

**LABORATORY EVALUATION AND RANKED PREFERENCE ASSESSMENT  
OF SUBTERRANEAN TERMITES *COPTOTERMES FORMOSANUS* SHIRAKI  
(ISOPTERA: RHINOTERMITIDAE) ON PECAN CULTIVARS OF *CARYA  
ILLINOINENSIS* (WANGENH.) K. KOCH IN TEXAS**

A Thesis

by

CHRISTOPHER RANIER SWAIN

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

MASTER OF SCIENCE

May 2010

Major Subject: Entomology

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Approved by:

Chair of Committee,	Roger E. Gold
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## ABSTRACT

Laboratory Evaluation and Ranked Preference Assessment of Subterranean Termites  
*Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) on Pecan Cultivars of *Carya*  
*illinoensis* (Wangenh.) K. Koch in Texas. (May 2010)

Christopher Ranier Swain, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

Feeding preferences of *Coptotermes formosanus* Shiraki were evaluated on 60 field-collected pecan *Carya illinoensis* (Wangenh) cultivars. The Moneymaker cultivar of *C. illinoensis* was most preferred by *C. formosanus*, and the degree of feeding on this cultivar was significantly different ( $P < 0.05$ ) from all other cultivars tested. Creek was the least preferred cultivar, but the degree of feeding was not significantly different from other cultivars. There was a trend for lower consumption by *C. formosanus* on commercially versus native cultivars.

In a multiple-choice test, the Desirable pecan cultivar, was significantly ( $P < 0.05$ ) more preferred than southern yellow pine (*Pinus palustris*), chinaberry (*Melia azedarach*), and the pecan cultivar, Barton, respectively. The significance is that Formosan termites fed on both pecan cultivars and southern yellow pine which is a commercially important wood. They also fed on chinaberry, which is a commonly used tree in landscape.

*Coptotermes formosanus* were significantly ( $P < 0.05$ ) more attracted to green leaf material from the Creek cultivar as compared to the other 50 cultivars tested. However, the Creek cultivar was the least preferred in the consumption test. This suggested that Formosan termites may be attracted to pecan trees and chemicals associated with the wood. It is evident that Formosan termites feed on various types of pecans in agro-ecosystems, this may be attributed to

leaf characteristics as well as other factors such as random foraging and swarming behavior.

These results further demonstrate that pecan cultivars are at risk to *C. formosanus* feeding.

## DEDICATION

This research is dedicated to my father, Porter, my wife, Shavon, and my son, Jordan. Thank you father for teaching me to believe in myself and never lose faith no matter how large the obstacles may seem. Thank you for teaching me to be compassionate and to love all God's creations. I know you are still watching over me. Shavon, thank you for being patient and so loving. You are a true blessing. My Dear son Jordan who is with God now, we love you and we'll see you soon.

*Yea, though I walk through the valley of the shadow of death, I will fear no evil: for thou art with me; thy rod and thy staff comfort me. Thou preparest a table before me in the presence of mine enemies: thou anointest my head with oil; my cup runneth over. Surely goodness and mercy shall follow me all the days of my life: and I will dwell in the house of the LORD forever.*

Psalm 23: 4, 6

## ACKNOWLEDGEMENTS

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## INTRODUCTION AND LITERATURE REVIEW

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has been considered an urban pest in its invasive range. Since its introduction to the continental United States, it is estimated that Formosan termite damage exceeds \$1 billion annually as the result of feeding on houses, other buildings, utility poles, railway structures, boats, paper, and living trees (Edwards and Mill 1986, Su and Tamashiro 1987, Su and Scheffrahn 1990). A *Coptotermes* species was first discovered in 1956 at the Houston Ship Channel in Pasadena, Texas (Harris County). The first established *C. formosanus* population was confirmed in Beaumont, Texas in 1962. By 1967, Formosan termites were found in three states in the continental United States, and are presently established in 14 states in addition to Hawaii. This species is more destructive than native subterranean termites (*Reticulitermes* spp.) because of the larger colonies and foraging territories (Su and Tamashiro 1987, Su and Scheffrahn 1988; Gold et al. 1996). One of the unique characteristics of *C. formosanus* is its ability to attack living trees. Unlike native termites, Formosan termites attack at least 50 known plant species in the United States. Infestations in living trees often go undetected because of the cryptic and concealed nature of the feeding termites (La Fage 1987).

Control of termite populations in urban eco-systems is critical. Formosan termites weaken trees causing them to fall and, as a result, destroy the aesthetic and historic importance of trees. They also threaten urban structures as a result of being in close association with infested trees (Osbrink et al. 1999). The vast economic impact of *C. formosanus* was most evident in the aftermath of hurricanes in the coastal areas of the United States over the past two decades.

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This thesis follows the format of the Journal of Economic Entomology.

High winds produced by these storms caused trees to fall because of structural weakening. After hurricane Andrew in 1992, 60% of the 360 examined trees in New Orleans were found to be infested with Formosan termites. Investigations of coastal trees felled by Hurricane Rita determined that 40% of large trees marked for deposition in landfills were infested with Formosan termites (Mcquaid 2009).

Control of Formosan termites in agro-ecosystems is important because of the termite's ability to feed on living trees and, as a result, causing structural damage and eventually economic loss due to the tree falling over.

*Coptotermes formosanus* are in the family Rhinotermitidae and are considered lower termites because they do not have a sterile worker cast. They have three castes including pseudergates, soldiers and reproductives. Pseudergates, often referred to as workers, are the most numerous in the colony, and form tunnels, locate cellulose, maintain the nest, and feed other members of the colony through trophylaxis (Noirot & Noirot-Timothee 1969). Pseudergates are not considered true workers, as in the higher termites (Family: Termitidae), because of their ability to develop into other termite castes including workers, soldiers and reproductives. In the family Termitidae, workers are a terminal stage, and do not molt into any other termite castes. Pseudergates of *C. formosanus* are responsible for the damage done in urban structures and trees. The digestion of cellulose is accomplished by enzymes produced by protozoa and bacteria in the termite gut. The digested cellulose and resulting sugars are regurgitated, by the foragers for consumption by pseudergates, soldiers and reproductives (Miller, 1969). While some understanding of the biology and behavior of the Formosan termite has been achieved, much remains to be learned about their dietary needs and cellulose preferences.

Multiple choice-tests have been conducted on wood species that are preferred by *C. formosanus*. Results showed differences in feeding and survival rates of subterranean termite workers in multiple-choice tests in which termites were fed different species of wood (Smythe and Carter 1970, Mannesmann 1973, Waller et al. 1990, Morales-Ramos and Rojas 2001). Although Formosan termites have been shown to feed on almost any type of wood, and at least 50 plant species, field and laboratory studies showed preference for pecan, *Carya illinoensis* (Morales-Ramos and Rojas 2001). These researchers hypothesized, that Formosan termite feeding preferences may be determined by the nutritional value of the food source. It was further suggested that foraging preferences of the Formosan subterranean termite could follow an optimal foraging theory, wherein the termites choose their food according to specific nutritional needs (Emlen 1973). Additional studies at Louisiana State University demonstrated that of 16 tree species surveyed, pecan had the highest percentage of Formosan termite infestations (Ring et al. 2007).

