeability allows body water content to be passively lost through transpiration, maintaining an environmental equilibrium with RH (Grube et al. 1997, Grube and Rudolph 1999, Zukowski and Su 2019). However, subterranean termites are prone to desiccation due to thinly sclerotized cuticle on the majority of their body as well as having high body fat content, high metabolic rates, and a large surface area to volume ratio (Gibbs et al. 1997, Hoffmann and Harshman 1999, Woon et al. 2019). A series of surface lipids composed of cuticular hydrocarbons (CHCs), sterols, esters, fatty acids, and alcohols coat the surface of insect cuticle, create a hydrophobic barrier reducing desiccation stress with the majority of desiccation resistance believed to be linked to methyl-branched alkanes and linear alkanes (Neville 1975, Gibbs and Pomonis 1995). These CHCs become more hydrophobic as carbon chain length increases, with the majority of CHCs on insect cuticle linked to desiccation resistance being over 20 carbons (Lockey 1988, Blomquist and Bagnères 2010). The CHCs profiles of termites vary with species, colony, caste, as well as season (Howard and Blomquist 1982, Haverty et al. 1996). Soldiers of *C. formosanus* expressed an increase in a methyl-branched alkane closer to swarm season when soldiers are more likely exposed to environmental conditions while guarding alates (Haverty et al. 1996). Other insects

also exhibit seasonal changes in CHC profiles in response to spatiotemporal variation, such as *Drosophila* across the eastern coast of the United States (Rajpurohit et al. 2017). When looking at acclimation assays of the drywood termite, *Cryptotermes brevis* (Blattodea: Kalotermitidae), CHC profiles expressed an increase in longer chain linear alkanes under lower RH independent of temperature (Woodrow et al. 2000). The plastic response of CHCs to environmental conditions allows insects to improve desiccation resistance, as evidenced by studies in various species (Stinziano et al. 2015, Arcaz et al. 2016, Menzel et al. 2018, Sprenger et al. 2018, Yang et al. 2020).

1.4. Research Objectives

Until now, no study has analyzed CHC profile alterations of *C. formosanus* in response to water availability, which may vary in case of climate change or being introduced to different localities (Haverty and Howard 1981, Howard and Blomquist 1982, Su and Haverty 1990, Haverty et al. 1996, Cao and Su 2016). Additionally, previous studies have correlated substrate moisture and temperature to seasonal changes in foraging activity of *C. formosanus* (Cornelius and Osbrink 2011, Gautam and Henderson 2011a). However, no study has observed either species' behavior in a forced exposure assay with constant soil moisture conditions. Therefore, the objective of this study was to investigate the influences that soil moisture conditions had on foraging behavior, survivorship, and consumption in workers of *C. formosanus* and *R. flavipes* to examine how the species differed between each other and at different moisture conditions (Chapter 2). Then, after behavioral investigations were made, a second experiment focused on workers of *C. formosanus*

and their response to varying moisture regimes. CHC composition was analyzed and a desiccation assay was performed to determine if *C. formosanus* workers expresses any physiological acclimation to varying soil moisture (Chapter 3).

Chapter 2.

Effects of sand moisture on tunneling, survivorship, and food consumption of the Formosan and eastern subterranean termites (Blattodea: Rhinotermitidae)

2.1. Introduction

Geographic distribution of *Coptotermes* species (Blattodea: Rhinotermitidae) spans most tropical and subtropical regions worldwide (Evans et al. 2013). The Formosan subterranean termite, *C. formosanus* Shiraki, imposes high risks as a pest species against wood structures and forestry crops (Evans et al. 2013, Krishna et al. 2013, Evans et al. 2019). Endemic to east Asia, its invasion to the United States was mediated through wood and agricultural products transported via maritime activity (Kistner 1985, Scheffrahn and Crowe 2011, Evans et al. 2013, Blumenfeld et al. 2021). Since *C. formosanus* was introduced to the continental United States over 70 years ago, its distribution encompasses temperate, subtropical and tropical climates (Su 2003). It ranges as high north as Tennessee and has not been found farther west than southeast Texas, with a few isolated colonies in California (Beal 1967, Su and Tamashiro 1986, Howell 1987, Tseng et al. 2021). In contrast, the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is a native termite species widely distributed across most temperate regions of North America and as high north as Ontario, Canada (Su and Scheffrahn 1990).

Both *C. formosanus* and *R. flavipes* are sympatric to a large portion of the central and eastern United States (Cao and Su 2016). However, *R. flavipes* inhabits a broader geographical

range with greater variations in temperature and precipitation than *C. formosanus* (Cao and Su 2016). In addition to temperature, soil moisture is an important abiotic factor that affects the behavior and survival of subterranean termites, thus determining the geographic distribution of termite species (Su and Puche 2003, Woon et al. 2019). One crucial aspect of water availability in subterranean termites is soil moisture (Collins 1969). Both *C. formosanus* and *R. flavipes* have been observed expressing behavioral adaptations that assist in water retention (Grube and Rudolph 1999, Gallagher and Jones 2010). In soil conditions relatively low in relative humidity (RH), *R. flavipes* workers eject water from their salivary glands, increasing soil moisture, assisting tunnel formation, and maintaining favorable RH (Grube and Rudolph 1999). Additionally, subterranean termites increase soil moisture and RH by using fecal matter in tunnel formation (Grube et al. 1997). However, environmental augmentation is not always possible and subterranean termites would require seasonal changes in behavior to accommodate for extremes in water availability (Cornelius and Osbrink 2011, Gautam and Henderson 2011c). In *C. formosanus*, both low water availability and colder temperatures during winter months are correlated to a decrease in foraging activity and food consumption (Cornelius and Osbrink 2011). In *R. flavipes*, tunneling behavior and foraging activity were positively correlated to soil moisture, suggesting reduced foraging and tunneling in dry seasons and areas with lower soil water availability but a subsequent increase in activity following precipitation or when tunneling close to water sources (Houseman et al. 2001, Houseman and Gold 2003).

Previous studies have revealed the effects of temperature on seasonal changes in foraging activity of both *C. formosanus* and *R. flavipes*. Significantly less wood was consumed during winter months when soil temperature was below 15–19°C, suggesting that *C. formosanus* workers forage at higher rates with increasing soil temperature (Cornelius and Osbrink 2011, Gautam and

Henderson 2011c). In *R. flavipes*, food consumption increased in warmer months of the year when ground temperatures in shaded locations averaged between 22-24° C, also correlating *R. flavipes* foraging behavior to increasing temperatures (Harahap et al. 2005). Laboratory studies have also demonstrated the positive correlation of temperature with foraging behavior of termites within the range of tolerant temperature in *C. formosanus* and *R. flavipes* (Cao and Su 2014). In response to water availability, laboratory studies complemented field observations on seasonal changes in foraging activities (Houseman et al. 2001, Cornelius and Osbrink 2011), and revealed that higher water content in wood or tunneling substrates enhanced feeding and/or tunneling (Su and Puche 2003, Gautam and Henderson 2011c). Those studies conducted via seasonal observations or multi-choice assays allowed termites to choose among a range of moisture gradients. In the field, drought and flooding occur depending on the geoclimatic region or due to extreme climate events, causing prolonged extremes in water availability in the soil. However, the effects of constant soil moisture conditions on foraging behavior or survivorship of subterranean termites were poorly understood.

Determining the adaptability of *C. formosanus* or *R. flavipes* to water availability is important for predicting potential shifts in their distribution ranges. In this study, we hypothesized that foraging behavior and colony survival in both *C. formosanus* and *R. flavipes* are influenced by substrate moisture conditions, and that the effects of substrate moisture are different between the two species. To test these, a behavioral assay was conducted to analyze the tunneling activity, food consumption and survivorship of termites in both species under constant conditions with water content in sand varying from 0% (water deprivation) to 30% (water saturation).

2.2. Materials and Methods

2.2.1. Insects

Termites from three colonies of *C. formosanus* were collected at Brechtel Park in New Orleans, Louisiana (29°54'18.5"N, 90°00'44.3"W) at least 200 meters apart. Pinewood blocks were tied together in milk crates and buried as termite traps for collection of *C. formosanus* foragers. Termites from three colonies of *R. flavipes* were collected at Bluebonnet Swamp Nature Center in Baton Rouge, Louisiana (30°22'12.3"N, 91°06'26.0"W). These colonies were collected from using corrugated cardboard rolls placed above ground near or under infested wood. Collection samples from both species were maintained in an incubator at $27 \pm 1^\circ\text{C}$ and 80-100% RH in complete darkness and were maintained in lab at Louisiana State University. The colonies were provisioned with kiln-dried pine wood blocks and provided with moist organic soil (Miracle-Gro All Purpose for In-Ground Use, ScottsMiracle-Gro, Marysville, OH).

2.2.2. Experimental Setup

For our substrate moisture assay, experimental arenas were adapted from a previous publication (Chouvenc et al. 2011). Arenas comprised of two transparent acrylic sheets (14×14 cm) with four internally spaced black acrylic strips ($14 \times 2 \times 0.16$ cm for two pieces and $10 \times 2 \times 0.16$ cm for the other two), creating an internal termite tunneling area ($10 \times 10 \times 0.16$ cm) (Figures 2.1, 2.2). A small piece of black acrylic (0.16 cm thick) was tightly bolted through the center of the arena to ensure that an internal tunneling height of 0.16 cm was uniformly maintained.

Additionally, a release chamber (3.5 cm in diameter and 1.0 cm in height) was hot glued with a 0.2 cm hole drilled through the release chamber and edge of the first sheet of clear acrylic. Each arena was inserted with a stack of 0.16 cm thick cellulose filter paper (3.0 cm in diameter, Whatman grade 1, Cytiva, Marlborough, MA, USA), which was oven dried at 80°C for 4 hours then pre-weighed and suspended 1.0 cm in front of the arena wall most distal to the release chamber. Arenas were then filled with dry sand (Quikrete Premium Play Sand, Atlanta, GA, USA) as a substrate while leaving a 1.0 cm space free of sand parallel to the release chamber (Figure 2. 1).

Groups of 50 termites from each colony (49 workers and one soldier for *R. flavipes*, 45 workers and five soldiers for *C. formosanus*) were maintained inside 3.5 cm Petri dishes with moistened filter paper for 12 h at room temperature ($24 \pm 1^\circ\text{C}$) for acclimatization, and then each group was released to an arena through the release chamber. The lids of release chambers were sealed using parafilm (Parafilm M, Neenah, WI, USA). Arenas were then placed suspended in sealed plastic containers ($32 \times 25.5 \times 10$ cm, Pioneer Plastics, Dixon, KY, USA) with distilled water at the bottom to maintain 100% RH at room temperature ($24 \pm 1^\circ\text{C}$). For each species, nine replications were conducted for each moisture condition (0%, 1%, 5%, 15%, 25%, and 30%) composed of three replications per colony and three colonies tested, totaling 54 arenas per species. The arenas were maintained for 28 days and were photographed using an iPhone fixed to a tripod (iPhone 8, Apple Inc., Cupertino, California) at 3, 6, 12, and 24 h, then every 24 h for the first 144 h (7 days), and at 216 h (9 days) to analyze area tunneled using ImageJ (National Institutes of Health, Bethesda, MD). After 28 days, arenas were broken apart, cleaned, and the cellulose filter paper was dried at 80°C in Heratherm™ General Protocol mechanical convection,

100L, 208-240VAC 60Hz, Oven (Thermo Fisher Scientific, Waltham, MA) overnight then weighed for consumption between moisture conditions.

