RETICULITERMES FEEDING PREFERENCE AND EXPERIMENTAL DESIGN

by

GRETCHEN DENISE PERKINS

(Under the Direction of Brian T. Forschler)

ABSTRACT

Subterranean termites are a prevalent urban pest that cost billions of dollars in damage, control, and repair costs in the United States annually. In order to understand how to better control these pests, accurately measuring their food preference is critical. We examined three bioassay designs (multiple-choice, paired choice, and no choice) with four species of wood (*Sequoia sempervirens*, *Pinus* sp., *Liriodendron tulipifera*, and *Quercus* sp.) using three sympatric *Reticulitermes* species, *R. flavipes*, *R. malletei,* and *R. virginicus*. Results indicated that no choice and four choice bioassay designs should be used simultaneously to accurately establish a preference hierarchy. The amount of wood in a trial affects the wood consumption rate: 12 blocks of wood inspires more feeding than 3 blocks.

INDEX WORDS: *Reticulitermes flavipes, Reticulitermes malletei, Reticulitermes virginicus,* subterranean termite, wood preference, bioassay design

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DEDICATION

This is dedicated to my awesome parents for their unwavering support and solid belief that I can accomplish anything. And to Gerald, Gabrielle, and Gunther, who should always remember they can achieve anything with hard work, a little stubbornness, and a good attitude. Sorry I didn’t visit more.

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CHAPTER 1

INTRODUCTION AND REVIEW OF RELEVANT LITERATURE ON TERMITE WOOD PREFERENCE

Introduction

Termites, members of the order Isoptera, are xylophagous insects that have earned the reputation of being voracious pests (Snyder 1954). Forty-five species of termites are found in the United States, 30 of which are known to consume wooden structures or live plants (Su and Scheffrahn 1990). The family Rhinotermitidae contains five described species in the Southeastern United States (Snyder 1954, Weesner 1969, Su and Scheffrahn 1990, Austin et al. 2007, Lim and Forschler 2012) including *Reticulitermes flavipes,* considered to be the most economically important termites in the United States (Snyder 1954). Up to $11 billion dollars in damage per year to structures in the United States is attributed to termites (Su 2002, Green et al. 2011). Termites are also considered ecologically important yet little is known about how these sympatric species partition food resources in order to share the same niche (Houseman et al. 2001).

This research looks at three sympatric subterranean termite species, *R. flavipes*, *R. malletei,* and *R. virginicus*, relative to their food preference using three objectives.

1. The first objective is to identify the wood consumption rate for three subterranean termite species, *R. flavipes*, *R. malletei*, and *R. virginicus* for four wood species, *Sequoia sempervirens* (Redwood), *Pinus* sp. (Southern Yellow

Pine), *Liriodendron tulipifera* (Yellow Poplar), and *Quercus* sp. (Red Oak).

2. The second objective is to illuminate the effect of bioassay design on subterranean termite wood preference as indicated by wood consumption.

3. The third objective is to compare various measures of wood consumption to determine their value in assessing termite wood preference.

Biology

Members of the Isoptera are eusocial insects with three castes: workers, soldiers, and reproductives. Colonies of subterranean termites can be quite large, individual colonies have been estimated to include up to 7 million individuals (Lenz 1994). Workers forage for food resources and provide for the other members of the colony (Lenz 2009). The soldier caste defend the colony from invertebrate predators, but must be fed by the workers, while the adult reproductives are long lived - > 10 y - and along with the sexually mature, but immature, neotenics ensure continuation of the population (Abe et al. 2000). Together these castes are organized into groups, or colonies, that lead a cryptic lifestyle. Termites are capable of eating numerous types of cellulose-containing materials including sound wood in structures, furniture, and fallen trees; decayed wood; processed materials containing cellulose such as paper and cardboard (Weesner 1969, Thorne 1999, Abe et al. 2000). They can also chew through materials that without nutrition, such as plastic, insulations, and rubber (Weesner 1969, Thorne 1999).

A recent review by Lenz (2009) outlined factors that contribute to a healthy laboratory colony while highlighting the overarching need for moisture in these fragile, soft-bodied insects. One of the factors indicative of a "healthy" colony is a high percent moisture: groups with $\leq$ 75% body moisture have shown reduced survivorship (Arquette et al. 2006).

Wood Preference Influences

A number of studies have examined the factors thought to influence wood consumption in subterranean termites. Among these are: environmental factors, wood dynamics, colony composition, moisture, and termite body size (Thorne 1999, Houseman et al. 2001, Green et al. 2005, Lenz 2009).

Environmental factors, such as temperature and moisture, have been shown to influence wood consumption in termites. Subterranean termites need to maintain contact with a moist substrate or be feeding on blocks of wood with high moisture content in order to survive since they are prone to desiccation (Gautam and Henderson 2011). Low mortality of *Coptotermes formosanus* was observed in bioassays conducted at 19°C, but low wood consumption rates were found when compared to the higher temperatures 28°C and 30°C (Gautam and Henderson 2011). Houseman et al. (2001) demonstrated that *R. hageni* foraged during seasons with drier soil, while *R. flavipes* foraged at the soil surface in seasons with moister soil. However, Waller (2007) collected *R. flavipes* and *R. virginicus* in different logs, finding that wood temperature did not differ between the species.

Likewise, wood properties influences wood consumption. Dry wood cannot provide the moisture that subterranean termites need for survival, so the moisture content of the wood is imperative to the success of the bioassay (Gautam and Henderson 2011). *Coptotermes formosanus*, when offered *Pinus* sp. of different moisture contents in no choice trials, mortality was significantly higher in dry (0-3%) and low (22-24%) blocks of wood (Gautam and Henderson 2011). Initial wood moisture content seems to be very important to the termites when picking their first food source. In Gautam and Henderson (2011), *C. formosanus* continued to eat the blocks of wood first attacked even as the moisture content in these blocks dropped. Higher mortality was associated with dry blocks and higher temperatures (28°C & 30°C) than in the same temperatures with wood with medium to high percent moisture content (Gautam and Henderson 2011). *Reticulitermes speratus* preferred blocks of wood with medium (79-103%) or high moisture (140 – 189%) content (Nakayama et al. 2005). Additionally, wood size has been shown to influence wood consumption rates of Rhinotermitids. Larger wood blocks inspire higher feeding rates in bioassays. Waller (1988) demonstrated that 200 worker *Reticulitermes* sp. ate nearly three times more wood from larger blocks (5 cm^3) than from the small blocks (2 cm^3) of Douglas fir. Smaller wood blocks caused termites to feed at lower, constant rates (Lenz 2009). It has been suggested that wood density and specific gravity are contributing factors to termite wood consumption (Esenther 1977, Waller 1988, Arango et al. 2006a). Arango et al. (2006a) even proposed a link between specific gravity and termite mortality, suggesting that as specific gravity increases, termite mortality also increases. Many factors are known to influence termite survivorship, however (Arquette et al. 2006, Lenz 2009, Gautam and Henderson 2011).

The number of termites in a bioassay also influences wood consumption rate. When more individuals are present, there is a significant amount of inactive for each individual even though the colony is always working (Lenz 2009). While the size of the colony is important, so is the body size of the termites. Each colony of termites, even within the same species, can vary in average body size. Su and La Fage (1984a) found a negative correlation between mean worker body size and wood consumption rate. The larger the mean worker size, the smaller the wood consumption rate is (Su and La Fage 1984a). Worker and soldier weights are higher in the winter months and lower in the spring (Waller and La Fage 1987). The differences in these weights between colonies and in different seasons can have an effect on the wood consumption rate if not standardized throughout the experiment.

Wood Preference Bioassays

Wood preference bioassays are most commonly used to show the resistance of a wood type to feeding of a candidate species of termite (Peterson and Gerard 2009). The American Society for Testing and Materials (ASTM) has developed a set of standards for testing resistivity in wood species (ASTM 1999). These bioassays consist of force-feeding (no choice) tests where one type of wood is offered to a group of termites for a specific period of time (4 weeks according to the ASTM) (ASTM 1999). Some researchers have modified this method to include multiple choice trials (Hapukotuwa and Grace 2011).