Several factors could contribute to the difference in consumption rates of certain wood species. Studies of fungal species associated with *C. formosanus* bodies, carton and infested wood, suggested a possible nutritional association with at least three species including *Curvularia lunata*, *Aspergillus fumigates* and *Aspergillus nomius* (Morales-Ramos and Rojas 2001). Wood infected with *C. lunata* was consumed by Formosan termites in termite bait matrices at a higher rate than uninfected wood (Morales-Ramos and Rojas 2001). *Curvularia lunata* is often found in the carton material of Formosan termite's nests, but it is not yet clear which precedes the other.

Research conducted by Chen and Henderson (1996) suggested that the presence of amino acids such as D-aspartic or L-aspartic, increased consumption of treated filter paper by *C. formosanus*. Studies of amino acids and other chemical constituents of preferred wood species

showed *C. formosanus* consumed more of the simulated food source than the other wood species tested (Morales-Ramos and Rojas, 2001). Therefore, the higher consumption of some wood species as compared to others, by *C. formosanus* was likely based on multiple factors.

Controlling termites in infested trees is usually accomplished through exposure to slow acting, non-repellent insecticides either as liquids, foams or applied as baits. This is usually accomplished via foaming or in ground bait systems. Termite baits delivered via ingestion, depended upon the consumption of the bait by the termites (Grace et al. 1996,). Control of termites in pecan trees has several additional factors that compound these efforts. First, there are no insecticides labeled to treat the interior of nut or fruit bearing trees. Second, available treatments such as slow-acting baits may take months, years or may never achieve control (Glenn and Gold, 2003). Third, the termite bait matrix may not induce termites to feed when in the presence of optimal alternative food sources. Termites tend to ignore baits when an optimal native food source, like pecan, is present (Morales-Ramos and Rojas, 2001). Fourth, the bark patterns on most pecan trees make visual identification of termite infestations very difficult. Identification of infested trees was most often done by visual inspection. Mud tubes formed on the outside of a tree, mud filled pruning scars, or the presence of termites at the base of a tree, are all signs of an infestation. Pecan bark morphology is arranged in a shingle-like overlapping pattern, making it difficult even to the trained eye, to find all termite infestations. Other methods of identifying termite infestations include audio acoustic and movement sensory devices but they are expensive and not readily available.

For nut improvement, pecan trees have been selected for particular traits through grafting or cross-breeding pollination. These traits are often associated with cultivars that have resistance or tolerance to insect feeding and high yields. It is these cultivars that are usually of commercial

importance. Historic or relic cultivars are of aesthetic value, and are often used as seed banks, and may not have commercial value.

There is a wide distribution of pecan trees in the continental United States, with a high concentration in the coastal and southern states including Texas and Louisiana. Production of pecans in both Texas and Louisiana is estimated at a combined annual production of between 50 to 75 million pounds. In Texas, pecans are considered an important commercial nut crop placing the state in the top 5% of pecan producers in the country (Austin and Glenn 2008). Many studies indicate that pecans are a preferred food source of *Coptotermes* species and show that high densities of *C. formosanus* and *C. illinoensis* overlap in the southern United States. The potential for great economic losses from these voracious termite pests in pecan agro-ecosystems is apparent. Research should be initiated to identify 'at risk' pecan cultivars, as well as those that have potential for natural resistance to termite feeding.

The first objective of this research was to determine whether *C. formosanus* showed feeding preferences for several different pecan cultivars. Determination of *C. formosanus* consumption rates among cultivars was valuable in evaluating whether cultivars were at high or low risk to Formosan termite feeding. The second objective was to determine if there were preferences for pecans as compared to two additional wood species. Tests by Ramos and Rojas (2001) identified several preferred wood species and pecan was clearly shown to be highly preferred by Formosan termites based on the growth and survival of incipient colonies (Morale-Ramos and Rojas, 2001). Additional testing is needed to determine if southern yellow pine and chinaberry would be preferred relative to two pecan cultivars by *C. formosanus*. The third objective was to determine if green leaf material from pecans collected from the field were an attractant to foraging *C. formosanus* workers.

**Objectives.** There were three objectives in this study. The first objective was to determine if *C. formosanus* had preferences among 56 pecan varieties and to identify low and high risk cultivars. The second objective was to determine if *C. formosanus* has a preference for pecan as compared to southern yellow pine and Chinaberry. The third objective was to determine if green leaf materials collected from pecan cultivars were attractive to foraging *C. formosanus* termite workers.

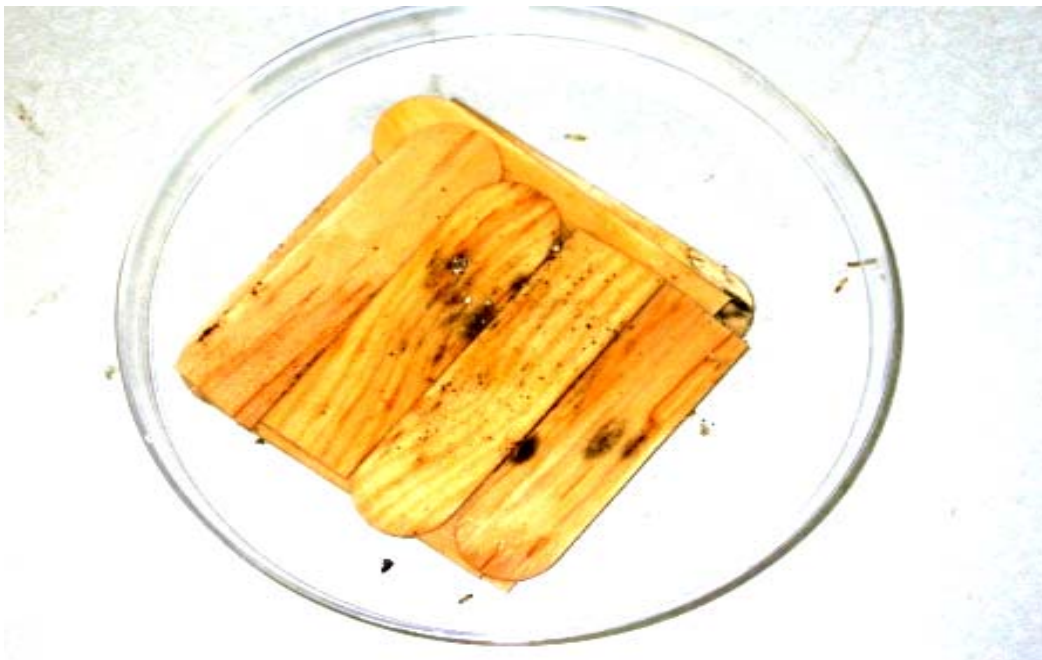
**Null Hypotheses.** There were three hypotheses in this study. The first null hypothesis was that *C. formosanus* will not show a significant feeding preference on wood from different pecan cultivars. The second null hypothesis was that *C. formosanus* would not show a significant feeding preference for wood of pecans when compared to southern yellow pine and chinaberry. The third null hypothesis was that *C. formosanus* workers would not show a significant attraction to green leaf material from several pecan cultivars.