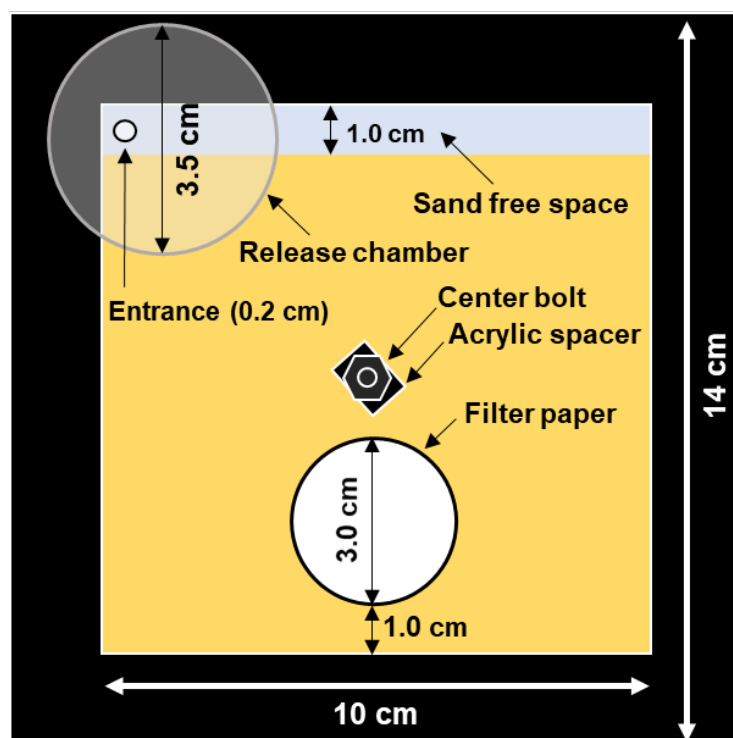


Figure 2.1. Experimental setup. The termite arena provides an internal tunneling space of $10 \times 10 \times 0.16$ cm with a release chamber for termite introduction, cellulose filter paper as a food source, and sand as the tunneling substrate.

2.2.3. Sand moisture treatments

Before completely sealing the arenas with hot glue and binder clips, distilled water was added to sand to achieve six moisture conditions: 0% (no water), 1%, 5%, 15%, 25%, and 30% (water saturation) (wt [%dH₂O]: wt [%sand]). For water saturation, 30% of the sand weight was the

maximum weight of water that can be added, filling all free space between sand particles without leaving sanding water in the arena.

2.2.4. Data analysis

Statistical analysis was performed using R software version 4.1.2 (The R Foundation, Vienna, Austria) (Team 2013), and data were visualized using PRISM version 9.3.1 (GraphPad Software, San Diego, CA, USA). To test for normality of all dataset distributions and homogeneity of variances, Shapiro-Wilk test and Levene's test for equality of variances were performed respectively. The data on tunnel area at different time points in each moisture regime, as well as the mortality data, did not meet the assumptions for parametric analysis ($P < 0.05$ for Shapiro-Wilk and Levene's tests), and Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test was used. For food consumption, the data met the assumptions for parametric analysis ($P > 0.05$ for Shapiro-Wilk and Levene's test), and generalized linear mixed model followed by the least squared means test (GLMM followed by lsmeans) was used.

2.3. Results

2.3.1. Tunneling activity

In both *C. formosanus* and *R. flavipes*, no tunneling activity was observed at 0% sand moisture during the course of experiments. At 3, 6, 24, and 144 h, tunnel area at 0% was significantly different from the other moisture conditions (mean \pm SE, Kruskal-Wallis followed by Dunn's test, $P < 0.05$, $n = 9$), while there was no significant difference between 1%, 5%, 15%, 25%, and 30% for either species (mean \pm SE, Kruskal-Wallis followed by Dunn's test, $P > 0.05$, $n = 9$) (Fig. 2). At each of the four time points, no significant difference was detected between *C. formosanus* and *R. flavipes* at each moisture condition (mean \pm SE, Kruskal-Wallis followed by Dunn's test, $P > 0.05$, $n = 9$).

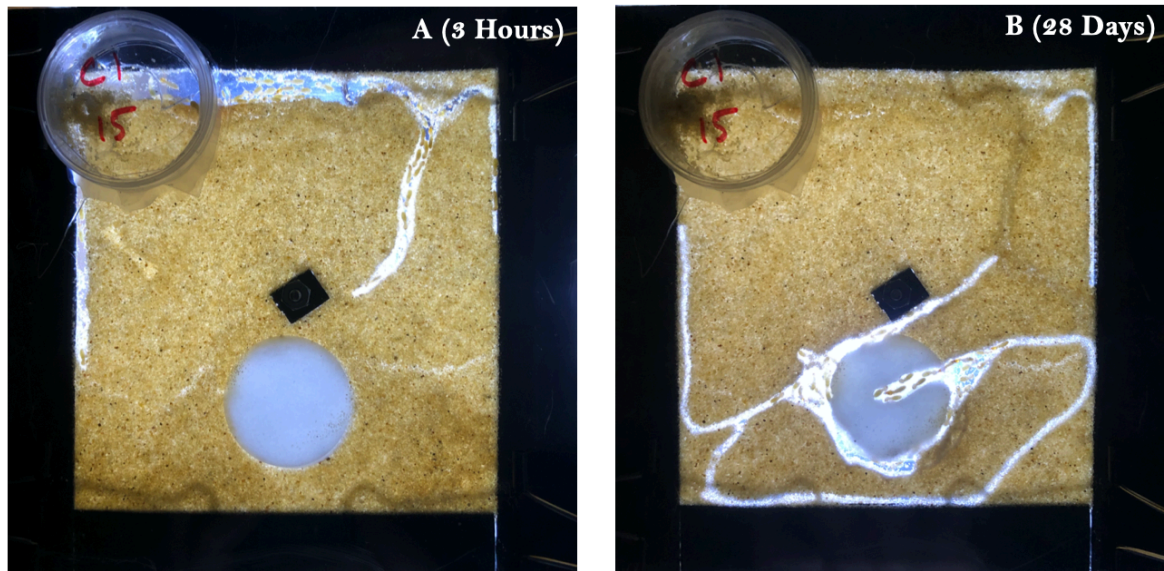
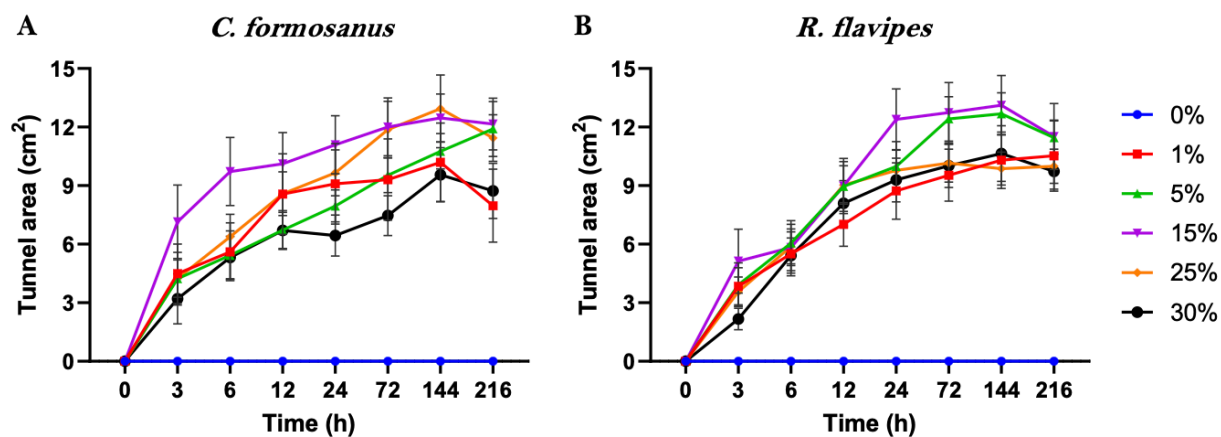


Figure 2.2. Example substrate moisture arena for *C. formosanus* colony 1 at 15% substrate moisture after 3 hours (A) and 28 days (B).

Figure 2.3. Area tunneled over time for *C. formosanus* (A) and *R. flavipes* (B) under six moisture conditions with mean \pm SE shown for each time point.

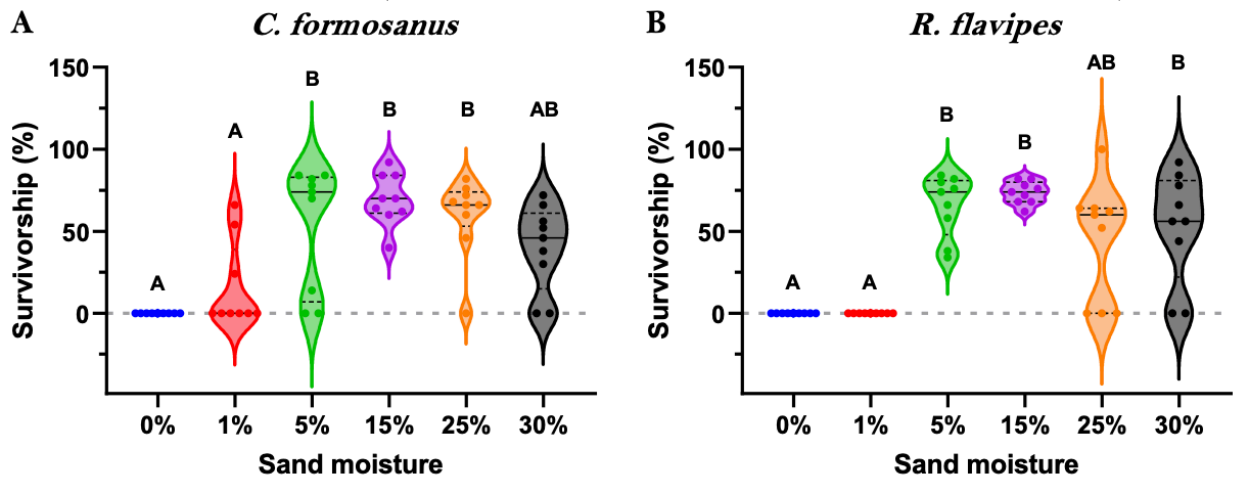


2.3.2. Survivorship

In both *C. formosanus* and *R. flavipes*, no termite survived for 28 days at 0% sand moisture, and survivorship at 1% was not significantly different from that at 0% (Kruskal-Wallis followed by Dunn's test, $P < 0.05$, $n = 9$) (Figure 2.4). For *C. formosanus*, survivorship was significantly higher at 5%, 15%, and 25% compared to 0% and 1% sand moisture (Kruskal-Wallis followed by Dunn's test, $P < 0.05$, $n = 9$) with no significant difference found for 30% when compared to any other moisture gradients (Kruskal-Wallis followed by Dunn's test, $P > 0.05$, $n = 9$) (Figure 2.4A). With *R. flavipes*, survivorship was significantly higher at 5%, 15%, and 30% compared to 0% and 1% sand moisture (Kruskal-Wallis followed by Dunn's test, $P < 0.05$, $n = 9$) with no significant difference found for 25% when compared to any other moisture gradients (Kruskal-Wallis

followed by Dunn's test, $P > 0.05$, $n = 9$) (Figure 2. 4B). No significant difference was found between *C. formosanus* and *R. flavipes* at each moisture condition (0%: $P = 1.0000$; 1%: $P = 0.5429$; 5%: $P = 0.8034$; 15%: $P = 0.8027$; 25%: $P = 0.4454$; 30%: $P = 0.4721$; Kruskal-Wallis followed by Dunn's test, $n = 9$).