Thorne (1999) identified three different variables that are typically manipulated in wood preference experiments: termites, experimental conditions, and wood. Experimental conditions manipulated can include type of bioassay (no choice or multiple choice), caste composition, or physical conditions (as in season) (Thorne 1999). The majority of wood preference studies manipulate the wood variable in their experiments (Esenther 1977, Cornelius et al. 2002, Morales-Ramos and Rojas 2003, Cornelius et al. 2004, Katsumata et al. 2007). Because scientists are focused on determining termite preference in relation to wood properties, sometimes wood food resources are treated before testing. Esenther (1977) compared *R. flavipes* and *R. virginicus* consumption of 21 wood species with 2 oil-based and 2 water-based preservatives. He found that the majority of the termite resistant properties were due to the qualities of the wood itself and not the preservatives (Esenther 1977). Katsumata et al. (2007) found that wood species with low termite-resistant properties, like *C. japonica* and *Pseudotsuga menziesii* (Douglas fir), had greater consumption by *C. formosanus* after gamma irradiation while the same treatment did not affect wood consumption rates for woods possessing high termite-resistant properties. Research has shown that termites prefer fungi-infected sawdust in laboratory bioassays over clean sawdust of multiple wood species (Cornelius et al. 2002, Cornelius et al. 2004). Morales-Ramos et al. (2003) demonstrated that the black-staining fungus, *Chamacyparis nootkatensis*, can decrease the termite-resistant properties of Alaskan yellow cedar and increase wood consumption by *C. formosanus*. Other studies have investigated termite wood preferences for various wood types. Only a handful of these have focused on *Reticulitermes* species, however (Arango et al. 2006a, Peterson and Gerard 2007).

Methods

The International Research Group (IRG) of wood protection uses a prescribed set of protocols to determine wood species resistance to subterranean termite feeding (ASTM 1999). It is important that we have comparable methods for studying these subjects because we need to know more about the susceptibility and resistant properties of economically important woods. The majority of termite wood preference studies have been conducted using a no-choice bioassay design. These forced-feeding studies are by design not enough to convincingly show preference, although no choice assays demonstrate that a termite species will eat certain types of wood in the absence of alternate food resources (Ngee et al. 2004, Peterson and Gerard 2007). Ngee (2004), using no choice assays, found that *C. gestroi* and *C. formosanus* consumed more of certain wood species compared to choice tests. Smythe and Carter (1970) noted that *R. flavipes* consumed more redwood in force feeding tests than in multiple choice trials.

Additionally, studies that examined individual *Reticulitermes* species are not always comparable, though they use similar methods and species. Arango et al. (2006) compared the feeding preferences of *R. flavipes* among twelve wood species; Peterson and Gerard (2007) used three wood species. Arango et al. (2006) found that balsa and pine had no-to-low termite resistance, while juniper had very little mass lost throughout the course of the experiment and was therefore considered highly resistant to termites. Peterson and Gerard (2007) tested *Quercus nigra* (water oak) and *Juniperus virginiana* (red cedar) against yellow pine, *Pinus* sp., which they used as a reference wood, to conclude that water oak is more resistant to termite damage than yellow pine, but less resistant than red cedar.

The design of the bioassay can make a difference as well. The length of time termites are allowed to feed has as low as 17 days and as many as 16 weeks (Thorne 1999). Extended periods of time can affect termite survivorship. The number of termites at the start of the trial varies. Some researchers report the weight of the group of termites instead of the number, while others just report the number of termites in the trial (as low as 100 and as high as 1000) (Thorne 1999). By reporting the weight of the group, but not the average weight of an individual, it is impossible to tell how many individuals were used in the trial.

Wood consumption is also calculated and reported differently. Wood consumption is also calculated and reported differently. Visual scales have been used for quantifying wood consumption (McMahan 1966, ASTM 1999, Hapukotuwa and Grace 2011). This scales use a numbering system where the wood is observed and the amount eaten is ranked. McMahan (1966) used a scale from 0 – 4 to quantify wood consumption, while the ASTM (1999) protocol used a 0 – 10 scale. In the ASTM (1999), a ranking of 10 means that there was very little feeding, 9 demonstrates a light attack, 7—a moderate attack, 4—a heavy attack, and 0—a failure of the wood. These scales are a good visual representation, but not an exact science for quantifying wood consumption. Other measures of wood consumption use various calculations for wood consumption (Smythe and Carter 1969, 1970, Waller 1988, Peterson and Gerard 2007, Waller 2007, Green et al. 2011, Hapukotuwa and Grace 2011). For example, Peterson and Gerard (2007) calculated a wood resistance index (WRI) and a termite preference index (TPI). The TPI was derived by subtracting the mass consumed from the reference wood (pine) in a choice test from the mass consumed of the test wood and then dividing this number by the mass consumed from the reference wood in a no choice test added to the mass consumed in the test wood and multiplying the result by 100 (Peterson and Gerard 2007). The WRI was calculated by subtracting the mass consumed in the reference wood in a no-choice test to the mass consumed in the test wood in a no-choice test and dividing the resulting number by the mass consumed in the choice test for the reference wood added to the mass consumed in the test wood choice test and multiplying by 100 (Peterson and Gerard 2007). Arango et al. (2006) weighed wood blocks before and after the experiments and calculated mass percent lost. Calculating mass loss is the percentage of mass lost over the course of the trial that does not account for the number of termites, the length of time in bioassay, or mortality. Mortality is the only measure that cannot be controlled in an experiment. The most prevalent method of calculating wood consumption, however, is calculating mass (mg) consumed by subtracting the weight of the wood at the end of the trial from the starting weight (Smythe and Carter 1969, 1970, Waller 1988, Green et al. 2005, Waller 2007, Green et al. 2011, Hapukotuwa and Grace 2011). The use of different units for measuring wood consumption makes it difficult to compare between studies. Moreover, these units of measure do not account for the differences between the trials. Unless the wood consumption rate accounts for the number of termites in each trial, the body size of the workers, the survivorship, and the length of time in the trial, they cannot be accurately compared. Su and La Fage (1984b) derived a formula to account for body size, number of days in bioassay, survivorship in bioassay, and amount consumed expressed in mg of wood consumed/g of termite/day. They found that when they did not account for mortality, estimations of wood consumption rates were much lower (Su and La Fage 1984b).

Resource Partitioning

The genus *Reticulitermes* (subterranean termites) is represented by at least 5 species in the Southern United States (Snyder 1954, Weesner 1969, Su and Scheffrahn 1990b, Austin et al. 2007, Lim and Forschler 2012). The ecological role of subterranean termites is to breakdown the carbon sequestered in the cellulose of woody plants (Krishna 1969, Houseman et al. 2001). According to niche theory, species may occupy the same biotope, but not the same ecotope, meaning that they do not share the same niche although they share environmental space (Price 1997). Price (1997) suggests that sympatric species with similar needs will be unable to coexist for long due to competition for resources. Therefore species that serve the same ecological role are known to partition available resources (Price 1997). Termite species endemic to the SE USA (Snyder 1954, Weesner 1969, Su and Scheffrahn 1990b, Austin et al. 2007, Lim and Forschler 2012) might partition the cellulose found in the forest ecosystem by displaying wood preference. Competing species must divide resources, such as food or substrate, so that each utilizes the same type of resources in a different way. This division of resources is known as niche, or resource, partitioning (Price 1997, Thain et al. 2004).

Several methods of niche partitioning have been suggested for subterranean termites (Houseman et al. 2001, Waller 2007). Houseman et al. (2001) suggested that *R. flavipes* and *R. hageni* partitioned resources by season and soil temperature gradient in Texas. *R. flavipes* tended to follow cooler and moister soil gradients, while *R. hageni* was found in warmer and drier gradients (Houseman et al. 2001). *R. flavipes* was found most often infesting homes in Missouri, while *R. hageni* was found in forested areas (Pinzon and Houseman 2009). In Virginia, Waller (2007) found that *R. virginicus* inhabited logs with larger diameters than did *R. flavipes* suggesting that log diameter could be a factor in resource partitioning (Waller 2007). Green et al. (2005) found a significant species effect on consumption of filter paper between *R. flavipes, R. tibialis,* and *R. virginicus*: *R. flavipes* and *R. tibialis* consumed 1.5X more filter paper than *R. virginicus* over the course of seven days. Resource partitioning by wood preference has not been examined in *Reticulitermes* species.