## MATERIALS AND METHODS

**Collecting, Sorting and Culturing of Formosan Subterranean Termites.** Formosan subterranean termites were collected from three different locations. Two collection sites were in Baytown, TX (29.73°32,80"N,-94.97°70,64"W) (29.73°59,98"N,-94.99°69,45"W), and one was in Beaumont, TX (30.04°07,15"N,-94.06°85,46"W). Colonies were collected by using in-ground wood blocks made of southern yellow pine (*Pinus taeda* L.). Three wood blocks, 2.54 x 15.2 x 15.2 cm, were connected by running all-thread rod 9 cm in length and 0.6 cm in diameter inserted through a 0.6 cm hole drilled in the center of each block. The blocks were then held in place by using wing nuts to attach both ends of the all-thread rod to form the feeding substrate. The assembled blocks were housed in a 3.8 m<sup>3</sup> plastic bucket with the bottom removed to allow the block to have direct contact with the soil. A hole was dug in the soil to accommodate the size of the bucket, and then wooden blocks were placed in the center. The lid of the bucket was then placed on top, and covered with soil. This trap was left in the ground for a period of 1 month before being checked for the presence of Formosan termites. After termites were found in the traps, the blocks were removed and placed in individual 3.8 m<sup>3</sup> buckets, labeled with the location, collection time and date, and then brought back to the laboratory where they were stored at room temperature until sorting. After a period of no more than 3 d, the termites were tapped out of the blocks onto a plastic sorting tray. The sorting device consisted of a 40.50 x 30.50 cm plastic tray tilted at a 20° angle which allowed the termites to move downward. At the bottom of the plastic tray, six 0.60 cm holes spaced 3.8 cm apart were connected to plastic tubing (0.60 cm in diameter and 7.60 cm in length). Through the tubes, termites dropped down into a 33.00 x 19.10 x 11.40 cm acrylic plastic shoe box (Pioneer Plastics at USA Highway 41A North, Dixon Kentucky, 42409). Professional Choice tongue depressors (15.2 cm) (Solon Company, Skowhegan, Maine, 04976) were soaked in water for 1 h to saturate them and leach out soluble

chemicals. They were then placed on paper towels to dry for 5 minutes and then cut into 3.80 cm pieces and stacked in a 14.25 cm Petri dish (Nagle Nunc International at 75 Panorana Creek Drive, Rochester, New York, 14625). The 3.80 cm pieces were stacked in the Petri dish, one on top of the other, in a horizontal pattern. Two 7.60 cm cut tongue depressors were placed on top of the arrangement in the Petri dish to form a square. Two additional pieces were placed in the center as fillers (Fig. 1). This was done to simulate the galleries termites create in wood. This pattern was continued until the depressors were stacked four rows high. The arrangement of tongue depressors in Petri dishes was referred to as the standard culturing arenas. The termites were gently removed from the sorting system and placed in the arenas with a lid cover. These dishes were then stacked in an acrylic plastic box also containing a lid. These colony units were then stored in an environmental chamber at  $29.4 \pm 2^\circ\text{C}$  and  $85 \pm 4\%$  RH until initiation of the study.



**Fig. 1.** Photograph of standard culturing arena for *Coptotermes formosanus*.

**Experimental Design for the No-Choice Test.** A no-choice test was designed to compare *C. formosanus* feeding preference on 56 different pecan cultivars. This test was designed to test the null hypothesis that *C. formosanus* would not show a significant preference to different pecan cultivars. Pruning shears were used to cut branches (cross-sections containing bark and heartwood) measuring  $22.31 \pm 3.48$  cm in length and  $2.52 \pm 0.34$  cm in dia from 56 pecan cultivars (*Carya illinoensis*), grown at the USDA Pecan Breeding and Genetics Program, Southern Plains Agricultural Research Center, 10200 FM 50, Somerville, TX . Cultivars were selected based on historical and commercial importance (Thompson and Young, 1985). The wood samples were then placed in labeled Zip Loc® bags and brought back to the laboratory where they were stored at room temperature ( $25 \pm 2^\circ\text{C}$  and  $55 \pm 4^\circ\text{RH}$ ) until the final samples were cut. The final samples were cut on a band saw using a fence to ensure that all the sample sizes were similar in thickness. Samples were cut into wood wafers that were 0.42 cm thick, and then dried in a Fisher Scientific Isotemp oven (Fisher Scientific 2000 Park Lane Drive Pittsburgh, PA 15725) at  $51^\circ\text{C}$  for 8 hrs. To minimize the loss of the chemical make-up of the wood wafer, they were dried at this low temperature for an extended drying time. After drying, they were then allowed to cool to room temperature ( $25^\circ \pm 2^\circ\text{C}$ ) then weighed to the nearest 0.1 mg, labeled and stored in labeled plastic bags until initiation of the no-choice tests. This was done to get a pre-weight for each wood wafer.

A no-choice test was designed to allow the availability of only one food source to the termites tested. To accomplish this, termites were placed in a single Petri dish with only one wood wafer. The wood wafers were individually rehydrated by placing them in a 100 ml glass beaker containing 50 ml of distilled water. Each wafer was allowed to soak for a period of 5 minutes after which it was placed in individual Petri dishes. Each Petri dish contained 95.1 g of sand and 20 ml of water to provide moisture to keep the wood wafer damp and to preserve the

termites which were added in the next step. Termites were removed from the environmental chamber 1 wk prior to testing and allowed to acclimate to laboratory conditions. A total of 250 termites were hand counted, 25 of which were *C. formosanus* soldiers, and placed a Petri dishes containing hydrated sand (Fig. 2). In nature, Formosan termite soldiers make up 5 to 15% of a termite colony, therefore 25 soldiers were used in this study since foraging behavior was dependent upon the numbers of soldiers present in the colony (Mao, et al. 2005). After *C. formosanus* were added to the Petri dish arenas, a lid was placed on top to retain the termites, and to keep the wood wafers moist (Fig. 3). An untreated control with a wood wafer and no termites, was used to provide data on weight gain or loss. Additional untreated controls, each containing 250 termites, utilizing the standard culturing arena design (Fig. 3), were placed in proximity to the testing array. These controls were monitored through time to determine if there was unanticipated termite mortality. This experiment was replicated three times. The study was done in a darkened laboratory at  $25 \pm 2^\circ \text{C}$  and  $55 \pm 4\% \text{RH}$ . This study ran for 8 d after which the wood wafers were removed, cleaned, dried and reweighed to get a post-weight to determine the amount of wood consumed by the termites.