Figure 2.4. Survivorship for *C. formosanus* (A) and *R. flavipes* (B) at six different moisture conditions after 28 days. In each plot, all data points are shown, dash lines indicate first and third quartiles, and solid line indicates the median. Different letters denote significant differences between moisture conditions (Kruskal-Wallis followed by Dunn's test, $P < 0.05$, $n = 9$).

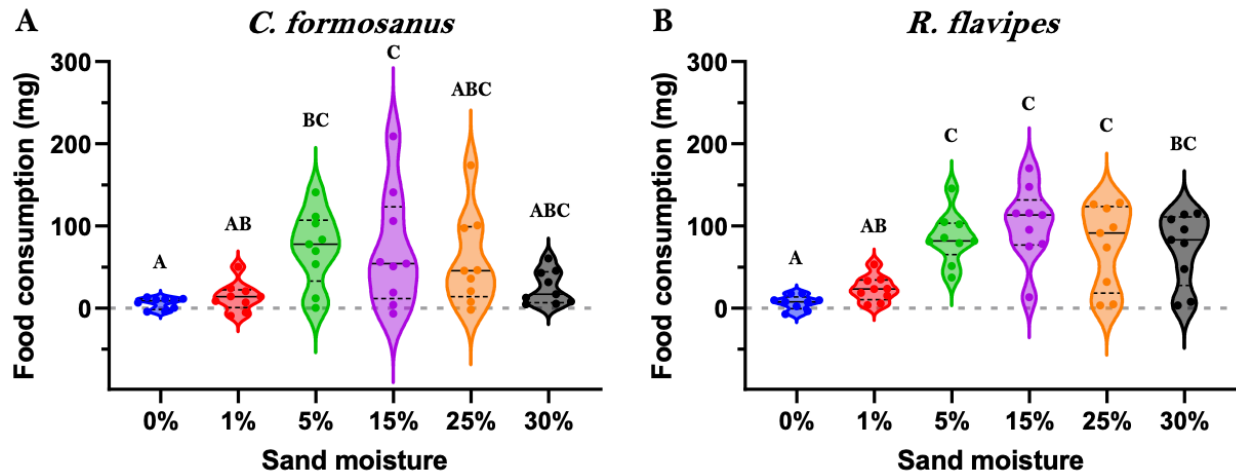


2.3.3. Food Consumption

For both *C. formosanus* and *R. flavipes*, no food consumption took place at 0% sand moisture, and the highest mean consumption was observed at 15% moisture (Figure 2. 5). Consumption by *C. formosanus* termites was significantly lower at 0% and 1% when compared to 15% sand moisture (GLMM followed by lsmeans, $P < 0.05$, $n = 9$), while no significant difference was observed between moisture conditions of 5%, 15%, 25% and 30% (GLMM followed by lsmeans, $P > 0.05$,

$n = 9$) (Fig. 2. 5A). In *R. flavipes*, food consumption was significantly lower at 0% and 1% when compared to 5%, 15% and 25% (GLMM followed by lsmeans, $P > 0.05$, $n = 9$), while no significant difference was found between 5%, 15%, 25% and 30% (GLMM followed by lsmeans, $P < 0.05$, $n = 9$) (Figure 2. 5B). When food consumption was compared between *C. formosanus* and *R. flavipes*, no significant difference was found at each moisture condition (0%: $P = 1.0000$; 1%: $P = 0.9972$; 5%: $P = 0.9900$; 15%: $P = 0.5854$; 25%: $P = 0.9645$; 30%: $P = 0.1499$; GLMM followed by lsmeans, $n = 9$).

Figure 2. 5. Violin plots represent consumption for (A) *C. formosanus* and (B) *R. flavipes* at six different moisture gradients after 28 days. In each plot, all data points are shown, dash lines indicate first and third quartiles, and solid line indicates the median. Different letters denote significant differences between moisture conditions (GLMM followed by lsmeans, $P < 0.05$, $n = 9$).



2.4. Discussion

2.4.1. Water balance in subterranean termites

Our study demonstrated the effects of constant substrate moisture content on foraging behavior and survivorship of *C. formosanus* and *R. flavipes* over 28 days. Subterranean termites can adjust their microhabitat by using fecal matter as insulation in carton nests and mud tubes, excrete liquids orally to increase RH, or even augment the food source to be more moist (Grube et al. 1997, Gallagher and Jones 2010). Substrate moisture is a critical aspect of water availability that assists in maintaining water balance in subterranean termite (Woon et al. 2019). This study showed that sand moisture $\leq 1\%$ was a water deficit condition for both *C. formosanus* and *R. flavipes* (Figure 2.4, 2.5). Sand moisture at 0% resulted in 100% mortality in termites due to desiccation, and it was insufficient for tunneling because sand does not hold shape when completely dry. A minimum of 1% moisture content in sand was sufficient for tunnel formation in both species (Figure 2.3). However, 1% was insufficient in maintaining necessary microclimatic conditions needed to prevent complete colony mortality by desiccation (Figure 2.4). And 5% was the lowest moisture content in sand that maintained colony survival, resulting in comparable food consumption and tunneling activity to moisture conditions of 15% and 25% in both species (Figure 2.4, 2.5). This was possibly because 5% moisture content allowed termites to maintain an environmental equilibrium of RH within the tunneling area in both species without behaviorally releasing too much body water content or passively losing it through their soft cuticle (Grube et al. 1997, Grube and Rudolph 1999, Zukowski and Su 2019). In field site where

soil moisture is variable and different moisture gradients can be accessed, termites may exhibit a preference on higher moisture for microclimatic modification. Previous studies showed that, in choice tests, tunneling of *C. formosanus* was positively correlated to soil moisture where termites would seek higher soil moisture conditions or augment it using available water from neighboring soil with higher moisture contents (Green et al. 2005). In a natural setting, soil moisture influences how subterranean termites excavate through various types of soil, expressing preference to soil with higher moisture content and less organic matter or dry soil with large amounts of organic matter (Cornelius and Osbrink 2010). Subterranean termites also express preference to granule size of their substrate, preferring smaller soil granule size over larger while being unable to move soil granules larger than 1.7 mm in diameter (Tamashiro 1991, Jouquet et al. 2002).

Additionally, neither species under these experimental parameters showed any significant difference in total tunnel area at any time point among 1%, 5%, 15%, 25%, and 30%, with the exception of 0% moisture since no tunneling took place in either species. (Figure 2.3). A previous study in *C. formosanus* and *R. flavipes* showed that daily distance traveled decreased as moisture percentages increased, but the inverse was observed for tunnel area in sand moisture levels 4% up to 16% where tunneling increased as moisture conditions increased (Su and Puche 2003). The discrepancies between these findings may be attributed to the means by which data were collected. In that choice assay where soil moisture reduced as distance from the water source increased, the daily amount of sand moved per individual day in each grid section of the arena was analyzed. Termites of *C. formosanus* and *R. flavipes* had the option of tunneling through various moisture gradients and may have expressed preferential treatment to certain soil moisture conditions. In our study, which was a forced exposure assay with constant and

homogenous soil moisture conditions in each arena, the cumulative area tunneled over seven days was measured, eliciting behavior pertinent only to that moisture condition. Based on the results from our experiment, the total area tunneled over a seven-day period was not significantly different between moisture conditions. The insignificance of soil moisture content on tunneling after seven days has been previously mentioned (Su and Puche 2003).

2.4.2. Comparisons between *C. formosanus* and *R. flavipes*

Both *C. formosanus* and *R. flavipes* can sustain colony survival down to 5% soil moisture for at least 28 days, and there were no significant differences between species in foraging behavior or survivorship under the same moisture condition, suggesting that soil moisture under the tested temperature is unlikely a major contributing factor to the difference in geographic distribution of the two species. Two major factors that could affect the northern distribution of *C. formosanus* is a dependence on temperature and humidity outside the subterranean environment. Parts of the United States that both *C. formosanus* and *R. flavipes* are sympatric to constitute higher average humidities and temperatures in comparison to the north-western parts of the United States inhabited only by *R. flavipes* (Cao and Su 2016). Temperature preference between the two species may contribute to the differences in their distribution considering *C. formosanus* preferred higher temperatures compared to *R. flavipes* in a choice assay (Cao and Su 2016). Both species tunneling increased with increasing temperature suggesting that activity increases in warmer soil conditions (Cao and Su 2014). Preferences to higher RH in *C. formosanus* are also expressed where almost all individuals aggregated in 98% RH (Gautam and Henderson 2011b). The humidity preference of *R. flavipes* remains unknown since previous research only

observed seasonal foraging variation (Idham et al. 2005). Nuptial flights of both species are also influenced by temperature and RH, which only take place between May through June, around sunset, and with mean RH and temperature of 60-70% and 25°C for *C. formosanus* (Higa and Tamashiro 1983). Alates of *R. flavipes* are far less specific in their conducive conditions, taking flight mid to late spring and again in early fall depending on the geographic population, with a broad range of temperatures but only during daylight hours (Dietz 1919, Nutting 1969). However, these conducive environmental conditions are potentially only preferences (Higa and Tamashiro 1983, Green et al. 2005, Cornelius and Osbrink 2011, Gautam and Henderson 2011b). The preferred environmental conditions could be altered over time if previously preferred conditions never arise given that there are slight variations in time of year, temperature and humidity conditions correlated to nuptial flights of *C. formosanus* dependent on geographic location (Higa and Tamashiro 1983, Leong et al. 1983).

To focus only on natural means of dispersal is to ignore *C. formosanus* as being invasive to the United States and transported worldwide via human interference, like almost all invasive species (Scheffrahn and Crowe 2011, Simberloff 2013). After assisted introduction to a non-native area, the invading colony may eventually produce new colonies centered around their initial introduction site. In the case of two unique populations of *C. formosanus* in California, both were well established, and one had undergone nuptial flights even though temperature and RH were 30-35°C and 55-65% for that day (Tseng et al. 2021). That is outside the mean swarming temperature of 25°C and 60-70% RH previously mentioned (Higa and Tamashiro 1983). Additionally, how *C. formosanus* responds to temperature or humidity conditions may be more complex in the climate change era, as global warming is projected to continue for the future decades based on historic weather data (Hansen et al. 2006, Stott et al. 2006, Fyfe et al. 2011).

Research suggests that if trends continue, global average atmospheric temperatures could be over 2-4°C higher than 1990 (or 1-2°C higher than 2010) by the year 2100 (Stott et al. 2006). This minute change in temperature may result in drastic influences on precipitation given that every 10°C increase in temperature correlates to a doubling effect in vapor pressure (Fowler and Hennessy 1995). This implies that increasing global atmospheric temperatures correlates to an increase in both precipitation event duration and magnitude of precipitation events but a decrease in total number annually (Fowler and Hennessy 1995). In addition, extreme climate events, including droughts and flooding, are predicted to become more frequent depending on the geographic locations (Easterling et al. 2000). Under the impacts of climate change, areas currently uninhabitable to *C. formosanus* may also be at risk of invasion, and some current distribution ranges may become no longer suitable in the future. If northern ecologies increase in temperature and gain more precipitation, it could provide more hospitable living conditions for species limited to southern climates with those conditions, like *C. formosanus*. Likewise, some southern ecologies could become uninhabitable to *C. formosanus* if southern climates are too warm, decreasing the total number of precipitation events and creating extended periods of drought (Easterling et al. 2000).