We designed a series of bioassays to address food preference in three species of *Reticulitermes*: *R. flavipes, R. virginicus,* and *R. malletei.* We examined subterranean termite wood preference in different units of measure (mg of wood consumed/g of termite/day; mg consumed; mg consumed/day) in three bioassay designs: no choice, paired choice, and multiple-choice. Several studies report that no-choice bioassay designs result in higher feeding rates in non-preferred wood species compared to multiple-choice designs (Ngee et al. 2004, Peterson and Gerard 2007). It is necessary to determine the most effective combination of bioassay designs and measures of determining wood consumption rates relative to a preference ranking in order to resolve the importance of termite wood preference in terms of ecological studies or management tactics.

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CHAPTER 2

WOOD PREFERENCE OF THREE RETICULITERMES SPECIES IN NO-CHOICE, FOUR-CHOICE, AND PAIRED CHOICE BIOASSAY DESIGNS[1]

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Abstract

Wood preference bioassays with subterranean termites can provide considerable ecological and practical management insights. Researchers have previously used a variety of designs and employed different units of measure when comparing wood consumption. In the present study, we use three bioassay designs (no-choice, paired choice, four-choice) with three species of *Reticulitermes* (*R. flavipes, R. malletei, R. virginicus*) commonly found in the SE United States to determine relative wood preferences for four wood species (*Sequoia sempervirens, Pinus* sp., *Liriodendron tulipifera*, *Quercus* sp.). In addition, a comparison of the units found in the literature (mg of wood consumed; mg of wood consumed/day; mg of wood consumed/g of termite/day) was conducted. Bioassay design had an effect on the wood consumption rates for all species of what; no choice designs inspired more feeding on blocks that were non-preferred in paired or multiple choice designs. Feeding rates were affected when more wood was available.

Introduction

Subterranean termites are considered the most economically important urban pest (Su and Scheffrahn 1990), with up to $11 billion annually in damage, repair, and control costs in the United States (Su 2002, Green et al. 2011). In addition to their pest status, subterranean termites play an important role in the ecosystem, breaking down carbon sequestered cellulose (Weesner 1969, Thorne 1999, Abe et al. 2000). It is critical to understand the foraging and feeding preferences of these pests as an integral part of efforts to develop economically appealing and environmentally conservative management options.

A number of factors are believed to influence wood preference by subterranean termites, including season, temperature, wood properties such as moisture content and resource size, soil moisture, and termite body size (Thorne 1999, Houseman et al. 2001, Green et al. 2005, Lenz 2009, Gautam and Henderson 2011). Sympatric *Reticulitermes* species foraged during different seasons, effectively portioning resources with *R. flavipes* feeding on wood when soil moisture was greatest and *R. hageni* feeding during seasons with less soil moisture (Clément 1986, Houseman et al. 2001). Despite the apparent partitioning of resources based on wet/dry seasonal cycles it is possible that, with subterranean termites, food choice and partitioning of preference for wood type may also occur in climate zones that experience a more equitable distribution of annual precipitation.

Moisture, whether in the wood or the water-bearing substrate included in a bioassay, is critical to interpreting data from bioassays of termite wood preference (Green et al. 2005, Nakayama et al. 2005, Gautam and Henderson 2011). It has been suggested that *R. flavipes* can manipulate the moisture content by moving the particles around using water sacs, a reservoir in the labial gland designed for carrying water (Grube and Rudolph 1999). As the wood block size increases, wood consumption increases as well (Ahktar and Jabeen 1981, Waller 1988, Lenz 1994).

Bioassay design also can influence wood consumption (Smythe and Carter 1969, 1970, Waller 1988, Ngee et al. 2004, Peterson and Gerard 2007). The American Standards for Testing and Materials (ASTM 1999) promote a no-choice protocol examining different materials exposed to termites. No-choice bioassays are force feeding experiments, where termites are provided only one type of wood for a given period of time. Interpretation of data from no-choice protocols have been questioned the use of no-choice designs when examining wood preference, because termites feed on a greater variety of wood species compared to multiple-choice designs (Smythe and Carter 1969, 1970, Waller 1988, Ngee et al. 2004, Peterson and Gerard 2007). Smythe and Carter (1970) reported that *R. virginicus* consumed more walnut in choice trials than in no-choice, while *R.flavipes* consumed similar amounts in both designs. *Reticulitermes flavipes* consumed more redwood in no-choice trials then in the choice trials, indicating a preference when offered a choice (Smythe and Carter 1970). In no choice trials, *C. formosanus* and *C. gestroi* consumed more types of wood than in a multiple-choice design (Ngee et al. 2004). Recently researchers have begun using multiple-choice designs, modified from the ASTM protocol (Hapukotuwa and Grace 2011).

Analysis of food preference with subterranean termites is made more difficult because of the different calculations used for wood consumption (Smythe and Carter 1969, 1970, Su and LaFage 1984, Waller 1988, Thorne 1999, Ngee et al. 2004, Green et al. 2005, Arango et al. 2006, Peterson and Gerard 2007, Waller 2007, Green et al. 2011, Hapukotuwa and Grace 2011). Originally, visual scales were used to quantify wood consumption in wood preference bioassays (McMahan 1966). Visual scales are also employed in the ASTM protocol (ASTM 1999) and are still used today (Hapukotuwa and Grace 2011). This subjective method involves scoring feeding activity based on a picture and written description of percent amount of wood missing from a block of wood. The scales also differ; McMahan (1966) used a scale 0 – 4, while ASTM (1999) calls for a 0 – 10 ranking. Weight loss (in mg) is a popular choice for determining the feeding rate (Smythe and Carter 1969, 1970, Waller 1988, Green et al. 2005, Green et al. 2011, Hapukotuwa and Grace 2011). Other studies have used mg consumed/day, which accounts for the length of the trial, but not mortality or termite size (Arango et al. 2006, Terzi et al. 2011). Su and LaFage (1984) determined a calculation that accounts for all of these factors, giving a wood consumption rate of mg of wood consumed/g of termite/day. This formula standardizes wood consumption rates, by averaging the starting number and surviving termites in the trial and also calculating the average weight per termite (Su and LaFage 1984). It is not frequently used by researchers, though there is nothing to indicate why. In addition, certain researchers have derived their own indices such as the wood resistivity index (WRI) and termite preference index (TPI) (Peterson and Gerard 2007). It would be advantageous to have a consensus on the best consumption rate calculation so that studies can be easily compared.

An examination of the feeding rates and food preference by subterranean termites is necessary to ensure that bioassays reflect accurate feeding rates. In this study, I used three sympatric subterranean termite species found in the southeastern United States, *R. flavipes, R. malletei*, and *R. virginicus*, in an examination of relative food preference. There were three objectives:

1. Demonstrate effect of bioassay design: no-choice, dual-choice and 4-way choice, (or combination of designs) on wood preference rankings from laboratory bioassays with subterranean termites.
2. Identify if there is a hierarchy of wood preference shown by *R. flavipes*, *R. malletei*, and *R. virginicus* for four wood species: *Sequoia sempervirens*, *Pinus* sp., *Liriodendron tulipifera*, and *Quercus* sp.

3. Compare various measures of wood consumption to determine their value in assessing termite wood preference.

We hypothesized that the three termite species would feed equally on all types of wood in the three bioassay designs and that wood consumption rates would be equal across bioassay design regardless of wood type, wood amount and termite species. We also hypothesized that the relative ranking for wood preference would be the same regardless of the method used to quantify consumption.

Materials and Methods

Termite Collection

Logs infested with *Reticulitermes* species, collected from Whitehall Forest, in Clarke County, Georgia, were brought back to the laboratory and processed according to the method of Forschler and Townsend (1996). Moistened rolls of corrugated cardboard fit snugly in a PVC pipe (17 cm in length with 10-cm diameter) were briefly moistened and placed beside the logs to collect termites as described in Forschler and Townsend (1996). Collected termites were kept in plastic boxes (26 cm x 19 cm x 9 cm) containing thin (4 cm x 12 cm x 2 mm) slats of *Pinus* sp. wood and maintained in complete darkness inside an environmental chamber at 26°C for no more than 4 weeks before inclusion in a bioassay.

Three methods of species identification were used after termites were collected. Soldier mandibles were extracted and examined using the criteria of Lim and Forschler (2012). Alates, when present, were preserved in 100% ethanol, and compared to specimens using the key of Lim and Forschler (2012). Analysis of COII gene sequence was conducted on specimens and matched with reference sequences for the third method (Lim and Forschler 2012).