**Fig. 2.** A no-choice arena with a pecan wood wafer and 250 Formosan termites.



**Fig. 3.** An array of replicated no-choice arenas with pecan wood wafers and 250 Formosan termites.

**Experimental Design for the Multiple-Choice Test.** A multiple-choice test was designed to compare *C. formosanus* feeding preferences on three tree species including *Carya illinoensis* (two cultivars), *Pinus taeda* L., and *Melia azedarach* (Table 1).

**Table 1.** Wood species tested for *C. formosanus* feeding preferences on samples collected from Brazos County, TX.

Common Name	Scientific Name
Southern yellow pine <sup>a</sup>	<i>Pinus Palustris</i>
Chinaberry <sup>b</sup>	<i>Melia azedarach</i>
Pecan (Desirable) <sup>b</sup>	<i>Carya illinoensis</i>
Pecan (Barton) <sup>b</sup>	<i>Carya illinoensis</i>

<sup>a</sup> heartwood

<sup>b</sup> sapwood and heartwood

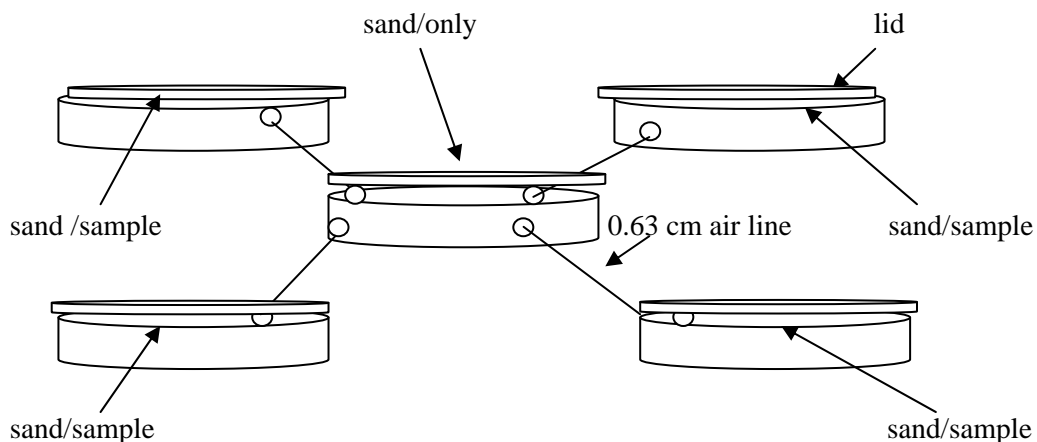
This test was designed to test the null hypothesis that *C. formosanus* would not show a significant preference for pecan compared to two other wood species. Pruning shears were used to cut branches (cross-sections containing bark and heartwood) measuring between  $22.31 \pm 3.38$

cm in length from Desirable and Barton pecan cultivars from the USDA Pecan Breeding and Genetics Program, Southern Plains Agricultural Research Center, 10200 FM 50, Somerville, TX. The samples were placed in labeled bags and transported to the laboratory until the initiation of the study. Pruning shears were used to cut one branch from a chinaberry tree in Robertson County, TX. The branch was labeled and transported to the laboratory until initiation of the study. An untreated southern yellow pine wood board 5.1 x 10.2 x 15.2 cm was purchased from Home Depot in College Station, TX and transported and held in a laboratory at  $25 \pm 2^\circ\text{C}$  and  $55 \pm 4\%$  RH until the study began. A multiple-choice test was designed to allow the termites, the availability of multiple food sources. To accomplish this, termites were placed in a center Petri dish that attached to four outer Petri dishes, which all contained different wood types. Wood wafers were cut from the Desirable and Barton cultivars and chinaberry branches using a band saw and a wedge to ensure similar thicknesses. These wafers measured  $2.52 \pm 0.34$  cm in dia and 0.42 cm in thickness. Wood wafers of the southern yellow pine board were sized by multiple types of saws. The finished wafers measured  $2.71 \pm 0.41$  cm in dia and 0.42 cm in thickness, and were weighed to the nearest 0.1 mg.

Wood consumption was not measured in this experiment; therefore, the wood wafers were not dried prior to weighing. For this experiment, the numbers of termites present in each outer Petri dish arena were counted. There were 60 (5 per replicate) multi-choice arenas (14.50 cm Petri dishes) used for this experiment. There were four outer Petri dishes and one center Petri dish (Fig. 4). One hole was drilled in the side of each of the four outer Petri dishes using a 0.64 cm drill bit. The four outer Petri dishes were connected to the center Petri dish by 0.64 cm in dia and 3.50 cm long plastic tube (IPSD Inc. in San Diego, CA) (Fig. 4). The center Petri dish had four 0.64 cm holes uniformly drilled in the side, for the attachment of the tubing which

connected to the four outer Petri dishes. The distance between each side hole was 3.50 cm. The test arenas were set up 1 d prior to testing.

Termites were removed from the environmental chamber 1 wk prior to testing and allowed to acclimate to laboratory conditions. A total of 250 termites were hand counted, including 25 soldiers, and placed into the center Petri dish which contained only hydrated sand, but no food source. The assumption was that the 250 *C. formosanus* would leave the center dish and forage to one of the four outer Petri dishes which contained one of the four food sources. An untreated control was used in the multiple-choice test. This control utilized the standard culturing arena with 250 *C. formosanus* that were placed in proximity to the testing array. These controls were monitored through time to determine if there was unanticipated termite mortality. Both controls and treatment were kept in a closed room in complete darkness at  $25 \pm 2^\circ\text{C}$  and  $55 \pm 4\%$  RH for a period of 3 d. The numbers of termites present in each of the outer arenas were counted at intervals of 1, 4, 8, 24, 48, and 72 hrs. All termites remaining in the air lines between Petri dishes were considered to be in the center Petri dish.



**Fig. 4.** Diagram of Multi-Choice arenas with hydrated sand, pecan wood wafers, and 250 Formosan termites.

**Experimental Design for the Green Leaf Material Test.** This experiment was done to determine if *C. formosanus* were attracted to green leaf material from 48 pecan (*Carya illinoensis*) cultivars, 1 chinese wing nut (*Pterocarya stenoptera*) tree, 1 black walnut (*Juglan J. Major*) tree and 1 hybrid [*Carya aquatica* (*C. x lecontei* Little)] tree. Leaf samples were chosen from the same cultivars represented in the no-choice test; however, samples from some cultivars were not available at the time of testing. *Coptotermes formosanus* were offered leaf samples to test the null hypothesis that these termites would not show significant attraction to green leaf materials. Leaf samples (of similar color and size) were cut with pruning shears from the branches of 51 different trees from the USDA Pecan Breeding and Genetics Program, Southern Plains Agricultural Research Center, 10200 FM 50, Somerville, TX. Once cut, the leaf samples were placed in labeled plastic bags and transferred to the laboratory and stored in a refrigerator at  $18 \pm 2^\circ \text{C}$  until initiation of the study.