2.4.3 Future directions

Future directions on substrate moisture may incorporate temperature as an additional factor to determine how desiccation tolerance, food consumption, tunneling, and survivorship are affected in subterranean termites. In addition to behavioral aspects, it is important to investigate the physiological mechanisms of desiccation resistance in subterranean termites and their plastic

response to changes in environmental water availability. The drywood termite, *Cryptotermes brevis* (Blattodea: Kalotermitidae), exhibits plastic changes in cuticular hydrocarbons that constitute a desiccation barrier in response to RH, suggesting its acclimation capacity to environmental stress (Woodrow et al. 2000). The physiological underpinnings in subterranean termites warrant further investigation. A comprehensive understanding of behavioral and physiological mechanisms is critical for determining the potential shifts in distribution range of *C. formosanus*, an economically devastating invasive species worldwide.

Chapter 3.

Effects of sand moisture on cuticular hydrocarbon profiles and desiccation resistance in the Formosan subterranean termite (Blattodea: Rhinotermitidae)

3.1. Introduction

The *Coptotermes* species (Blattodea: Rhinotermitidae) worldwide geographic distribution spans primarily tropical and subtropical regions (Evans et al. 2019). Endemic to continental China and Taiwan, the Formosan subterranean termite, *C. formosanus* Shiraki, was transported to the United States via maritime activity and mediated through wood and agricultural products (Kistner 1985, Scheffrahn and Crowe 2011, Evans et al. 2013). The species now imposes high risks as a pest species against forestry crops and wood structures worldwide (Evans et al. 2013, Krishna et al. 2013, Evans et al. 2019). The species has occupied temperate, subtropical and tropical regions in the United States for over 70 years dispersing north into Tennessee but has not been detected farther west than southeast Texas, except for three isolated populations in California (Beal 1967, Su and Tamashiro 1986, Howell 1987, Tseng et al. 2021). This could be because *C. formosanus*, potentially, has physiological limitations preventing it from establishing in more arid regions with lower soil moisture conditions across central and western parts of the country.

Subterranean termites (family Rhinotermitidae) are prone to desiccation in low relative humidity outside of their subterranean environments, like most small ectotherms, because of high body fat content, high metabolic rates, and a large surface area to volume ratios (Gibbs et al.

1997, Hoffmann and Harshman 1999, Woon et al. 2019). Most ancestrally, cuticular hydrocarbons (CHCs) act as an external hydrophobic barrier on insect cuticle to regulate transpirational water loss (Blomquist and Bagnères 2010). These CHCs are accompanied by multiple lipids like sterols, esters, fatty acids, and alcohols on the external cuticular surface (Gibbs and Pomonis 1995).

Cuticular hydrocarbon synthesis is strongly associated with oenocytes which are located within the fat body or embedded in the epidermal layer of insects (Figure 3.1) (Schal et al. 1998). It is unknown in termites if oenocytes are located in the fat body or epidermal layer (Figure 3.1). But, its location is believed not to affect the general biochemical process of CHC synthesis (Schal et al. 1998). Synthesis of methyl-branched alkanes, linear alkanes, alkenes or alkadienes begins with one of three precursor enzymes, methylmalonyl-coenzyme A (methylmalonyl-CoA), malonyl-coenzyme A (malonyl-CoA), or propionyl-coenzyme A (propionyl-CoA) (Juarez et al. 1992, Gu et al. 1997, Blomquist and Bagnères 2010, Holze et al. 2021). Synthesis of linear alkanes starts with acetyl-coenzyme A (acetyl-CoA), a derivative of fatty acids and coenzyme A in the citric acid cycle, binding to malonyl-CoA, derived from melonic acid and coenzyme A, during fatty acid synthesis (FAS) which produces acyl-CoA (Figure 3.2) (Howard and Blomquist 2005, Blomquist and Bagnères 2010, Holze et al. 2021, Moris et al. 2021). The resulting acyl-CoA is continually bound to malonyl-CoA, for even carbon chain lengths, or propionyl-CoA, for uneven carbon chains, during FAS creating long chain fatty acyl-CoA (Blomquist and Bagnères 2010, Holze et al. 2021). These long chain fatty acyl-CoAs can be further elongated with the addition of a malonyl-CoA using fatty acid elongases which are then reduced to aldehydes by fatty acyl-CoA reductases (Howard and Blomquist 2005, Holze et al. 2021). Finally, the resulting aldehyde from fatty acyl-CoA reductase undergoes oxidative decarbonylation by a cytochrome p450

decarbonylase which removes one carbon from the aldehyde creating a linear alkane (Reed et al. 1995, Qiu et al. 2012). Methyl-branched alkanes are produced under similar parameters except the precursor enzyme is methylmalonyl-CoA added to acetyl-CoA during a specialized FAS (Juarez et al. 1992). Alkene and alkadiene synthesis are also similar to linear alkane synthesis except fatty acid desaturases insert double bonds into the long chain fatty acyl-CoAs before interaction with fatty acid elongase, creating unsaturated hydrocarbons with double bonds (Blomquist and Bagnères 2010, Holze et al. 2021). Once produced, CHCs are transported via the lipoprotein, lipophorin, from the oenocyte, through hemolymph, then released into pore canals that evaginate the cuticular layers allowing for release of CHCs on the epicuticular surface (Figure 3.1) (Haruhito and Haruo 1982, Schal et al. 1998).

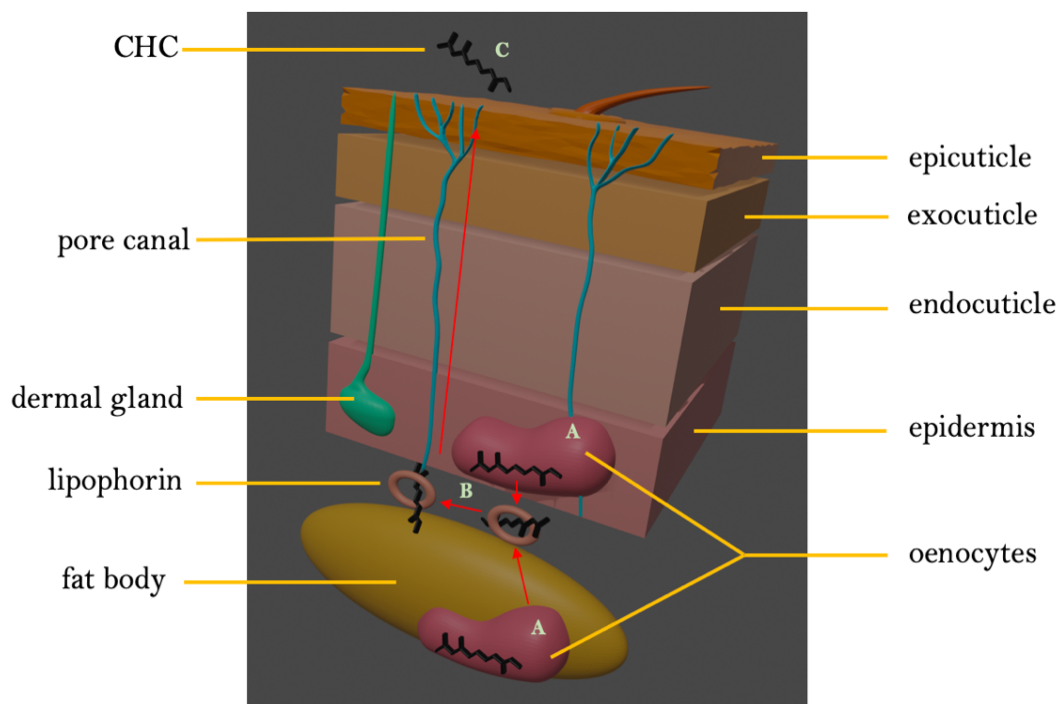


Figure 3.1. Hydrocarbon pathway from production by oenocytes (A), transportation by lipophorin to the pore canal (B), and excretion from the pore canal to the epicuticular surface (C). Modeled using Blender 3.1 (Blender Institute, Amsterdam, Netherlands).

Greater concentrations of longer chain hydrocarbons and saturated hydrocarbons (alkanes), are closely correlated with desiccation resistance because of their higher melting temperature compared to shorter chain hydrocarbons and unsaturated hydrocarbons (alkenes) (Neville 1975, Gibbs and Pomonis 1995, Gibbs et al. 1997, Blomquist and Bagnères 2010). Longer chain CHCs with more than 20 carbons are the compositional majority of lipids on insect cuticle (Blomquist et al. 1987, Lockey 1988). Specifically, only linear alkanes and methyl-branched alkanes were detected in *C. formosanus* using gas chromatography mass-spectrometry analysis (GC-MS) (Haverty et al. 1996). In addition to desiccation resistance, CHCs evolved as communication signals in insects, resulting in distinct CHC profiles between species, colonies and sexes (Howard and Blomquist 1982). Although the qualitative composition of CHCs does not change between individuals of the same caste in a *C. formosanus* colony, the quantity of individual CHCs have been shown to fluctuate seasonally (Howard and Blomquist 1982, Haverty et al. 1996).

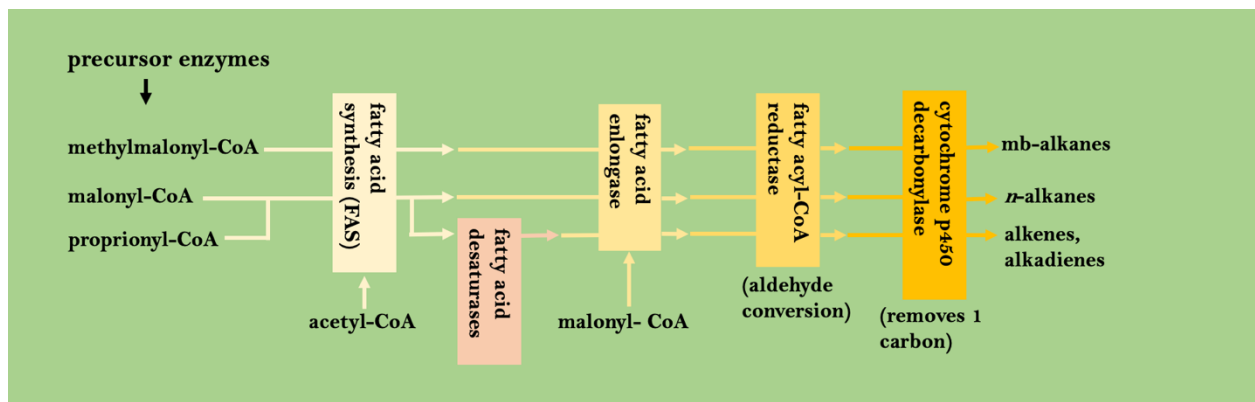


Figure 3.2. Illustration of general biosynthesis pathway for linear alkanes, methyl-branched alkanes, alkenes, and alkadienes.

In colonies of *C. formosanus*, 13-; 15-methylnanotricotane (13-; 15- meC29) and 15,17-dimethylnanotricotane (15,17- dimeC29) and 13,17-; 15,19-dimethylnanotricosane (13,17-; 15,19-dimeC29in) workers decreased from around 1.1% of the total CHCs in March to below 0.6% in May through June (Haverty et al. 1996). Soldiers also had a similar drop in 9-; 11-; 13-methylpentacosane (9-; 11-; 13-meC25) from February at 1.6% down to 1% in May, while, 9-; 11-; 13-methylheptacosane (9-; 11-; 13-meC27) rose from below 5% of the total CHCs during March to over 7% in May. These patterns in workers and soldiers regressed after June. The drop in worker CHCs during swarm season was most likely from workers differentiating into immatures, leaving behind the remaining younger workers which had different CHC profiles from the older workers (Haverty et al. 1996). The decrease in 9-; 11-; 13-meC25 in soldiers was suggested to be for similar reasons since more investment in colony populations are focused on soldiers during peak swarm seasons in subterranean termites (Haverty and Howard 1981). The increase in 9-; 11-; 13-meC25, a longer chain hydrocarbon, may be linked more to desiccation resistance since soldiers will be directly exposed to external temperature and humidity conditions outside the colony while guarding alates during dispersal (Nutting 1969).