Live weights were obtained for all termites used in bioassays by taking 50 randomly-selected workers divided into five groups of 10. The five groups were weighed and averaged to obtain the mean weight of ten individuals. This number was used in the determination of wood consumption described in the Data Analysis section. Three species of subterranean termites common to the southeastern United States were tested; *R. flavipes, R. virginicus* and *R. malletei*.

Wood Preparation

Four species of wood were used in these bioassays: *Sequoia sempervirens* (redwood), *Pinus* sp. (pine), *Liriodendron tulipifera* (yellow poplar), and *Quercus* sp. (red oak). Boards purchased at a local lumber retailer were cut into 1 cm – 1.2 cm^3 cubes. Wood cubes were dried overnight at 64°C, allowed to return to room temperature by placement in an environmental chamber with Drierite for twenty minutes, and weighed. Three cubes of each wood species being tested were then soaked in water overnight before inclusion in bioassay.

Bioassay

Three bioassay designs were used in this study that involved a four-choice, paired choice and no-choice. In the four-choice design, each of the four feeding chambers contained three blocks of a different species of wood: redwood, pine, poplar, or red oak. The paired choice had two feeding chambers, each with three blocks of a different type of wood in the combinations: redwood-pine, redwood-poplar, redwood-red oak, pine-poplar, pine-red oak, or poplar-red oak. No choice arenas were identical to the paired choice design but contained one species of wood in one chamber and an empty chamber that termites could enter.

Arenas consisted of round plastic cylinders (3.6 cm tall, 5.2 cm in diameter) which were connected by 7 cm lengths of Tygon tubing (external diameter = 5 mm, internal diameter = 3 mm) to form a bioassay arena (Fig. 2.1). The four-choice tests consisted of a central introduction chamber with 4 holes (5 mm diameter) spaced equidistant around the circumference approximately 1.7-cm from the cylinder base while the paired and no-choice arenas had introduction chambers with two holes. All feeding chambers contained a choice of three cubes of one of four wood species (or empty) and had one hole at the base of the container. Introduction chambers contained a saturated sand and vermiculite mixture (14:12) (Forschler 2009) that was filled to a depth that was sufficient to touch the edge of the Tygon tubing that was pushed through the holes to provide access for termites to a particular feeding chamber. Feeding chambers were cut off from the introduction chamber with 5-cm binder clips for 24-h after introduction of termites to allow acclimation prior to providing access to the peripheral chambers (Messenger and Su 2005, Schwinghammer and Houseman 2006). Three hundred worker (3^{rd} instar or higher) termites were added to each introduction chamber and 21 days later arenas were dismantled and the number of live termites counted in each arena. The wood blocks were removed, cleaned, and dried overnight, as previously described, before being weighed.

A replicate consisted of one four-choice, one of each of the no-choice [redwood (R), pine (Pi), poplar (Po), and red oak (O)], and one each of the paired choice arenas (R-Pi, R-Po, R-O, Pi-Po, Pi-O, and Po-O). Thirteen replicates were conducted on *R.*

virginicus, ten on *R. flavipes*, and four on *R. malletei*. Controls were conducted with replicates set up and run without termites present.

Statistical Analysis

Wood consumption rate (mg of wood consumed/g of termite/day) was calculated by dividing the amount consumed [original wood weight (mg) – final wood weight (mg) + mean difference in control] by the replicate factor [mean weight (in g) per termite x average of the 300 beginning termites and the number of surviving termites] divided by the number of days in bioassay (21). Resulting negative values were considered to be zero because they were within the standard deviation. Statistical analysis was conducted in SAS 9.2 software using analysis of variance (ANOVA) and protected LSD mean separation. Wood consumption rates were compared by wood type for each species and bioassay design using analysis of variance (ANOVA) and protected LSD mean separation. Wood consumption rates were compared by species using wood type and bioassay design and the interaction between the two. Again, LSD mean separation was conducted where tests were significant in ANOVA. Wood consumption rates were compared within species by test type. LSD mean separations were conducted as previously mentioned. Wood consumption rates also were compared by wood type, test type, and species as with the other tests. Wood consumption rates were added across replicates by species and ANOVAs conducted for overall wood consumption by bioassay design followed by protected LSD mean separation.

Wood consumption was calculated as a wood consumption rate (mg of wood consumed/g of termite/day), mass loss (mg), and mass loss/day (mg/day). Wood preference was defined more consumption of one wood, relative to the other woods. A wood preference ranking was created for the no choice and four choice bioassay designs, where woods were ranked based on their statistical differences.

Results

Bioassay Design

R.flavipes

Wood consumption rates by *R. flavipes* among test types across all bioassays was significantly different in redwood ($F = 6.39$; $P = 0.0039$), pine ($F = 3.16$; $P = 0.0517$), and red oak ($F = 11.19$; $P = 0.0001$) (Figure 2.2, Table 2.1). Consumption of pine was not significantly different between all three test types: no choice (27.98 ± 7.90), paired choice (27.00 ± 9.33), and four choice (19.02 ± 10.37) (Figure 2.2, Table 2.1). Red oak consumption also differed between no choice (25.36 ± 10.85), paired choice (16.91 ± 8.24), and four choice (7.41 ± 6.35) (Figure 2.2, Table 2.1). Redwood consumption differed significantly among no choice (14.57 ± 9.33) and four choice (1.65 ± 1.85) designs, as well as between no choice and paired choice (7.08 ± 8.85) designs, but not between four choice and paired choice designs (Figure 2.2, Table 2.1). Poplar consumption was similar across all designs: no choice (1.86 ± 5.09), paired choice (2.10 ± 5.06), and four choice (0.10 ± 0.94) (Figure 2.2, Table 2.1).

Overall wood consumption differed significantly ($F = 8.73$; $P = 0.0003$) between test types for *R. flavipes* (Figure 2.3). When *R. flavipes* was offered 3 blocks (no choice) of wood, consumption was lower (17.45 ± 13.39) than when offered 6 blocks (paired choice) (26.27 ± 9.40) or 12 blocks (four choice) (28.19 ± 9.57). Overall consumption did not differ significantly between paired choice (6 blocks) and four-choice (12 blocks).

R.malletei

Significant differences (F = 31.05; P = 0.0001) in *R. malletei* wood consumption between bioassay designs were observed in redwood no choice (20.67 ± 2.82) and four choice (3.29 ± 1.7) tests, as well as between no choice and paired choice (6.05 ± 4.03) designs (Figure 2.2, Table 2.1). Red oak consumption was significantly greater (F = 5.05; P < 0.0199) in no choice than four choice (28.06 ± 4.93 > 10.68 ± 7.97) (Figure 2.2, Table 2.1). Pine consumption in paired choice tests was also significantly greater than four choice (20.56 ± 8.38 > 10.68 ± 7.97), but no significant differences were observed between no choice and paired choice (Figure 2.2, Table 2.1). Consumption was not significantly different (F = 1.44; P = 0.2658) between the three designs with poplar (Figure 2.2, Table 2.1). When 12 blocks were offered to *R. malletei*, overall consumption was significantly higher than when only 6 blocks of wood were offered (34.32 ± 9.91 > 20.55 ± 12.65) (Figure 2.3).

R. virginicus

Wood consumption rates by test type, showed significantly different consumption (F = 21.51; P = 0.0001) in four choice, no choice, and paired choice bioassay designs comparing redwood and poplar, but not when pine and red oak were compared (Figure 2.2, Table 2.1). Additionally, the amount of the wood consumed increased with the amount of wood in the trial (Table 2.3). More wood was consumed in the four choice trials with 12 blocks of wood (mean of 37.47 ± 8.62) than in the paired choice with 6 blocks of wood (23.84 ± 9.37) and more wood is consumed in the paired choice trials than in the no choice trials with 3 blocks of wood (17.68 ± 11.71) (F = 25.39, P < 0.0001) (Figure 2.3).