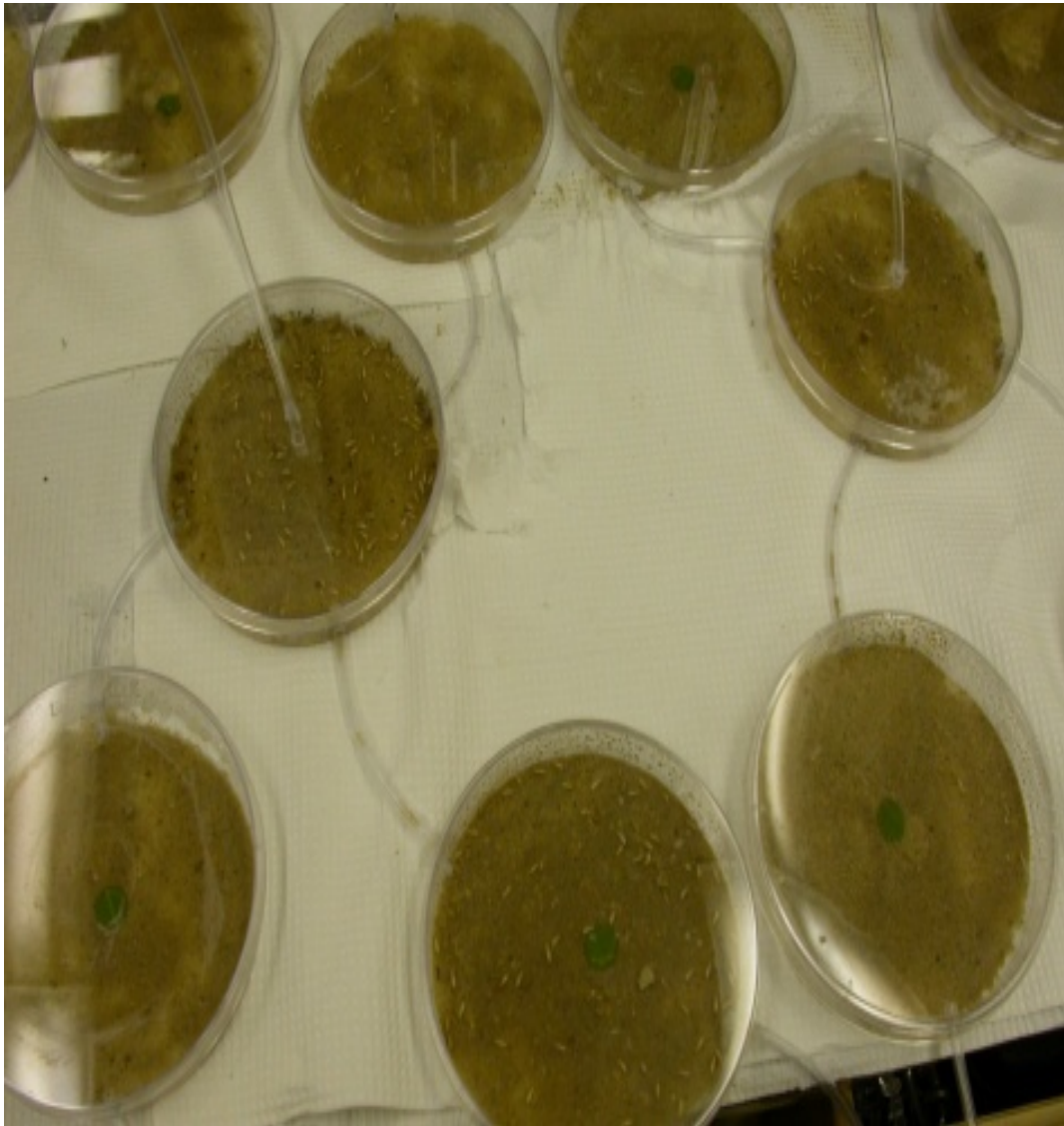


This experimental design contained an array of four outer Petri dishes and one center Petri dish with interconnecting tubes (Fig. 6). Two holes were drilled in the side of each of the four outer Petri dishes. The holes were drilled 7.20 cm apart using a 0.64 cm drill bit. The four outer Petri dishes were connected to a center Petri dish by 0.64 cm plastic tubing. The center Petri dish had four 0.64 cm holes uniformly drilled in the side. The distance between each side hole was 3.50 cm. One hole was drilled and centered on the lid of the center Petri dish. A tube, 0.64 cm in dia. and 15.24 cm in length connected the center hole to the lower intake hole of an air regulator (Cole Pramer at 625 East Bunker Court Vernon Hill, IL, 60061). The top outlet hole of the air regulator connected to a 455 cm long and manifold 1.90 cm in dia. main air line, which was then connected to a 15.24 cm long and 0.64 cm in dia. air line. The 455 cm long tube connected to a 115 volt air pump (Curtin Matheson Scientific Inc. in Fort Wayne, IN 46803). The air pump pulled air across samples at 0.90 m/sec and was then vented into a fume hood. The 0.64 cm air line tubing was cut in lengths of 15.24 cm and placed in the four holes of the center dish and connected to one of the two holes on the four outer dishes. The remaining hole on the outer dishes received an air line, 0.64 cm in dia. and 15.24 cm in length that attached to a manifold 1.90 cm in dia. main and 455 cm in length that contained a filter which cleaned the air and allowed it to be pulled into the test arenas. The air regulator (Cole Pramer 625 East Bunker Court Vernon Hill, IL, 60061) placed between the pump and the outer Petri dishes, allowed equal amounts of air flow (0.90 m per sec) to be pulled through the Petri dishes up through the vent hood (Figs. 5-6). This design was complete 1 d prior to testing, and was calibrated to ensure that uniform air movement was achieved.