Additionally, CHC profiles of *Drosophila* are also distinct among population of various geographic distributions and seasonal origins, with shorter chain hydrocarbons such as *n*-tricosane, (Z,Z)-7,11-pentacosadiene and (Z)-9-pentacosene more prevalent in cooler climates, and CHCs with longer chains such as (Z,Z)-5,9-heptacosadiene and heptacosene predominant in warmer climates (Rajpurohit et al. 2017). It is possible that both humidity and temperature played a significant role in changing CHC quantities since the localities used had notable differences in average RH corresponding to higher temperatures between collection sites (Rajpurohit et al. 2017). Additionally, the effect of temperature on CHCs has been demonstrated.

In *Drosophila*, the CHC profiles can be altered during pupal emergence with longer chain CHCs upregulated in warmer conditions (Rajpurohit et al. 2021). Similar observations were made in an acclimation study with the drywood termite, *Cryptotermes brevis* (Blattodea: Kalotermitidae), where notable changes in CHC profiles were expressed, particularly an increase in longer chain linear alkanes, under low RH independent of temperature (Woodrow et al. 2000). This suggests that CHC profiles of *C. brevis* and temperature are not correlated. However, unlike Rhinotermitidae that dwell underground and mediate humidity or temperature behaviorally, Kalotermitidae colonies have a different nesting ecology and are not known for the behavioral regulations, and physiological mediation may be important for that group. Little was known if similar physiological regulations through CHCs occurs in subterranean termites upon changes in environmental water availability.

Previous studies on CHCs of *C. formosanus* have been mainly focused on chemical communication (Su and Haverty 1990, Haverty et al. 1996, Florane et al. 2004, Chouvenec and Su 2017). No empirical study has been conducted to investigate CHC acclimation and its correlation with desiccation resistance, which is important for understanding physiological adaptation of these termites when they are introduced to localities with low water availability like arid environments in west Texas (Haverty and Howard 1981, Howard and Blomquist 1982, Su and Haverty 1990, Haverty et al. 1996). It is hypothesized that desiccation resistance and CHC profiles in *C. formosanus* change in response to water availability in the soil. To test CHC profile alterations and desiccation resistance, we acclimated *C. formosanus* colonies to three sand moisture conditions, and comparatively analyzed survival of workers under desiccation stress and their CHC profiles.

3.2. Materials and Methods

3.2.1. Termite Colonies

Termites from three colonies of *C. formosanus* were collected at Brechtel Park in New Orleans, Louisiana ((29°54'18.5"N, 90°00'44.3"W) at least 200 meters apart. Traps consisted of pinewood blocks tied together and buried. Three colonies of *C. formosanus* were maintained in an incubation fridge at $27 \pm 1^\circ\text{C}$ and 80-100% RH in complete darkness. Collection samples from both species were maintained in an incubator at $27 \pm 1^\circ\text{C}$ and 80-100% RH in complete darkness and were maintained in lab at Louisiana State University. The colonies were provisioned with Kiln-dried pine wood blocks and provided with moist organic soil (Miracle-Gro All Purpose for In-Ground Use, ScottsMiracle-Gro, Marysville, OH).

3.2.2. Experimental Design for Acclimation

To test how substrate moisture affects CHC profiles and desiccation resistance in *C. formosanus*, over a six-week acclimation period, three sand moisture conditions (5%, 15%, 25%) were used following sand moisture calculations used in Chapter 2. Field collections from three different colonies were used. In total, 3600 termites (including approximately 10% soldiers) were removed from each colony with 1200 termites placed in their respective containers. Eighteen sealed plastic containers ($32 \times 25.5 \times 10$ cm, Pioneer Plastics, Dixon, KY, USA) were filled with 1100 g of dry sand (Quikrete premium play sand, Atlanta, GA) as a substrate then mixed with distilled water,

equally distributing water content throughout the sand to achieve either 5%, 15%, or 25% sand moisture (wt [dH₂O]: wt [sand]). Approximately 20 g of pinewood popsicle sticks were used as a food source and were pre-dried at 80°C overnight in oven and again post experiment. All containers were sealed and wrapped using parafilm (Parafilm M®, Neenah, WI) to prevent water loss. Additionally, the containers were placed in an incubator and maintained at 27 ± 1°C with open tubs of water at the bottom to maintain over 90% RH.

3.2.3. Hydrocarbon Analysis

After six weeks of acclimation, 100 workers, divided into ten sets of ten workers, were extracted from each of the nine containers. Each sample of ten workers were pre-weighed, then frozen at -20°C for 10 min, and all ten termites were thawed in room temperature and completely submerged in a wash vial containing 100 µl of hexane with 500 ng *n*-nonadecane as an internal standard (MilliporeSigma, Burlington, MA). Samples were then gently swirled at 5 min and 10 min, then the solution was transferred to a separate clean vial. The CHC samples were stored at -20°C before analysis using gas chromatography–mass spectrometry (GC-MS) (GC: Trace 1310 GC in splitless mode; MS: ISQ 7000; column: TG-5MS, 30 m × 0.25 mm × 0.25 µm; Thermo Scientific, Waltham, MA). Helium was used as the carrier gas (1.0 ml/min). Column temperature was programmed to increase from 90°C to 150°C at 20°C/min, and then to 320°C at 10°C/min with 5 min hold at 320°C. Injection temperature was 280°C, MS source temperature was 310°C, and transfer line was 300°C. The MS was set to scan a mass range from 45 to 550. Standard mixture of linear alkanes (C7 to C40) (MilliporeSigma, Burlington, MA) were injected following the same temperature program. CHCs were tentatively identified based on electron ionization

mass spectra, Kovats indices, and previously published data in *C. formosanus* (Haverty and Howard 1981, Cvačka et al. 2006). The quantities of CHCs were calculated based on the internal standard and normalized to body weight to adjust for any differences in surface area between samples. Bodyweight normalization was done by dividing the absolute ng/mg (which is $1000 \times \text{area count of unknown} / \text{area count of n-19C}$) by the termite body weight of that sample. In the text and tables, CHCs are addressed using shorthand nomenclature to identify hydrocarbons.

3.2.4. Desiccation Assay

A desiccation chamber was designed using a transparent square container with inner dimensions $6\text{-}3/4'' \times 4\text{ } 13/16'' \times 2\text{-}3/8''$ (Pioneer Plastics, Dixon, KY, USA) with 27 small vials (4.45cm height by 1.27cm diameter), labeled and hot glued to the internal wall around the container. At the bottom of the container, a 5.5 cm wide Petri dish was hot glued to the center with 20g of desiccant containing 98% CaSO_4 and 2% CoCl_2 (Drierite, W.A. Hammond Drierite Company, Xenia, OH). An Onset's HOBO and InTemp Data Loggers (Onset Computer Corporation, Bourne, MA) was inserted into the chamber to ensure RH remained $\leq 1\%$. Five workers were placed in each vial (15 termites per soil moisture condition per colony) making a total of 45 replications for each acclimation condition. The desiccation chambers were kept in an incubator at $27 \pm 1^\circ\text{C}$ in complete darkness. Mortality of each individual (i.e., a complete lack of movement when shaken) was observed hourly, and desiccation resistance was determined by survival time of termites.

3.2.5. Statistical Analysis

Statistical analysis was performed using R software version 4.1.2 (The R Foundation, Vienna, Austria). To test for normality of all dataset distributions and homogeneity of variances, Shapiro-Wilk test and Levene's test were performed, respectively. A significance level of $\alpha = 0.05$ was used for all tests in this study. The desiccation data did not fit the generalized linear mixed model (GLMM), and they were analyzed using two-way analysis of variance with soil moisture, colony, and interaction between soil moisture and colony as factors, followed by Tukey's HSD test. Quantities of linear alkanes, methyl-branched alkanes, and total CHCs data met the assumptions for parametric analysis ($P > 0.05$ for Shapiro-Wilk and Levene's test), and GLMM followed by least squared means test was used. Body weight between moisture conditions did not fit the assumptions for parametric analysis and Kruskal- Wallis followed by lsmeans was used.

3.3. Results

3.3.1. Hydrocarbon Analysis

A total of 20 peaks were detected in GC-MS (9 linear alkanes and 12 methyl-branched alkanes) for workers of *C. formosanus* (Table 3.1, Figure 3.3, 3.4). No unsaturated alkanes or other cuticular lipids were detected. The quantity of total CHCs in workers acclimated to 5% sand moisture was significantly greater than to 25%, while no significant difference was found between 15% to any of the other two conditions (Figure 3.5, 15% - 25%: $P = 0.0545$; 15% - 5%: $P = 0.0630$; 25% - 5%: $P = 0.0001$; GLMM followed by lsmeans, $\alpha = 0.05$; $n = 30$). With methyl-branched alkanes, significant difference was present only between 25% when compared to 5% and 15% (Figure 3.6 A, 15% - 25%: $P = 0.0002$; 15% - 5%: $P = 0.0601$; 25% - 5%: $P = 0.0001$, GLMM followed by lsmeans, $\alpha = 0.05$; $n = 30$). For linear alkanes, significant difference was found between all three acclimation conditions with an increase in CHC quantity as soil moisture content decreased (Figure 3.6 B, 15% - 25%: $P = 0.0031$; 15% - 5%: $P = 0.0001$; 25% - 5%: $P = 0.0001$; GLMM followed by lsmeans, $P < 0.05$; $n=30$). For individual CHCs and their regulation at 5%, 15% and 25% soil moisture, *n*-heptacosane (*n*-C27), 3-methylheptacosane (3-meC27), *n*-octacosane (*n*-C28), 11-; 13-; 15-methyloctacosane (11-; 13-; 15-meC28), and *n*-nanocosane (*n*-C29) showed significant difference between all three moisture conditions where decreasing soil moisture corresponded to higher quantities (ng/mg) of each CHC (Figure 3.4, GLMM followed by lsmeans, $P < 0.05$, $n = 30$). Hydrocarbons *n*-hexacosane (*n*-C26), 2-methylhexacosane (2-meC26), 2-methylheptacosane (2meC27) + 9,13-dimethylheptacosane (9,13-dimeC27), 9-;11-;13-

;15-methylnanocosane (9-;11-;13-;15-C29), *n*-triacontane (*n*-C30), and *n*-hentriacontane (*n*-C31) all expressed significant difference between 5% and 25% soil moisture with higher quantities at 5% when compared to 25% (Figure 3.4, GLMM followed by lsmeans, $P < 0.05$, $n = 30$). No significant differences were observed in *n*-pentacosane (*n*-C25), 2-meC25, or 13,15-dimethylnanocosane (13,15-dimeC29) between 5% and 25% or within *n*-dotriacontane (*n*-C32) and 9-; 11-; 13-meC27 at any moisture condition (Figure 3.4, GLMM followed by lsmeans, $P > 0.05$, $n = 30$).