Wood Preference

R.flavipes

No choice bioassays showed *R. flavipes* consumed more (F = 18.58; P = 0.0001) pine (27.98 ± 7.90) and red oak (25.36 ± 10.85), and significantly different consumption of redwood (14.57 ± 9.93) and poplar (1.87 ± 5.09) (Table 2.1, Figure 2.2). In four choice tests, *R. flavipes* consumed significant amounts (F = 19.35; P = 0.0001) of pine (19.02 ± 10.37), then red oak (7.41 ± 6.35), but similar amounts of poplar (0.12 ± 0.94) and redwood (1.65 ± 1.85) (Table 2.1, Figure 2.2). In the paired trials, significantly different consumption occurred in all tests (Table 2.1). Significantly more pine was consumed in tests paired with redwood (29.78 ± 10.31 > 2.46 ± 2.6), poplar (26.82 ± 8.62 > 0 ± 1.3), and red oak (26.04 ± 8.69 > 7.70 ± 2.77). Red oak was consumed at significantly higher rates in tests paired with redwood (23.56 ± 5.75 > 2.23 ± 1.77) and poplar (19.75 ± 3.06 > 1.81 ± 3.05). Significantly more redwood was consumed when paired with poplar (15.67 ± 9.99 > 4.04 ±7.01).

R.malletei

R. malletei consumed significantly (F = 29.56; P = 0.0001) more pine (31.61 ± 7.37) in no choice bioassays, followed by red oak (28.06 ± 4.93) and redwood (20.67 ± 2.82), and significantly lower consumption of poplar (1.85 ± 2.98). In four choice designs, pine (21.17 ± 14.23) was consumed significantly more (F = 5.52; P = 0.0129) than redwood (3.29 ± 1.70) and poplar (0 ± 1.19). Among the paired tests, significantly more (F = 86.52; P = 0.0001) pine consumption was observed when pine was paired with redwood (29.37 ± 4.9 > 4.35 ± 2.22). Significantly lower poplar consumption was observed when paired with pine (1.53 ± 1.15 < 25.44 ± 1.86) and red oak (0.5 ± 0.6 < 25.43 ± 2.81).

R. virginicus

R. virginicus showed significantly different consumption of redwood (F = 21.51; P = 0.0001) in four choice (9.40 ± 4.09), no choice (20.19 ± 8.42), bioassay designs (Figure 2.2, Table 2.1). Poplar consumption was significantly different between the four choice design (2.57 ± 2.88) and when paired with redwood (1.85 ± 3.18) and when paired with red oak (1.98 ± 3.27) (Figure 2.2, Table 2.1). . but not when pine and red oak were compared (Figure 2.2, Table 2.1). Additionally, the amount of the wood consumed increased with the amount of wood in the trial (Table 2.3). More wood was consumed in the four choice trials with 12 blocks of wood (mean of 37.47 ± 8.62) than in the paired choice with 6 blocks of wood (23.84 ± 9.37) and more wood is consumed in the paired choice trials than in the no choice trials with 3 blocks of wood (17.68 ± 11.71) (F = 25.39; P = 0.0001) (Figure 2.3).

Species Interaction

No significant differences (F = 1.20; P = 0.3014) in wood consumption were observed among the species when they were separated by bioassay design, wood preference, or the interaction among the three. Wood consumptions were the same statistically among the species.

Discussion

Bioassay design influenced wood consumption rates by the *Reticulitermes* species included in these experiments. Bioassay design provided distinctly different wood preference rankings in each of the species. In *R. flavipes*, no choice designs showed pine and red oak to be consumed at the highest rates, followed by redwood and poplar (Table 2.1, Fig. 2.2). Pine was consumed at the highest rates in four choice designs, with red oak next, while redwood and poplar provided similar rates (Table 2.1, Fig. 2.2). In paired trials with *R. flavipes,* pine was always consumed at the highest rate compared to the other wood types (Table 2.1, Fig. 2.2). Red oak consumption was higher when paired with redwood and poplar, while redwood was only consumed at higher rates when paired with poplar (Table 2.1, Fig. 2.2).

Consumption of pine was highest with *R. malletei* in no choice designs, followed by redwood and red oak, with poplar being the lowest consumed (Table 2.1, Fig. 2.2). *R. malletei* consumed identical amounts of pine and red oak, as well as similar amounts of red oak, redwood, and poplar in four choice designs (Table 2.1, Fig. 2.2). Paired choice designs offered slightly more clarity: pine wood was consumed at higher rates when paired with poplar and redwood; higher consumption of red oak was observed in designs pairing it with redwood and poplar (Table 2.1, Fig. 2.2). The lack of a clear preference hierarchy in *R. malletei* could be due to the low number of replicates (n = 4) in the present study.

The no choice wood consumption data provided evidence for a hierarchy of preference by *R. virginicus* with pine most preferred followed by red oak and redwood with equivalent consumption rates in second place and poplar a distant third (Table 2.1, Fig. 2.2). The multiple choice bioassays likewise provided a preference ranking with pine most preferred and red oak and poplar in second place with equivalent consumption rates or was it third, where is poplar (Table 2.1, Fig. 2.2). The wood consumption data from the paired choice tests when provided that pine and red oak equivalent consumption rates but pine was clearly preferred over the other two wood species. Redwood was however preferred over red oak in the dual choice tests as indicated by a higher wood consumption rate (Table 2.1, Fig. 2.2). And redwood and red oak were both preferred over yellow poplar (Table 2.1, Fig. 2.2).

Research aimed at examining wood preference should utilize all three of the bioassay designs--no-choice, paired-choice, and multiple-choice— to get a clear picture of wood preference in these animals. Using these three designs, a hierarchy of preference with these four wood species can be determined; for *R. flavipes,* pine was most preferred, then red oak, redwood, and poplar. Consumption of pine and red oak was not statistically different in the no choice tests (Table 2.1, Fig. 2.2). In the multiple choice design, differences between consumption rates of redwood and poplar could not be determined. Therefore, these two tests can be used together to gather a clearer picture of termite wood preference. Likewise, the combination of bioassay designs helps to determine preferences in *R. virginicus*. No choice tests were unable to provide a clear indication of preference between red oak and redwood species, while consumption rates of pine and red oak could not be statistically separated in four choice designs.

Across all three species, pine was clearly preferred in the all was it really in all the bioassay designs, especially in *R. virginicus* when it was consumed the greatest amount in both the four choice and no-choice designs (Table 2.4). In the other two species, pine was always preferred but sometimes the amount of consumption was statistically similar to red oak (Table 2.4). Poplar was never consumed more than pine wood and always in the bottom of the food preference hierarchy, even when consumption was similar to another wood type.

When consumption rate (mg of wood consumed/g of termite/day) was compared to the mass loss (mg) and mass lost/day over the course of the experiment, similar statistically significant results were observed (Table 2.3). In individual studies, calculating only the mass loss and mass loss/day will give researchers similar preference ranking, especially in no choice bioassay designs. However, for the sake of comparing the results between studies and controlling variables (such as trial length, number of termites, and termite body size), wood consumption rate (mg of wood consumed/g of termite/day) is a better measure to use.

In all three species, overall wood consumption was higher in trials where more wood was offered to the termites (Figure 2.3). When 12 blocks were present in four choice designs, consumption was significantly greater than in paired choice (6 blocks) and no choice (3 blocks) for *R. virginicus* (Figure 2.3). For the species *R. flavipes*, overall wood consumption was significantly higher in four choice trials than in no choice, but significant differences were not observed between paired choice trials and the other two designs (Figure 2.3). *Reticulitermes malletei* consumed significantly more wood in the no choice and four choice designs than in the paired choice.

Conclusion

Many different factors influence food preference in subterranean termites (Waller 1988, Houseman et al. 2001, Waller 2007, Haifig et al. 2008, Wong and Lee 2010). It has been suggested that larger blocks of wood increase feeding (Waller 1988, Lenz 2009). Our study showed that the amount of wood offered to the termites also makes a difference in feeding. Waller (1988) proposed that more wood was consumed (mg) in larger blocks only when a preferred wood species is offered to *R. flavipes* because there were no significant differences between large and small block wood consumption (mg) in ponderosa pine and red oak trials. However, in the present study, we saw higher consumption (mg wood consumed/g of termite/day) in two species in four choice assays (12 blocks) than in no choice bioassays (3 blocks). When 12 cubes of wood were offered to *R. virginicus* and *R. malletei* consumed more than in trials with 6 cubes and 3 cubes.

Like in Smythe and Carter (1970), the present study demonstrates that *R. flavipes* consumed (mg of wood/g of termite/day) redwood when no other wood was present (no choice trials) or if the only other choice (paired poplar trials) was more unpalatable. We also saw wood consumption similar to that of Ngee et al. (2004) in that non-preferred species were consumed in the no choice trials, but not as heavily in the choice trials. Unlike the study with *Coptotermes*, we demonstrated that, overall, more wood was consumed in the choice trials than in the no choice designs.