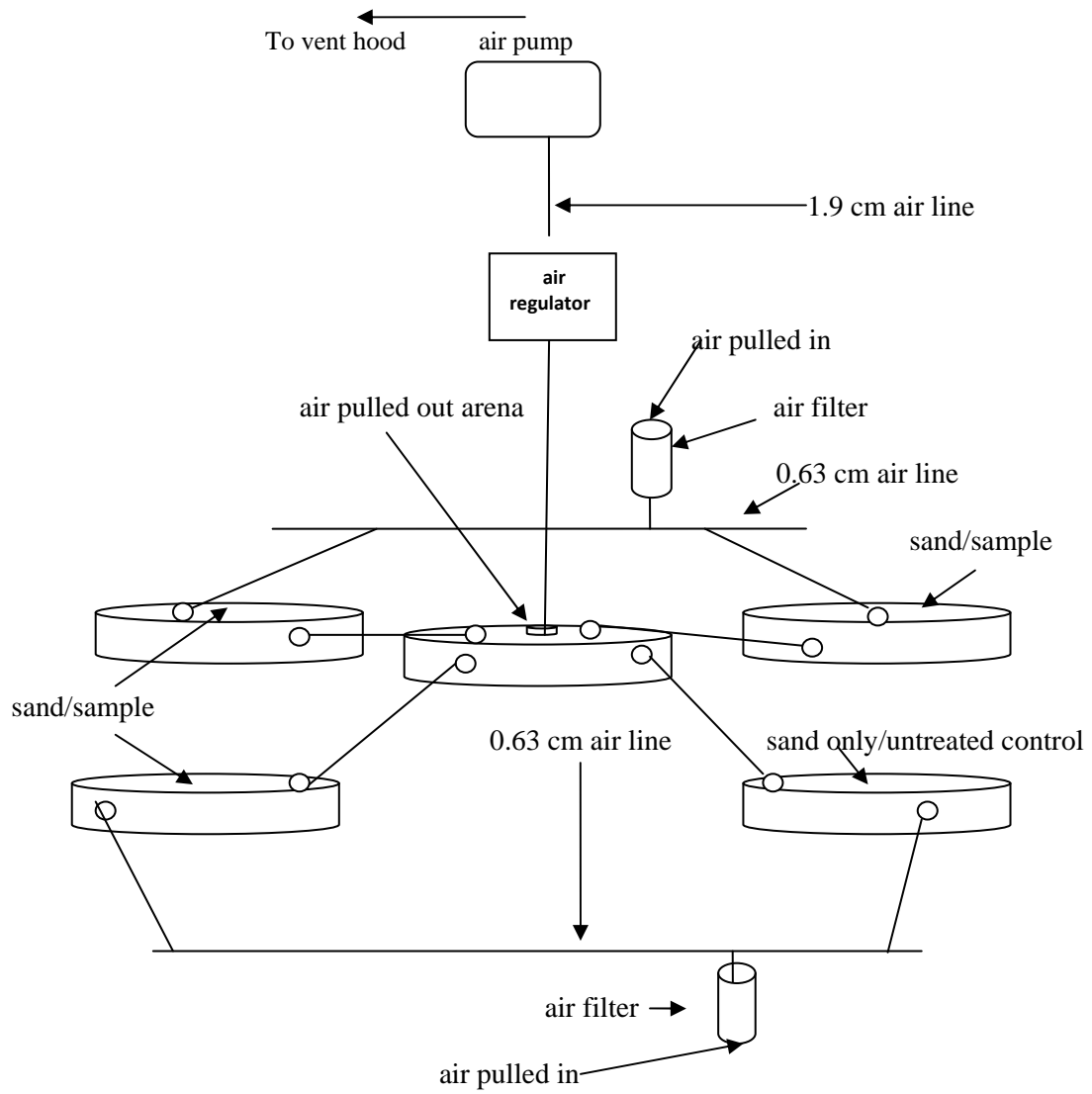
At the beginning of the study, leaf discs from each pecan cultivar were cut using a 3.0 cm in dia. circular hollow metal punch. The leaf disc was removed from the center of each leaf and then placed in three of four outer arenas, previously assembled with hydrated sand. The leaf

samples were randomly placed clockwise (starting from the lower left dish) into three of the four outer Petri dishes. Each Petri dish contained 95.1 g of sand and 20 ml of water to provide moisture to keep the wood wafer damp and preserve the termites. The leaf samples were randomly assigned to test arenas. The fourth Petri dish, in the array did not receive a leaf sample and acted as an untreated control. This was done to determine if *C. formosanus* were attracted to green leaf material of specific cultivars. The assumption was that there would be an equal number of termites present in each of the four Petri dishes, if foraging was random.

Untreated controls utilizing the standard culturing arena were placed in proximity to the testing array. These controls were monitored through time to determine if there was unanticipated termite mortality. Termites were removed from the environmental chamber 1 wk prior to testing and allowed to acclimate to laboratory conditions. Two hundred and fifty hand counted termites, including 25 soldiers, were placed in the center Petri dish containing hydrated sand, but with no green leaf material. The four outer Petri dishes also contained hydrated sand but three contained green leaf material. Termites were kept in a closed room in complete darkness at  $25 \pm 2^\circ\text{C}$  and  $55 \pm 4\%$  RH allowed to forage for a period of 3 d. The four outer Petri dishes and one center Petri dish represented a test group. There were a total of 17 groups, each replicated three times. The lights remained on only when counting termites in Petri dishes at intervals of 1, 4, 8, 24, 48 and 72 hrs. If termites were located in the tubing they were considered in the center Petri dish.



**Fig. 5.** Photograph of the green leaf material design.



**Fig. 6.** Diagram of the green leaf material experiment.

**Statistical Data Analysis.** Analysis of Variance (ANOVA) or application of the General Linear Model (GLM), with appropriate post hoc test (e.g., Tukey's Highly Significant Difference) was used in all three experiments ( $P < 0.05$ ) (SigmaStat, 1997).

## RESULTS

**No-Choice Test.** Consumption of pecan cultivar wood wafers by 250 Formosan subterranean termites through 8 d, differed significantly ( $F= 15.49$ ;  $df = 59,240$ ;  $P < 0.05$ ) based on GLM analysis of the 56 cultivars tested. The range of wood consumption by the termites was 0.0173 to 0.6375 g for the Creek and MoneyMaker cultivars, respectively (Table 2.). The MoneyMaker cultivar was the most preferred, while Creek was the least fed upon through 8 d. *Coptotermes formosanus* fed on MoneyMaker at a significantly greater ( $P < 0.05$ ) level than all other cultivars. When ranked, the commercially important cultivars occurred along, and were dispersed throughout, the continuum of cultivar preferences demonstrated in Table 2. However, it is important to note that five of the six cultivars most preferred by Formosan termites were considered commercially important. Termites in the untreated controls for this experiment had less than 10% throughout the 8 d period. The mean weight difference for the untreated controls of all 56 cultivars was 0.0300 g and post-weights did not differ significantly from pre-weights in the untreated controls. The mean consumption values of all 56 cultivars through five replications over an 8 d period are presented in Table 2. The overall mean consumption of Formosan termites feeding on 56 cultivars is presented in Figure 7.

**Table 2.** Formosan termite consumption of pecan cultivars. Summary of the mean consumption of wood (g) from different pecan cultivars by 250 Formosan termites in laboratory studies conducted over an 8 d period.