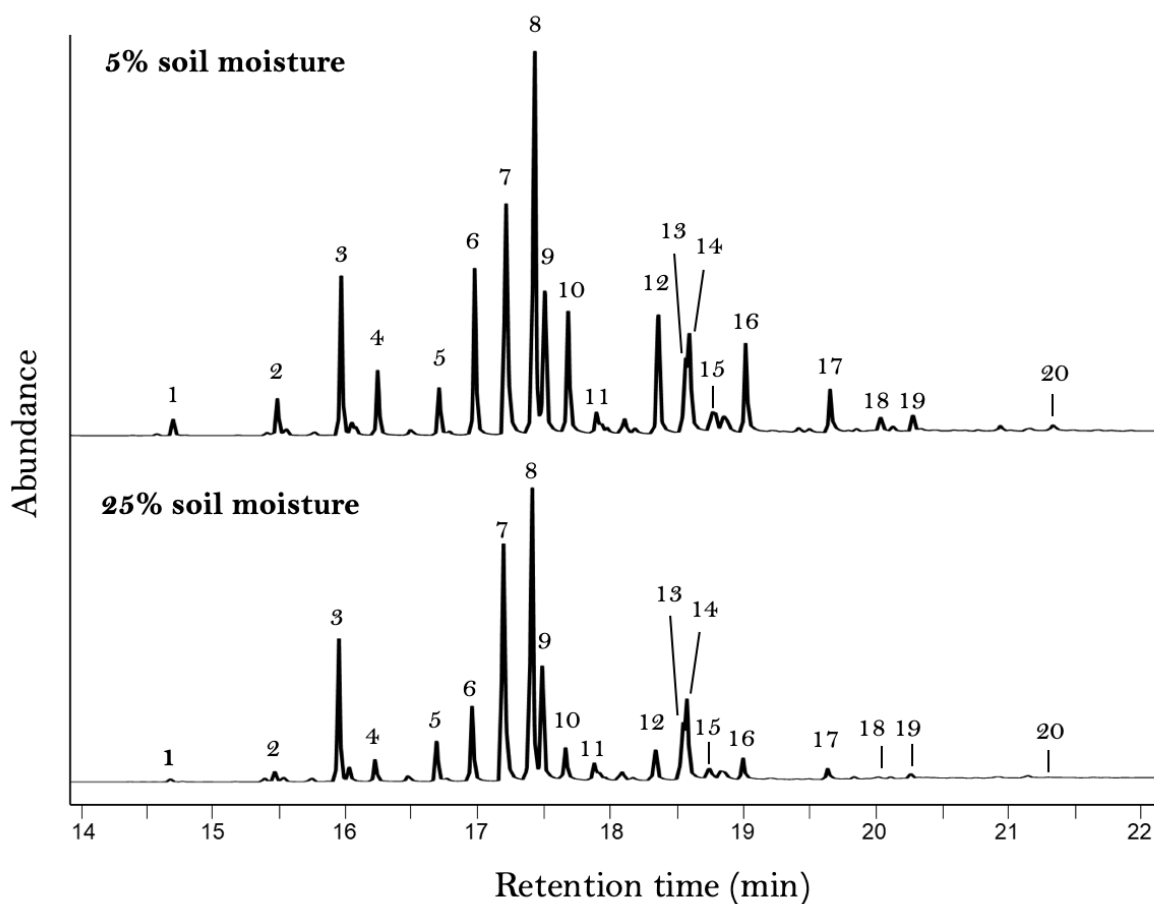


Figure 3.3. Example chromatograms for 5% and 15% soil moisture depicting CHC profile of *C. formosanus* by peak number.

Figure 3.4. Quantities of CHC peaks in *C. formosanus* workers acclimated to 5%, 15%, 25% soil moisture. In each peak, the quantity of CHC normalized to body weight is shown (mean \pm SE). Groups with no shared letters indicate significant difference between moisture conditions for that peak (GLMM + lsmeans, $P < 0.05$, $n = 30$).

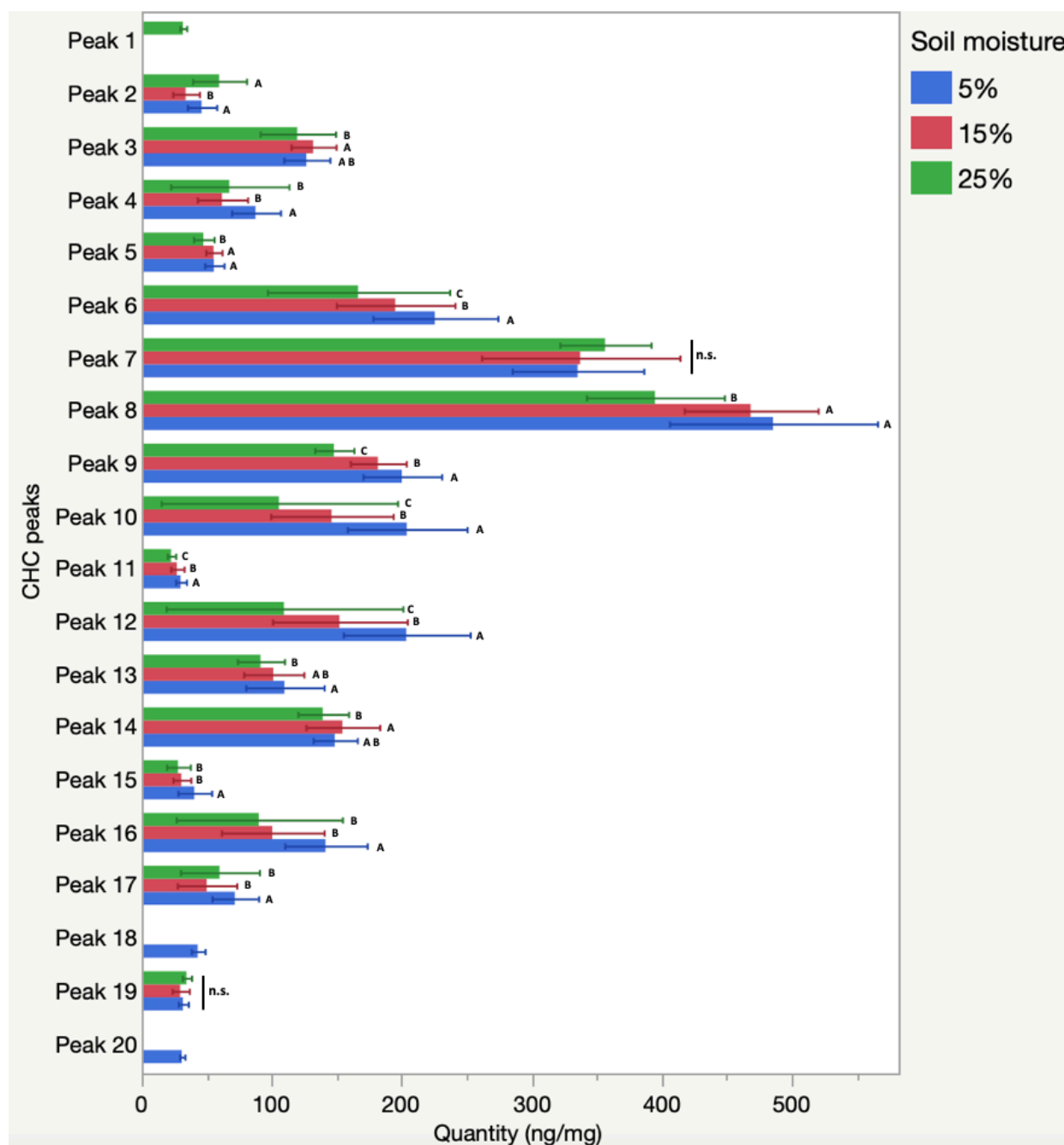


Table 3.1. Tentative identification of CHCs in *C. formosanus* as shorthand nomenclature with retention time and Kovats index. Peak numbers correspond to peaks in Figures 3.3 and 3.4.

Peak no.	Identity	Retention time (min)	Kovats index
1	n-C24	14.674	2400
2	n-C25	15.463	2500
3	2-meC25	15.946	2563
4	n-C26	16.221	2600
5	2-meC26	16.687	2663
6	n-C27	16.956	2700
7	9-; 11-; 13-meC27	17.194	2734
8	2-meC27 + 9,13-dimeC27	17.411	2764
9	3-meC27	17.486	2775
10	n-C28	17.663	2800
11	11-; 13-; 15-meC28	17.877	2831
12	n-C29	18.347	2900
13	9-; 11-; 13-; 15-meC29	18.554	2931
14	13,15-dimeC29	18.581	2935
15	2-meC29 or 9,13-dimeC29	18.758	2962
16	n-C30	19.007	3000
17	n-C31	19.646	3100
18	13,15-; 15,17-dimeC31	20.030	3161
19	n-C32	20.275	3200
20	11-; 13-; 15-; 17-meC33	21.333	3355

Figure 3.5. Total CHCs of *C. formosanus* workers acclimated to 5%, 15%, and 25% soil moisture. In each group, the quantity of all CHCs normalized to body weight is shown (mean \pm SE). Groups with no shared letters indicate significant difference (GLMM followed by lsmeans, $P < 0.05$, $n = 30$).