Wood density has been suggested as a contributing factor to termite wood preference; it is thought that higher wood density (or harder wood) is more resistant to termite damage (Behr et al. 1972, Esenther 1977, Waller 1988, Arango et al. 2006). The densities for redwood, pine, yellow poplar, and red oak are reported to be 0.436, 0.593, 0.427, and 0.657 g/cm^3, respectively (Seely 2002). In the present study, however, we saw all species consume pine (0.593 g/cm^3) and red oak (0.657 g/cm^3) at higher rates than redwood (0.436 g/cm^3)and poplar (0.427 g/cm^3) (Figure 2.2;Table 2.1,

Table **2.2**).

With the present study, we are unable to draw conclusions on the effect of food preference on niche partitioning in the genus *Reticulitermes* in the Southeast. All three species of *Reticulitermes* that we tested fed on the suitable food sources that they preferred (when it was available) (Table 2.3). This leads us to think that *Reticulitermes* species are opportunists and take advantage of whatever food resource is readily available to fulfill their role in the ecosystem.

No choice and multiple choice studies should not be used by themselves as an indicator of preference. For this study, we define preference as higher consumption rates (mg of wood consumed/g of termite/day) of one wood type over the others offered in the bioassay design. No choice bioassays show whether or not a species will eat a particular wood species, while multiple choice designs can indicate preference but are sometimes unable to distinguish clear preference between wood species. Multiple choice gives a clearer picture of termite preferences overall and can be verified with paired design.

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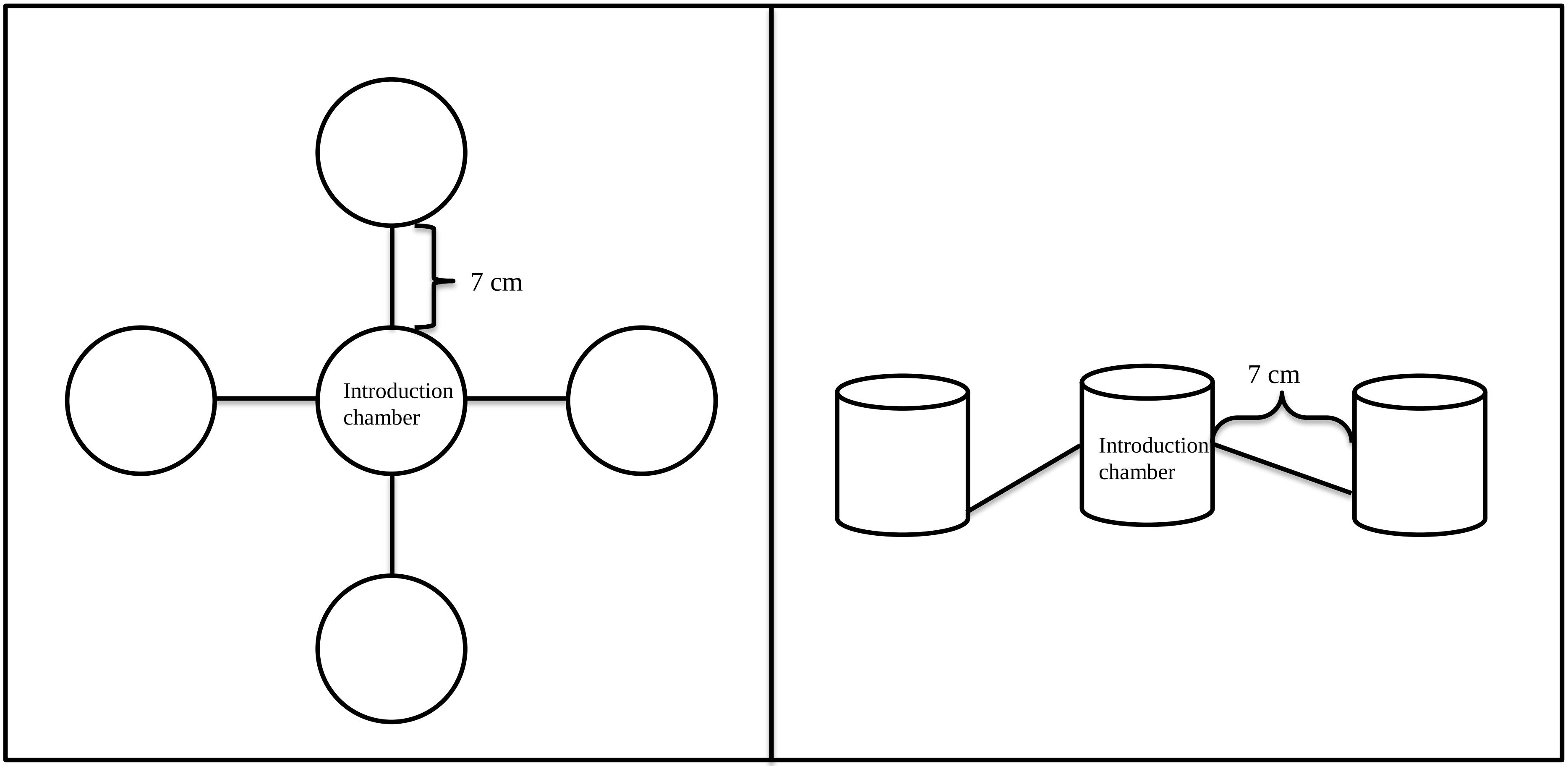

Figure 2.1. Illustration of four-choice bioassay design arenas. Left: top view. Right: side view & paired and no choice design illustration.

Table 2.1. Mean wood consumption rate (mg of wood consumed/g of termite/day) of each wood type tested in three bioassay designs by species.

R. flavipes

	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 10		n = 10		n = 11,11,11		n = 11,8,10		n = 11,8,9		n = 11,10,9	
Redwood	14.57 ± 9.93	Aa	1.65 ± 1.84	Ab			2.46 ± 2.60	***b	15.67 ± 9.99	***a	2.23 ± 1.77	***b
Pine	27.98 ± 7.90	Ba	19.02 ± 10.38	Bb	29.78 ± 10.31	***a			26.82 ± 8.62	***ab	24.06 ± 8.69	***ab
Poplar	1.85 ± 5.09	Ca	0.10 ± 0.94	Ab	4.04 ± 7.01	***ab	0 ± 1.30	***ab			1.81 ± 3.05	***ab
Red Oak	25.36 ± 10.85	Ba	7.41 ± 6.35	Cb	23.56 ± 5.75	***a	7.70 ± 2.77	***b	19.75 ± 3.06	***b		

R. malletei

	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 4		n = 4		n = 4,4,4		n = 4,3,4		n = 4,3,3		n = 4,4,3	
Redwood	20.67 ± 2.82	Aa	3.29 ± 1.69	Ab			4.35 ± 2.22	***b	9.40 ± 5.38	b	4.40 ± 1.80	***b
Pine	31.61 ± 7.37	Ba	21.17 ± 14.23	Bab	29.37 ± 4.90	***ab			25.44 ± 1.85	***ab	15.47 ± 5.15	b
Poplar	1.85 ± 2.98	Ca	0 ± 1.19	Ab	7.86 ± 5.55	***ab	1.52 ± 1.15	***ab			0.5 ± 0.59	***a
Red Oak	28.06 ± 4.93	ABa	10.68 ± 7.97	ABb	22.01 ± 11.89	***a	14.04 ± 8.30	a	25.46 ± 2.81	***a		

R. virginicus

	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 13		n = 18		n = 16,13,12		n = 16,12,12		n = 13,12,13		n = 12,12,13	
Redwood	20.19 ± 8.42	Aa	9.40 ± 4.09	Ab			2.54 ± 2.99c	***b	15.8 ± 8.65b	***a	19.65 ± 6.23c	***b
Pine	27.13 ± 9.00	B	22.38 ± 5.54	B	23.28 ± 8.64	***			24.96 ± 8.40	***	21.78 ± 10.49	***
Poplar	2.87 ± 6.79	Cab	2.57 ± 2.88	Ab	1.85 ± 3.18	***a	1.44 ± 3.03	***ab			1.98 ± 3.27	***a
Red Oak	21.79 ± 6.02	Aa	3.11 ± 3.54	Cb	5.26 ± 5.19	***a	6.31 ± 3.26	***b	18.48 ± 9.68	***a		

Notes –
Means with the same letter are not significantly different (α = 0.05). Capital letters indicate differences among wood type. Lowercase letters indicate differences among test type. Paired wood significance is indicated by *** (α = 0.05).