Cultivar	Consumption order least to greatest	Mean weight consumed (g)
Creek <sup>a</sup>	1	0.0173±0.0039
Clark II <sup>a</sup>	2	0.0192±0.0097
Wichita <sup>ab*</sup>	3	0.0282±0.0106
Navaho <sup>ab</sup>	4	0.0290±0.0067
Hopi <sup>ab</sup>	5	0.0297±0.0147
Chickasaw <sup>ab</sup>	6	0.0407±0.0167
Barton <sup>abc</sup>	7	0.0447±0.0200
Osage <sup>abc*</sup>	8	0.0453±0.0130
Kiowa <sup>abc*</sup>	9	0.0460±0.0229
Apache #5 <sup>abcd</sup>	10	0.0508±0.0223
Lucas <sup>abcde</sup>	11	0.0536±0.0275
Pawnee <sup>abcde*</sup>	12	0.0559±0.0353
Walnut <sup>abcde</sup>	13	0.0563±0.0297
Choctaw <sup>abcde</sup>	14	0.0583±0.0079
Candy <sup>abcde*</sup>	15	0.0588±0.0325
Kanza <sup>abcde*</sup>	16	0.0597±0.0079
Woodroof <sup>abcde</sup>	17	0.0611±0.0081
Shoshoni <sup>abcde*</sup>	18	0.0618±0.0253
Comanche <sup>abcde</sup>	19	0.0628±0.0185
Warren <sup>abcde</sup>	20	0.0672±0.0200
Shawnee <sup>abcde</sup>	21	0.0685±0.0237
Ocone <sup>abcde*</sup>	22	0.0688±0.0325
Forkert <sup>abcde</sup>	23	0.0695±0.0087
Apache <sup>abcde</sup>	24	0.0703±0.0105
Burkett <sup>abcde*</sup>	25	0.0714±0.0243
Caddo <sup>abcde*</sup>	26	0.0751±0.0210
Carter <sup>abcde</sup>	27	0.0754±0.0045
Mississippi <sup>abcdef</sup>	28	0.0812±0.0227
San Felipe <sup>abcdef</sup>	29	0.0960±0.0111
Nelson <sup>abcdef</sup>	30	0.0965±0.0087
Houma <sup>abcdef</sup>	31	0.0980±0.0207
Ramsey Mediums <sup>abcdef</sup>	32	0.1032±0.0098
Cherryle <sup>abcdef</sup>	33	0.1068±0.0062
Mohawk <sup>abcdef*</sup>	34	0.1090±0.0177
Van Deman <sup>abcdef</sup>	35	0.1122±0.0200
Sioux <sup>abcdef*</sup>	36	0.1174±0.0510

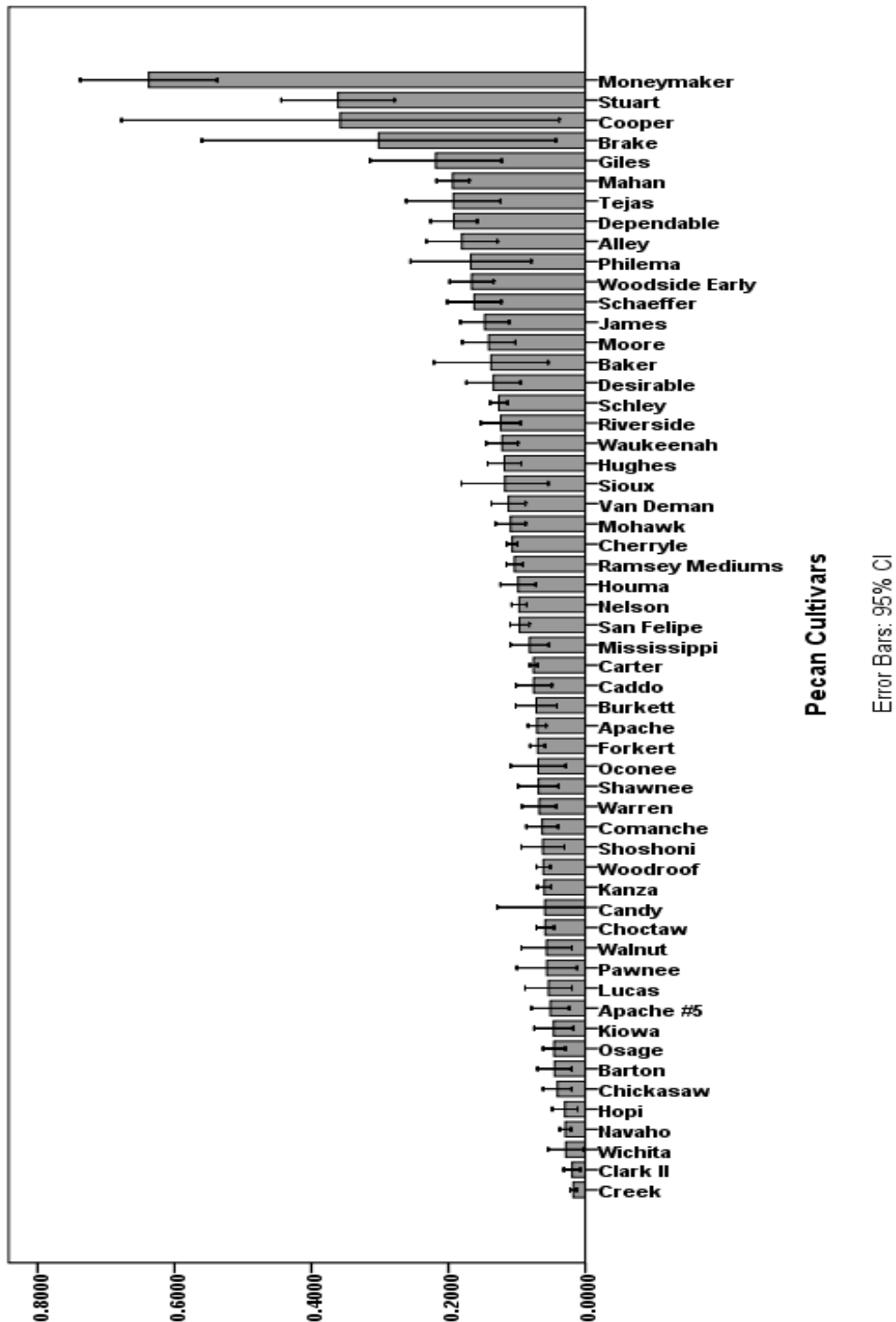
**Table 2. Continued.**

Cultivar	Consumption order least to greatest	Mean weight consumed (g)
Hughes <sup>abcdef</sup>	37	0.1181±0.0198
Waukeelah <sup>abcdef</sup>	38	0.1215±0.0186
Riverside <sup>abcdef</sup>	39	0.1233±0.0234
Schley <sup>abcdef</sup>	40	0.1261±0.0101
Desirable <sup>abcdef*</sup>	41	0.1340±0.0318
Baker <sup>abcdef</sup>	42	0.1376±0.0670
Moore <sup>abcdef</sup>	43	0.1408±0.0312
James <sup>abcdef</sup>	44	0.1467±0.0286
Woodside Early <sup>bcdefg</sup>	45	0.1660±0.0257
Schaeffer <sup>bcdefg</sup>	46	0.1622±0.0316
Philema <sup>bcdefg</sup>	47	0.1672±0.0709
Alley <sup>cdefg</sup>	48	0.1802±0.0415
Dependable <sup>defg</sup>	49	0.1917±0.0273
Tejas <sup>efg</sup>	50	0.1924±0.0553
Mahan <sup>efg*</sup>	51	0.1935±0.0190
Giles <sup>fgh*</sup>	52	0.2182±0.0773
Brake <sup>ghi*</sup>	53	0.3016±0.2081
Cooper <sup>hi</sup>	54	0.3578±0.2573
Stuart <sup>i*</sup>	55	0.3612±0.0665
Moneymaker <sup>j*</sup>	56	0.6375±0.0805

\*i indicates a commercially important cultivars (in production in 2009) based on the number of trees currently in production in Texas in 2009.