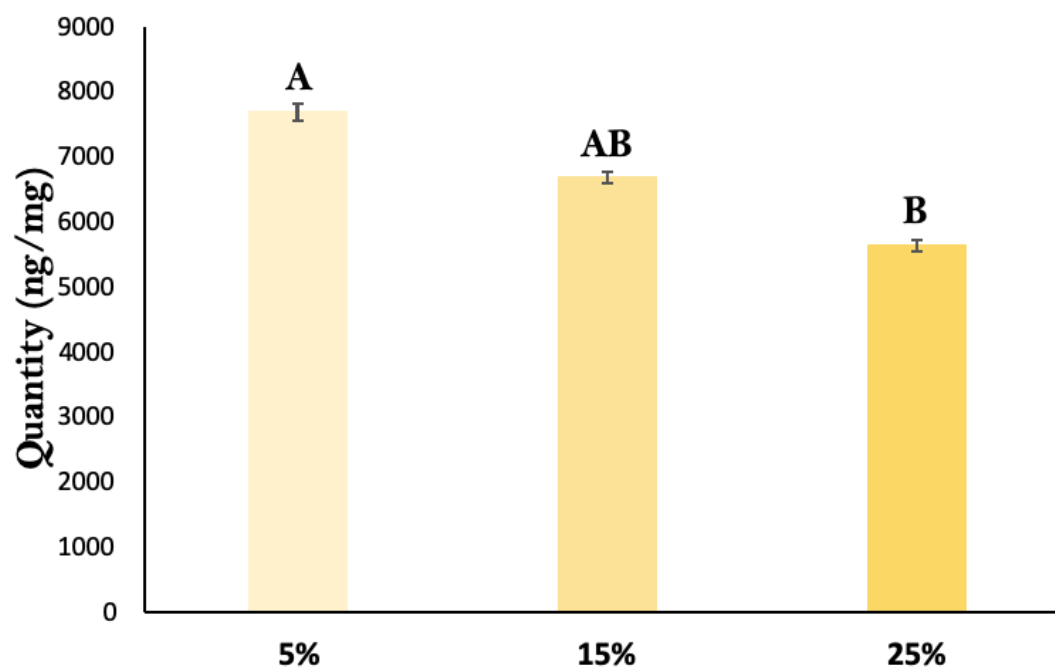
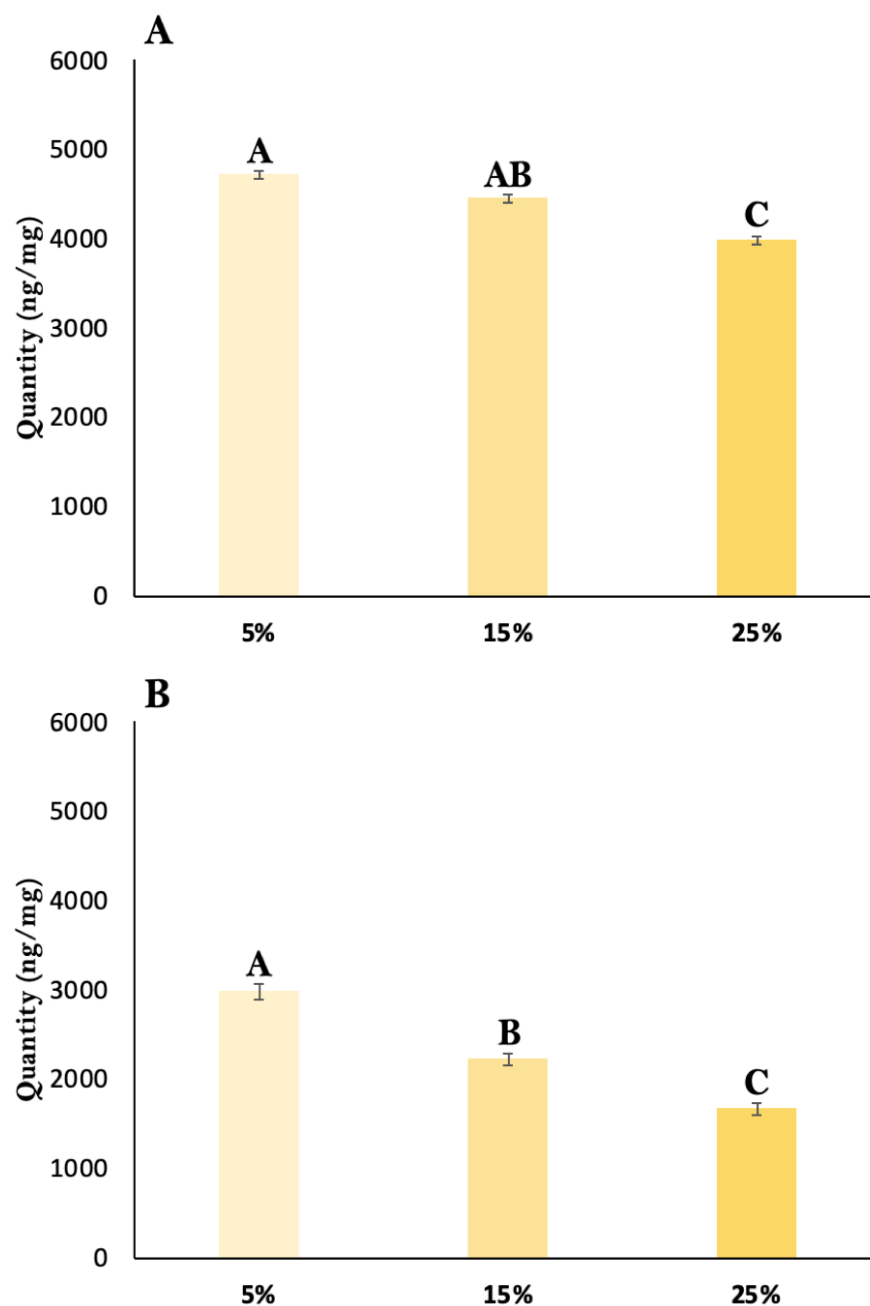


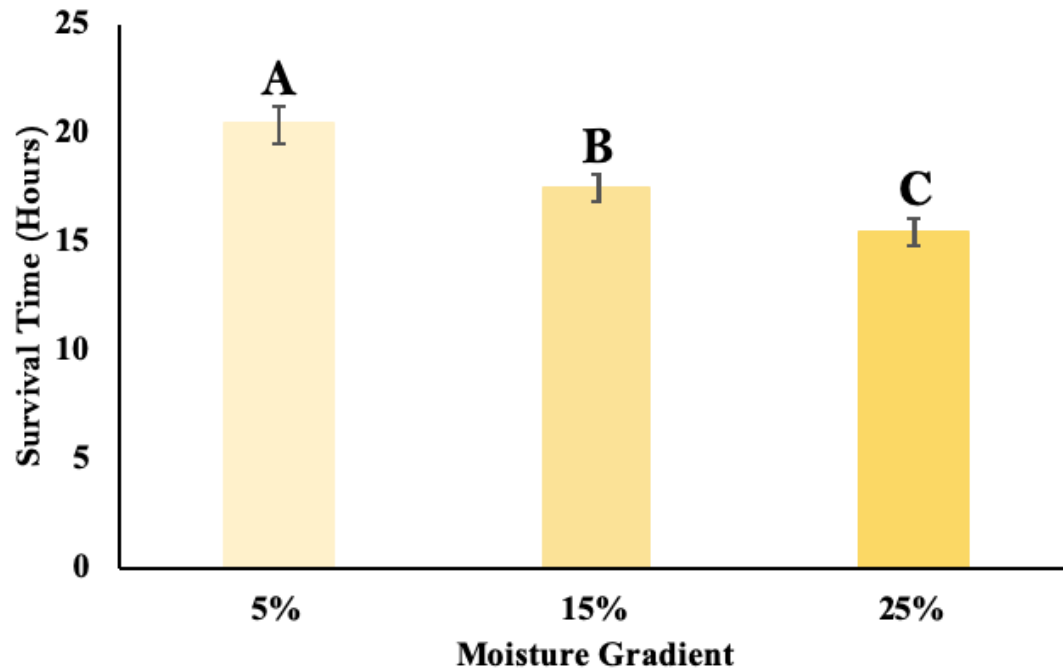
Figure 3.6. Total methyl-branched (A) and linear alkanes (B) of *C. formosanus* workers acclimated to 5%, 15%, and 25% soil moisture. In each group, the quantity of all alkanes normalized to body weight is shown (mean \pm SE). Groups with no shared letters indicate significant difference (GLMM followed by lsmeans, $P < 0.05$, $n = 30$).



3.3.2. Desiccation Assay

Workers of *C. formosanus* maintained at 5%, 15%, and 25% soil moisture for six weeks exhibited significant difference in mean survival time when compared to each other (Figure 3.7, 5% - 15%: $P = 0.0031$; 5% - 25%: $P < 0.0001$; 15% - 25%: $P = 0.0449$, two-way ANOVA followed by Tukey's, $P < 0.05$, $n = 45$). No significance between moisture conditions was found in average body weight of samples (5% = 0.0409 ± 0.001 g, 15% = 0.0398 ± 0.0009 g, 25% = 0.0394 ± 0.0007 g, mean \pm SE, Kruskal-Wallis, $n = 30$). There was an effect of colony on survival time ($P < 0.0001$, two-way ANOVA) and an interaction between colony and moisture ($P = 0.0262$, two-way ANOVA). The survival time of workers was 20.36 ± 0.79 hours (mean \pm SE) at 5% soil moisture, 17.47 ± 0.63 hours at 15% soil moisture, and 15.38 ± 0.64 hours at 25% soil moisture. One outlier survived for 25 hours before it was no longer able to flip itself over after being shaken. At 26 hours, no movement was observed.

Figure 3.7. Mean desiccation resistance of *C. formosanus* workers acclimatized to 5%, 15%, and 25% soil moisture where the survival time (hours) of workers under desiccation stress at 1% RH is shown. Groups with no shared letters indicate significant difference (Mean \pm SE, two-way ANOVA followed by Tukey's, $P < 0.05$, $n = 45$).



3.4. Discussion

Our hypothesis was supported in that CHC profiles did express a significant difference in quantities between moisture acclimation conditions. Until now, research on CHC alterations in response to soil moisture conditions in *C. formosanus* or any subterranean insect had not been directly investigated (Haverty et al. 1996). This is, in part, because most experiments dealing with *C. formosanus* and soil moisture focused on seasonal variation in thermal resistance or consumption and survivorship within choice assays or field studies (Hu and Appel 2004, Green et al. 2005). The results of this experiment suggest that regulation of CHC profiles in *C. formosanus* workers are strongly affected by soil moisture, and CHC quantities increased with decreasing soil moisture upon an acclimation period. Body weight was not affected by differing soil moisture conditions.

Both methyl-branched and linear alkanes had significant increases in total quantity when comparing 5% to 25% soil moisture (Figure 3.6). Interestingly, although previous studies suggested that 9-, 11-, 13-meC27 had environmental factors correlated to upregulation in soldiers during peak swarm season months (Haverty and Howard 1981), workers in this experiment did not express any significant difference in the regulation of 9-, 11-, 13-meC27 between moisture conditions (Figure 3.4). This could be because 9-, 11-, 13-meC27 has separate functions between castes or because it is correlated to communication rather than desiccation resistance (Sappington and Taylor 1990, Sugeno et al. 2006, Guédot et al. 2009). 2-MeC25 and 13,15-dimeC29 may have expressed no difference between 5% and 25% for similar communication functions (Ginzel et al. 2003, Murakami et al. 2015). *n*-C32 also expressed no

significant change under any moisture condition, possibly because it also correlates to communication within the colony (Sledge et al. 2004) (Figure 3.4).

Linear alkanes exhibited a consistent pattern of increasing with decreasing soil moisture, suggesting stronger plasticity to environmental conditions (Buellesbach et al. 2018) (Figure 3.6 B). Structurally, linear alkanes have higher melting points than branched alkanes, leading to a decrease in cuticular permeability, thus increasing desiccation tolerance by creating a stronger hydrophobic barrier (Neville 1975, Gibbs and Pomonis 1995, Gibbs et al. 1997, Blomquist and Bagnères 2010). However, the total quantities of methyl-branched alkanes were greater than linear alkanes and also increased with varying moisture conditions when comparing 5% to 25% soil moisture, suggesting that both linear alkanes and methyl-branched alkanes are plastic in response to water availability in soil (Figure 3.6).

Additionally, our results support the hypothesis with regard to changes in desiccation resistance, in that soil moisture influenced survival time of workers under desiccation stress. Body weight, which would correspond to an increase in external surface area, did not change between moisture conditions, suggesting that physical size was not a factor in desiccation pressure or CHC quantities. For 5% soil moisture and 1% RH, workers of *C. formosanus* survived for approximately 20 hours, which was significantly longer than 15% and 25% (Figure 3.7). This increase in survival time between moisture acclimation conditions was likely from the increase in CHCs of both linear and methyl-branched alkanes resulting in a less permeable barrier that decreases water loss (Gibbs and Pomonis 1995) (Figure 3.6). The causal effects of CHCs on desiccation resistance warrant further investigation.

The results of this experiment strongly support that soil moisture influences cuticular hydrocarbon profiles and desiccation resistance in *C. formosanus*. Further research could include

temperature into the soil moisture assay to determine if temperature and soil moisture have any effect on regulation of methyl-branched alkanes and linear alkanes. Additionally, investigating the influence of soil moisture and temperature and its interaction with CHCs on soldier or worker aggression (agnostic behavior) could give insight into how CHC profiles influence intercolonial interactions. This would be insightful considering previous studies suggest there is no link between agnostic behavior and CHC profiles (Su and Haverty 1990). Further research could also be done on 9-; 11-; 13-meC27, 2-meC25 and *n*-C32 and their effects on colony level communication or, specifically, agonistic behavior between soldiers. In addition, studies on CHC acclimation and desiccation resistance in other species will provide insights on the ecological adaptation of subterranean termites and help predict their future shifts in geographic distribution.