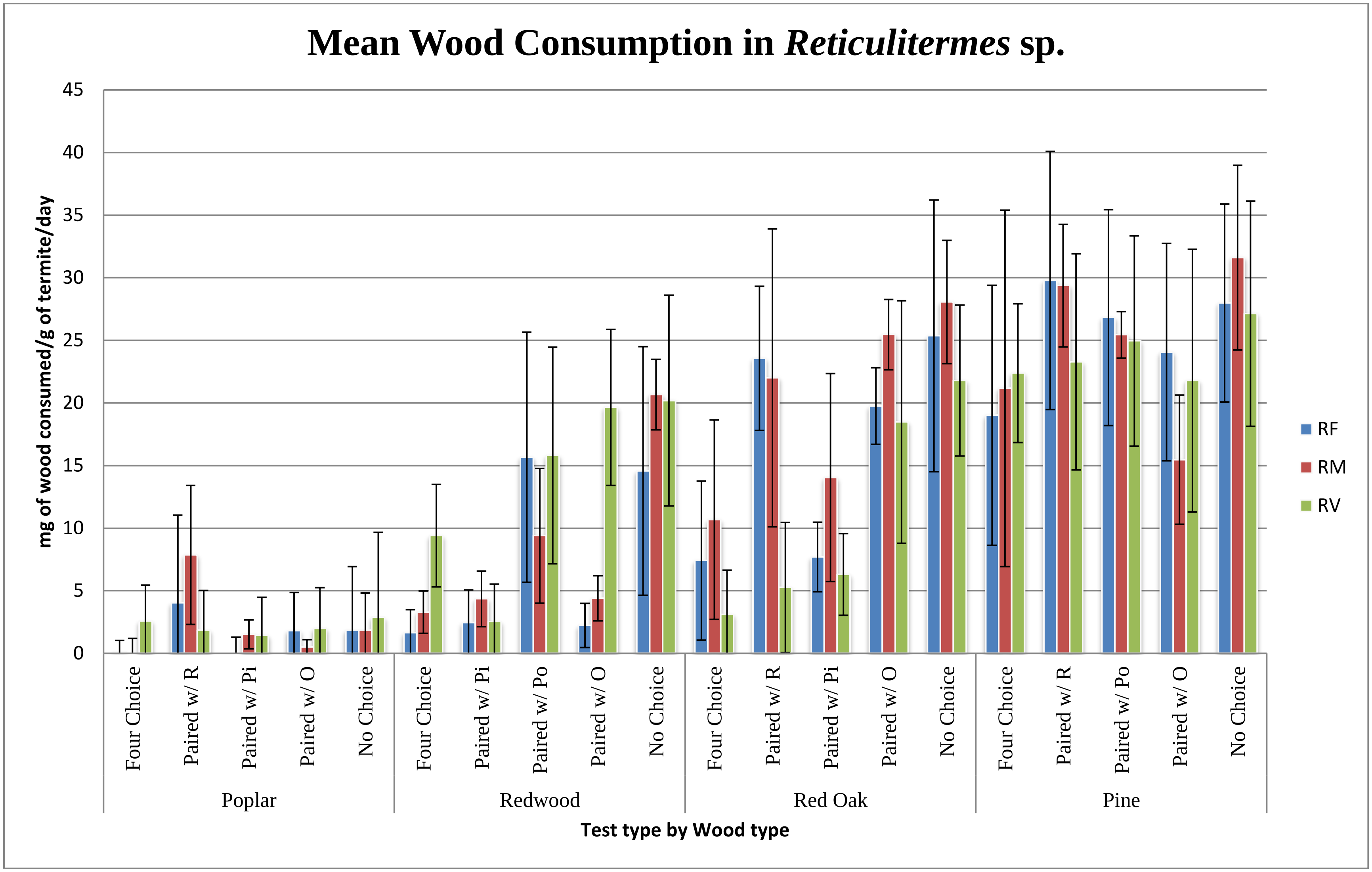

Figure 2.2. Mean wood consumption rates (mg of wood consumed/g of termite/day) for three species (*R. flavipes*, *R. malletei*, and *R. virginicus*) separated by test type and wood type.

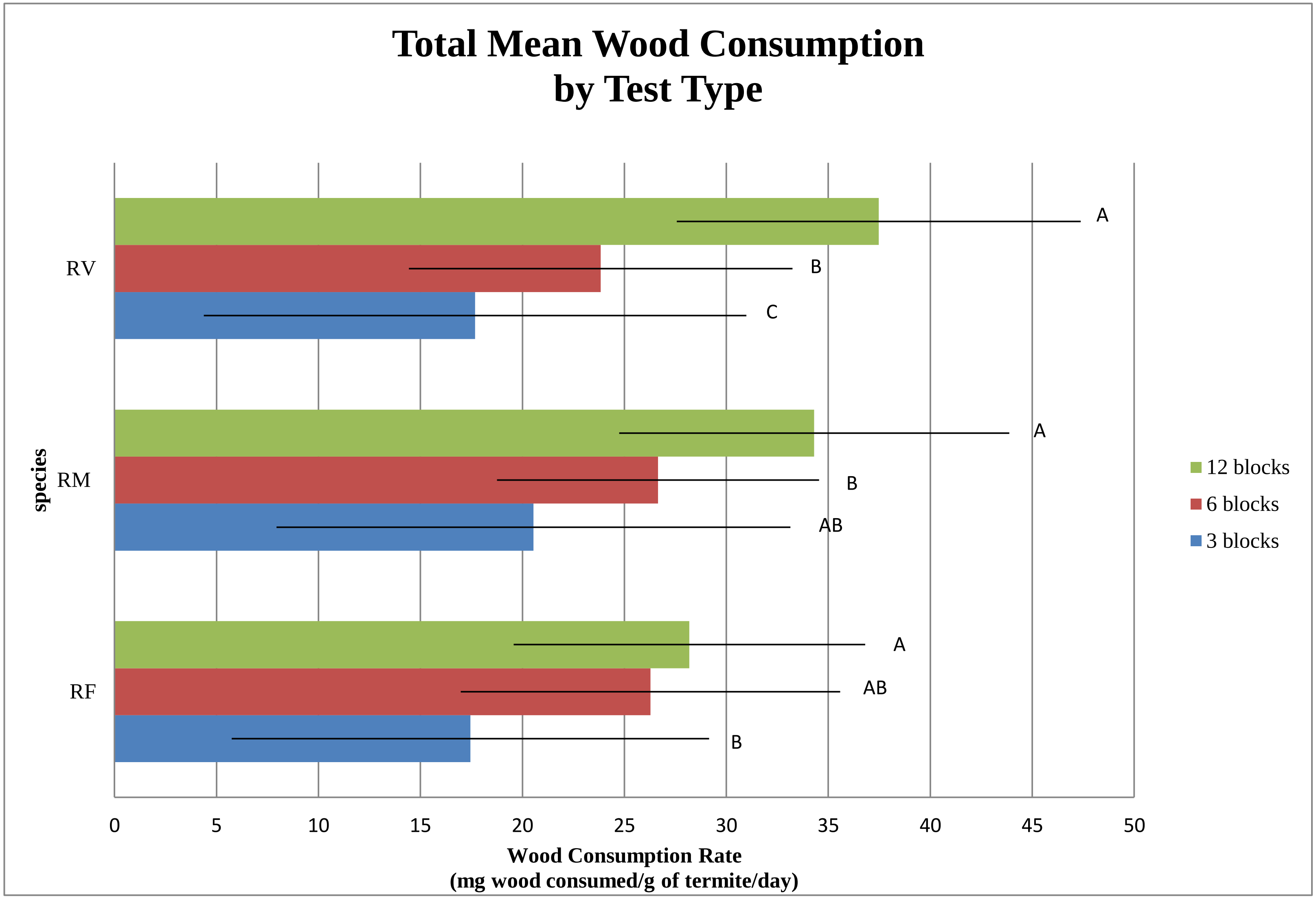

Figure 2.3. Combined mean wood consumption rates (mg of wood consumed/g of termite/day) in each test for *R. flavipes* (RF), *R. malletei* (RM), and *R. virginicus* (RV).