Chapter 4. Summary

4.1. Behavioral alterations

In chapter 2, both *C. formosanus* and *R. flavipes* were found to maintain above 50% survivorship down to 5% soil moisture for at least 28 days (Fig 2.4). And there were no significant differences between foraging behavior or survivorship under the same moisture conditions (Fig 2.3, 2.4, 2.5). This suggests that soil moisture, under the tested temperature, is unlikely the only contributing factor to the difference in geographic distribution of the two species. Two major factors that could affect the northern distribution of *C. formosanus* is a dependence on temperature and humidity outside the subterranean environment. An increase in tunneling was expressed by members of both species under warmer soil conditions suggesting that increased foraging is positively correlated to temperature (Cao and Su 2014). Preferences from members of *C. formosanus* to higher RH are also expressed where almost all individuals aggregated in 98% RH (Gautam and Henderson 2011b). Unfortunately, no research to date has presented what moisture conditions members of *R. flavipes* prefer but have only observed seasonal foraging variation (Idham et al. 2005). However, temperature and humidity conditions are potentially only preferences (Higa and Tamashiro 1983, Green et al. 2005, Cornelius and Osbrink 2011, Gautam and Henderson 2011b). And these preferred environmental conditions could be augmented over time if those conditions never occur. An example of this is that there are slight changes in flight patterns from alates of *C. formosanus* during swarming seasons, dependent on geographic location (Higa and Tamashiro 1983, Leong et al. 1983).

Additionally, *C. formosanus* response to changing temperature or humidity conditions could be more complex when taking into account global warming which is projected to be slightly elevated in future decades based on historic weather data (Hansen et al. 2006, Stott et al. 2006, Fyfe et al. 2011). If predicted trends continue, global average atmospheric temperatures could reach 2-4°C higher than 1990 (or 1-2°C higher than 2010) by the year 2100 (Stott et al. 2006). Although this temperature difference over a 100 year period might seem insignificant, an increase of 10°C correlates to a doubling effect in vapor pressure (Fowler and Hennessy 1995). This suggests that, although the total number of weather events occurring annually might decrease from increasing global temperatures, the duration and intensity of weather events is expected to increase (Fowler and Hennessy 1995). In addition, extreme climate events, including droughts and flooding, are predicted to become more frequent depending on the geographic locations (Easterling et al. 2000). Under the impacts of climate change, northern ecologies might become more hospitable for species limited to southern climates, like *C. formosanus* in the southern United States, and some southern ecologies could become uninhabitable to *C. formosanus* if southern climates are too warm, decreasing the total number of precipitation events and creating extended periods of drought (Easterling et al. 2000).

4.2. Physiological alterations

4.2.1. CHC alterations

Until now, alterations in CHC composition by soil moisture conditions had not been directly investigated in any insect (Haverty et al. 1996). It was found that alterations in both CHC composition and desiccation resistance took place in *C. formosanus*. A significant difference in CHC quantities were expressed between moisture conditions. The results of this experiment suggest that regulation of CHC profiles in *C. formosanus* workers are strongly affected by soil moisture, and CHC quantities increased with decreasing soil moisture upon an acclimation period. Body weight was not affected by differing soil moisture conditions.

Both methyl-branched and linear alkanes had significant increases in total quantity when comparing 5% to 25% soil moisture (Figure 3.6). Interestingly, although previous studies suggested that 9-, 11-, 13-meC27 had environmental factors correlated to upregulation in soldiers during peak swarm season months (Haverty and Howard 1981), workers in this experiment did not express any significant difference in the regulation of 9-, 11-, 13-meC27 between moisture conditions (Figure 3.4). This could be because 9-, 11-, 13-meC27 has separate functions between castes or because it is correlated to communication rather than desiccation resistance (Sappington and Taylor 1990, Sugeno et al. 2006, Guédot et al. 2009). 2-MeC25 and 13,15-dimeC29 may have expressed no difference between 5% and 25% for similar communication functions (Ginzel et al. 2003, Murakami et al. 2015). *n*-C32 also expressed no significant change under any moisture condition, possibly because it also correlates to communication within the colony (Sledge et al. 2004) (Figure 3.4).

Linear alkanes exhibited a consistent pattern of increasing with decreasing soil moisture, suggesting stronger plasticity to environmental conditions (Buellesbach et al. 2018) (Figure 3.6 B). Structurally, linear alkanes have higher melting points than branched alkanes, leading to a decrease in cuticular permeability, thus increasing desiccation tolerance by creating a stronger hydrophobic barrier (Neville 1975, Gibbs and Pomonis 1995, Gibbs et al. 1997, Blomquist and Bagnères 2010). However, the total quantities of methyl-branched alkanes were greater than linear alkanes and also increased with varying moisture conditions when comparing 5% to 25% soil moisture, suggesting that both linear alkanes and methyl-branched alkanes are plastic in response to water availability in soil (Figure 3.6).

4.2.2. Desiccation Resistance

Soil moisture also had a significant influence on desiccation resistance in *C. formosanus*. Interestingly, body weight did not change between moisture conditions, suggesting that physical size was not a factor in desiccation pressure or CHC quantities. For 5% soil moisture and 1% RH, workers of *C. formosanus* survived for approximately 20 hours, which was significantly longer than 15% and 25% (Figure 3.7). This increase in survival time was, most likely, from the increase in CHC quantities of both linear and methyl-branched alkanes on the extra cuticular surface under lower soil moisture conditions creating a more hydrophobic barrier which would decrease the rate of water loss and increase time until desiccation (Gibbs and Pomonis 1995) (Figure 3.6). The causal effects of CHCs on desiccation resistance warrant further investigation.

4.3. Future Directions

Future studies on soil moisture could incorporate temperature into the analysis to better determine how desiccation tolerance, CHC composition, food consumption, tunneling, and survivorship are affected in subterranean termites. Temperature may have an effect on regulation of methyl-branched alkanes and linear alkanes. Another insightful experiment would be to alter CHC profiles of workers along with soldiers under varying soil moisture conditions and pair up members of the same colony that acclimated under different moisture conditions (and pairing different colonies under differing moisture conditions) to observe their behavior, potentially giving insight into how CHC profiles influence intercolonial interactions. This experiment could be insightful considering previous studies suggest that CHC profiles and agonistic behavior (aggressing) in *C. formosanus* are not correlated (Su and Haverty 1990). Further research could also be done on 9-, 11-, 13-meC27, 2-meC25 and *n*-C32 and their effects on colony level communication or, specifically, agonistic behavior between soldiers and workers. In addition, studies on CHC acclimation and desiccation resistance in other species will provide insights on the ecological adaptation of subterranean termites and help predict their future shifts in geographic distribution. A comprehensive understanding of behavioral and physiological mechanisms is critical for determining the potential shifts in distribution range of *C. formosanus*, an economically devastating invasive species worldwide.

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Curriculum Vitae

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Professional Profile

'A commercially and academically knowledgeable entomology student with a passion for research and fieldwork who can illustrate insect morphology and translate research into presentable data.'

With a strong background in entomology, knowledge in IPM, field research experience within the laboratory, the United States and abroad; I have proven my ability to competitively succeed at a higher level of education in entomology. In addition to being focused on Entomology academically, I also have experience with entomology commercially through doing internships involving integrated pest management.

Core Skills

- Completed a Bachelor of Science in Entomology
- Experienced in 3D modeling and illustration

Education

- BS Entomology, Texas A&M University: 2015 — 2019
- MS Entomology, Louisiana State University: 2020 — 2022

Extracurricular Experience

June 2021

- Competed at the US Olympic Team Trials for swimming

Publications

Refereed Publications- Peer Reviewed Research Journals:

March 2019 *Zootaxa*

- Richardson, S. J., Trimm, T., Paredes, R., Koehl, J., Song, H. (2019). A new species of king cricket *Glaphyrosoma* Brunner von Wattenwyl, 1888

(Orthoptera: Anostostomatidae: Glaphyrosomatini) from Costa Rica with behavioral observations. *Zootaxa*. 4671 (1): 093–104

January 2021 Insects

- Shults, P., Richardson, S. J., Eyer, P. A., Chura, M., Barreda, H., Davis, R., Vargo, E. L. (2020). Area-wide Elimination of Subterranean Termite Colonies using a Novaluron Bait. *Insects*. DOI: 10.3390/insects12030192

Non-refereed Publications- University Affiliated Outreach Publications:

Fall 2020 LSU AgCenter BugBiz Series

- Richardson, S., Carlton, C. (2020) *Solenopsis invicta*, Red Imported Fire Ant (Hymenoptera: Formicidae). BugBiz.
- Richardson, S., Carlton, C. (2020). *Atta texana*, Texas Leaf Cutter Ant (Hymenoptera: Formicidae). BugBiz.
- Richardson, S., Carlton, C. (2020). *Gryllus texensis* and *G. rubens*, Field Crickets (Orthoptera: Gryllidae). BugBiz.
- Richardson, S., Carlton, C. (2020). *Oncopeltus fasciatus*, Large Milkweed Bug (Hemiptera: Lygaeidae). BugBiz.
- Richardson, S., Carlton, C. (2020). *Sphecius speciosus*, the eastern cicada killer (Hymenoptera, Crabronidae). BugBiz.

Spring 2021 LSU AgCenter Website

- Richardson, S., Qian, S. (2021) Managing Stored Product Pests of Homes and Businesses. LSU AgCenter. <https://www.lsuagcenter.com/profiles/qsun/articles/page1613620996926>
- Richardson, S., Qian, S. (2021) Secondary Pests of Stored Product. LSU AgCenter. <https://www.lsuagcenter.com/profiles/qsun/articles/page1613612203515>
- Richardson, S., Qian, S. (2021) Primary Pests of Stored Product. LSU AgCenter. <https://www.lsuagcenter.com/profiles/qsun/articles>

Research Presentations

Head Presenter/Author:

Spring-Fall 2021

- Richardson, S., Khadka, A., Sun, Q. Effect of soil moisture on survivorship, tunneling, and food consumption in *Coptotermes formosanus* and *Reticulitermes flavipes* (Blattodea: Rhinotermitidae), Poster Presentation, 11th Annual LSU Entomology Department Graduate Student Symposium, LSU, 2021

- Richardson, S., Khadka, A., Sun, Q. Effect of soil moisture on survivorship, tunneling, and food consumption in *Coptotermes formosanus* and *Reticulitermes flavipes* (Blattodea: Rhinotermitidae), Poster Presentation, Annual meeting of Entomological Society of America, 2021

Co-author:

- Khadka, A., Richardson, S., Sun, Q. Desiccation tolerance and influence of relative humidity on the survival of different castes in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, Poster Presentation, Annual meeting of Entomological Society of America, 2021

Extension Presentations

Head Presenter/Author:

Spring 2021 Louisiana State University

- Stored Product Pests- LSU/LPMA Winter Pest Control Institute

Grants/ Assistantships

Fall 2020 — Spring 2022

- Graduate Student Assistantship- Department of Entomology and LSU Residential Life, Louisiana State University

Scholarships

Spring 2022

- Roussel Scholarship Graduate Student Award in entomology

Technical Knowledge

- Photoshop: Adobe Photoshop Cs6 (Mac).
- Inkscape: (Mac)
- Adobe Illustrator Draw: i-pad
- Clip Studio Paint EX: i-pad
- Inkist: i-pad
- 3D Printing: Cura Ultimaker (Mac)
- 3D Modeling: Blender 3.1 (Mac)
- R
- JMP