Table 2.2. Mean mass loss (mg) of each wood type tested in three bioassay designs by species.

						R. flavipes						
	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 11		n = 11		n = 12,12,11		n = 12,9,11		n = 12,9,9		n = 11,11,9	
Redwood	0.22 ± 0.17	B	0.03 ± 0.03	BC			0.05 ± 0.06	***	0.26 ± 0.23	***	0.04 ± 0.03	***
Pine	0.53 ± 0.08	A	0.38 ± 0.26	A	0.56 ± 0.18	***			0.49 ± 0.26	***	0.48 ± 0.22	***
Poplar	0.02 ± 0.05	C	0.01 ± 0.02	B	0.08 ± 0.10	***	0.01 ± 0.02	***			0.04 ± 0.05	***
Red Oak	0.49 ± 0.18	A	0.13 ± 0.03	C	0.44 ± 0.19	***	0.16 ± 0.06	***	0.38 ± 0.07	***		
						R. malletei						
	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 3		n = 3		n = 3,3,3		n = 3,2,3		n = 3,2,3		n = 3,3,3	
Redwood	0.15 ± 0.03	B	0.03 ± 0.02	AB			0.05 ± 0.02	***	0.08 ± 0.04			
Pine	0.38 ± 0.03	A	0.19 ± 0.16	A	0.29 ± 0.08	***			0.24 ± 0.05	***	0.18 ± 0.05	
Poplar	0.02 ± 0.03	C	0 ± 0.01	B	0.04 ± 0.01		0.02 ± 0.01	***			0.29 ± 0.03	***
Red Oak	0.27 ± 0.04	AB	0.14 ± 0.11	B	0.19 ± 0.02		0.12 ± 0.08		0.01 ± 0.01	***		
						R. virginicus						
	No Choice		4-choice		w/ Redwood		w/ Pine		w/ Poplar		w/ Red Oak	
	n = 13		n = 13		n = 16,13,12		n = 16,12,12		n = 13,12,13		n = 12,12,13	
Redwood	0.14 ± 0.06	B	0.03 ± 0.03	C			0.02 ± 0.02	***	0.14 ± 0.11	***	0.04 ± 0.03	***
Pine	0.30 ± 0.12	A	0.22 ± 0.04	A	0.25 ± 0.12	***			0.35 ± 0.11	***	0.06 ± 0.02	***
Poplar	0.021 ± 0.04	C	0.02 ± 0.02	C	0.01 ± 0.02	***	0.01 ± 0.03	***			0.18 ± 0.12	***
Red Oak	0.19 ± 0.05	B	0.10 ± 0.05	B	0.19 ± 0.09	***	0.26 ± 0.15	***	0.01 ± 0.02	***		

Notes –
Means with the same letter are not significantly different (α = 0.05). Capital letters indicate differences among wood type. Lowercase letters indicate differences among test type. Paired wood significance is indicated by *** (α = 0.05).

Table 2.3. Wood preference rankings (from most to least preferred) by wood consumption rate, mass lost, and mass lost/day in no choice and four choice bioassay designs for *R. flavipes, R. malletei,* and *R. virginicus.*

<table>
<tr><th colspan="4">Wood consumption rate
(mg of wood consumed/g of termite/day)</th><th colspan="4">Mass Lost
(mg)</th><th colspan="4">Mass Lost per Day
(mg/day)</th></tr>
<tr><th colspan="2">No Choice</th><th colspan="2">Four Choice</th><th colspan="2">No Choice</th><th colspan="2">Four Choice</th><th colspan="2">No Choice</th><th colspan="2">Four Choice</th></tr>
<tr><td colspan="12">R. flavipes</td></tr>
<tr><td>1</td><td>Pine, Red Oak</td><td>1</td><td>Pine</td><td>1</td><td>Pine, Red Oak</td><td>1</td><td>Pine</td><td>1</td><td>Pine, Red Oak</td><td rowspan="3">1</td><td rowspan="3">Red Oak, Pine, Poplar, Redwood</td></tr>
<tr><td>2</td><td>Redwood</td><td>2</td><td>Red Oak</td><td>2</td><td>Redwood</td><td>2</td><td>Red Oak, Redwood</td><td>2</td><td>Redwood</td></tr>
<tr><td>3</td><td>Poplar</td><td>3</td><td>Redwood, Poplar</td><td>3</td><td>Poplar</td><td>3</td><td>Redwood, Poplar</td><td>3</td><td>Poplar</td></tr>
<tr><td colspan="12">R. malletei</td></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Pine, Red Oak, Redwood</td><td rowspan="2">1</td><td rowspan="2">Pine, Red Oak, Redwood</td><td rowspan="2">1</td><td rowspan="2">Pine, Red Oak</td><td rowspan="2">1</td><td rowspan="2">Redwood, Pine</td><td>1</td><td>Pine</td><td rowspan="2">1</td><td rowspan="2">Pine, Red Oak, Redwood</td></tr>
<tr><td>2</td><td>Red Oak</td></tr>
<tr><td rowspan="3">2</td><td rowspan="3">Poplar</td><td rowspan="3">2</td><td rowspan="3">Poplar, Red Oak, Redwood</td><td rowspan="2">2</td><td rowspan="2">Red Oak, Redwood</td><td rowspan="3">2</td><td rowspan="3">Redwood, Red Oak, Poplar</td><td>3</td><td>Redwood</td><td rowspan="3">2</td><td rowspan="3">Red Oak, Redwood, Poplar</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Poplar</td></tr>
<tr><td>3</td><td>Poplar</td></tr>
<tr><td colspan="12">R. virginicus</td></tr>
<tr><td>1</td><td>Pine</td><td>1</td><td>Pine</td><td>1</td><td>Pine</td><td>1</td><td>Pine</td><td>1</td><td>Pine</td><td>1</td><td>Pine</td></tr>
<tr><td>2</td><td>Redwood, Red Oak</td><td>2</td><td>Red Oak</td><td>2</td><td>Redwood, Red Oak</td><td>2</td><td>Red Oak</td><td>2</td><td>Red Oak, Redwood</td><td>2</td><td>Red Oak</td></tr>
<tr><td>3</td><td>Poplar</td><td>3</td><td>Redwood, Poplar</td><td>3</td><td>Poplar</td><td>3</td><td>Redwood, Poplar</td><td>3</td><td>Poplar</td><td>3</td><td>Redwood, Poplar</td></tr>
</table>

CHAPTER 3

CONCLUSION

Members of the termite family Rhinotermitidae are important economic urban pests in the United States. Wood preference bioassays are important for testing wood resistivity or substances that can help make a wood resistant to termites. In Chapter 1, a literature review revealed that wood preference bioassays can be conducted either as no choice or multiple choice designs. The different designs have been implicated to give different results. Additionally, many researchers measure wood consumption in different units of measurement making them difficult to compare. Therefore, in Chapter 2, we designed three bioassay types (no choice, four choice, and paired choice) and reported the results of a comparison of wood consumption amongst four wood species (*Sequoia sempervirens, Pinus* sp., *Liriodendron tulipifera,* and *Quercus* sp.) for three *Reticulitermes* species (*R. flavipes, R. malletei,* and *R. virginicus*).

We determined that, as previously suspected, bioassay design does effect wood consumption rates. No choice designs, where only one type of wood is offered to the termites, is a good measure of whether termites will eat the species if necessary. Four choice and paired choice designs are a better measure of preference, because non-preferred woods, consumed in the no choice bioassays, are consumed at much lower rates in choice designs. We compared various measures of consumption (mass lost (mg), mass lost per day (mg/day), and a wood consumption rate (mg/g of termite/day). We found that while determining a wood preference hierarchy, the measurements are very similar, but by using the wood consumption rate, we are able to account for all of the variables that differ between studies (length of trial, termite body size, and number of termites). We also supported previous data that suggested larger blocks of preferred wood were eaten at higher rates than lower blocks of wood. In our designs, when more blocks of wood were offered, regardless of type, wood consumption rates were higher.

It is hoped that this research will help future researchers better design wood preference bioassays that can be compared to add to the body of knowledge on termite food choice.