\*Means followed by the same letter are not significantly different using ANOVA, and Tukey HSD (Honestly Significant Difference) (SPSS v. 16.0).





Overall mean number of Formosan termites feeding on 56 pecan cultivars.

Fig. 7. Overall mean consumption of Formosan termites feeding of 56 pecan cultivars.

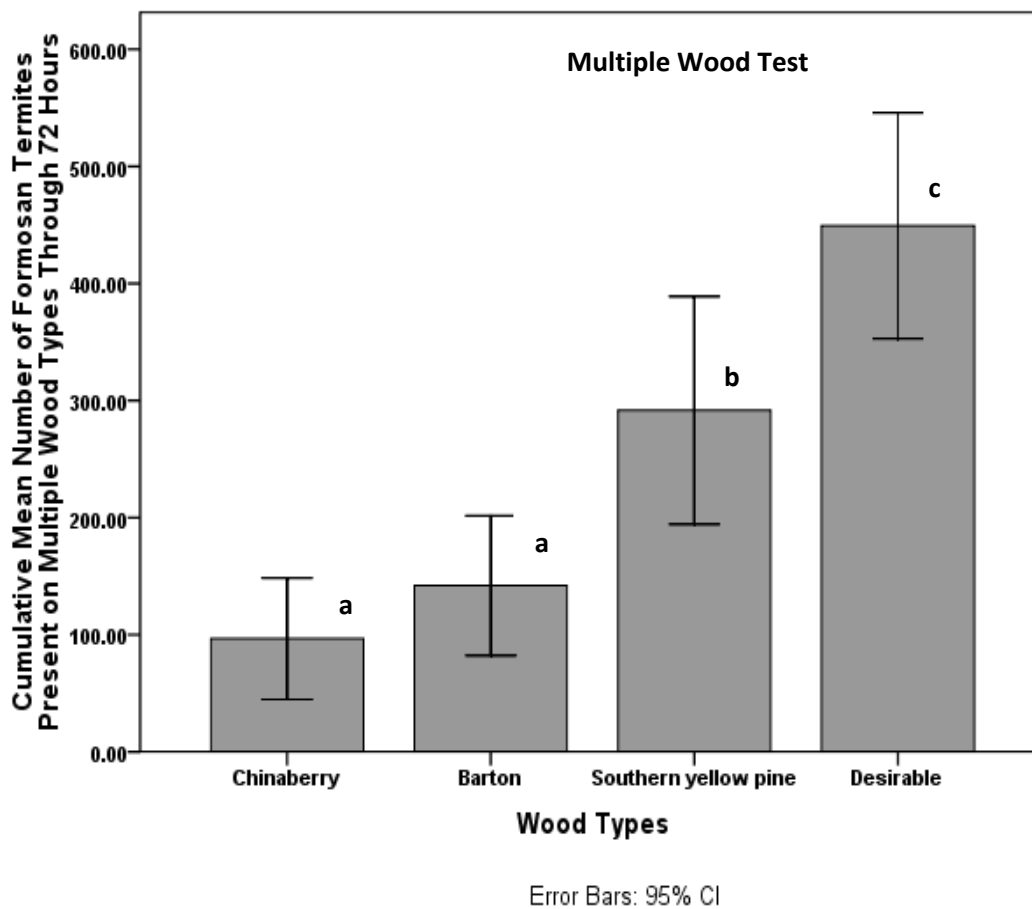
**Multiple-Choice Test.** Results from the ANOVA indicated that preferences for wood samples differed significantly ( $P < 0.05$ ) across the four woods tested ( $F = 25.482$ ;  $df = 3, 20$ ;  $P < 0.05$ ). The Desirable cultivar was the most consumed wood tested. Results of the Tukey post-hoc comparisons of the three wood species indicated that the mean number of *C. formosanus* present in the Desirable pecan cultivar arena was significantly ( $P < 0.05$ ) higher than the Barton cultivar, chinaberry and southern yellow pine. Results of the ANOVA of southern yellow pine indicated that the mean number of termites present in the southern yellow pine arena was significantly ( $P < 0.05$ ) different from both pecan cultivars and chinaberry. Southern yellow pine was ranked second in this study, based on the number *C. formosanus* termites present in the southern yellow pine arenas. Results of the ANOVA indicated that mean numbers of *C. formosanus* in chinaberry and the Baron cultivar arenas were not significantly ( $P < 0.05$ ) different from each other, but both were significantly ( $P < 0.05$ ) different from the Desirable cultivar and southern yellow pine. Termites in the untreated controls for this experiment did not experience mortality greater than 10% throughout the 3 d period. The mean number of termites present on each wood type through time is summarized in Table 3 and Figure 8.

**Table 3.** Mean number of termites present on multiple wood types (2 pecan cultivars, chinaberry and southern yellow pine) at 1, 4, 8, 24, 48 and 72 hrs.

Wood Type	1 hr	4 hrs	8 hrs	24 hrs	48 hrs	72 hrs
Desirable <sup>a1</sup>	38.58 <sup>a</sup> ±17.88	59.83 <sup>a</sup> ±24.18	74.50 <sup>a</sup> ±31.14	84.08 <sup>a</sup> ±35.11	91.67 <sup>a</sup> ±39.46	94.83 <sup>a</sup> ±75.85
Southern yellow pine <sup>b</sup>	23.17 <sup>b</sup> ±17.87	34.67 <sup>b</sup> ±23.50	47.33 <sup>b</sup> ±28.17	57.17 <sup>b</sup> ±28.96	55.33 <sup>b</sup> ±40.25	58.58 <sup>b</sup> ±39.37
Barton <sup>c1</sup>	15.58 <sup>c</sup> ±17.72	16.33 <sup>c</sup> ±17.37	20.08 <sup>c</sup> ±18.18	13.58 <sup>c</sup> ±11.43	14.16 <sup>c</sup> ±13.73	15.50 <sup>c</sup> ±4.61
Chinaberry <sup>c</sup>	11.42 <sup>c</sup> ±8.30	18.42 <sup>c</sup> ±18.64	20.08 <sup>c</sup> ±16.83	25.33 <sup>c</sup> ±18.60	24.33 <sup>c</sup> ±18.07	26.92 <sup>c</sup> ±14.61
<i>P</i> value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
<i>F</i> statistic	7.06	10.85	13.53	19.14	19.93	24.58
df	3,44	3,44	3,44	3,44	3,44	3,44

\*Means followed by the same letter are not significantly different using ANOVA, and Tukey HSD (Honestly Significant Difference) (SPSS v. 16.0).

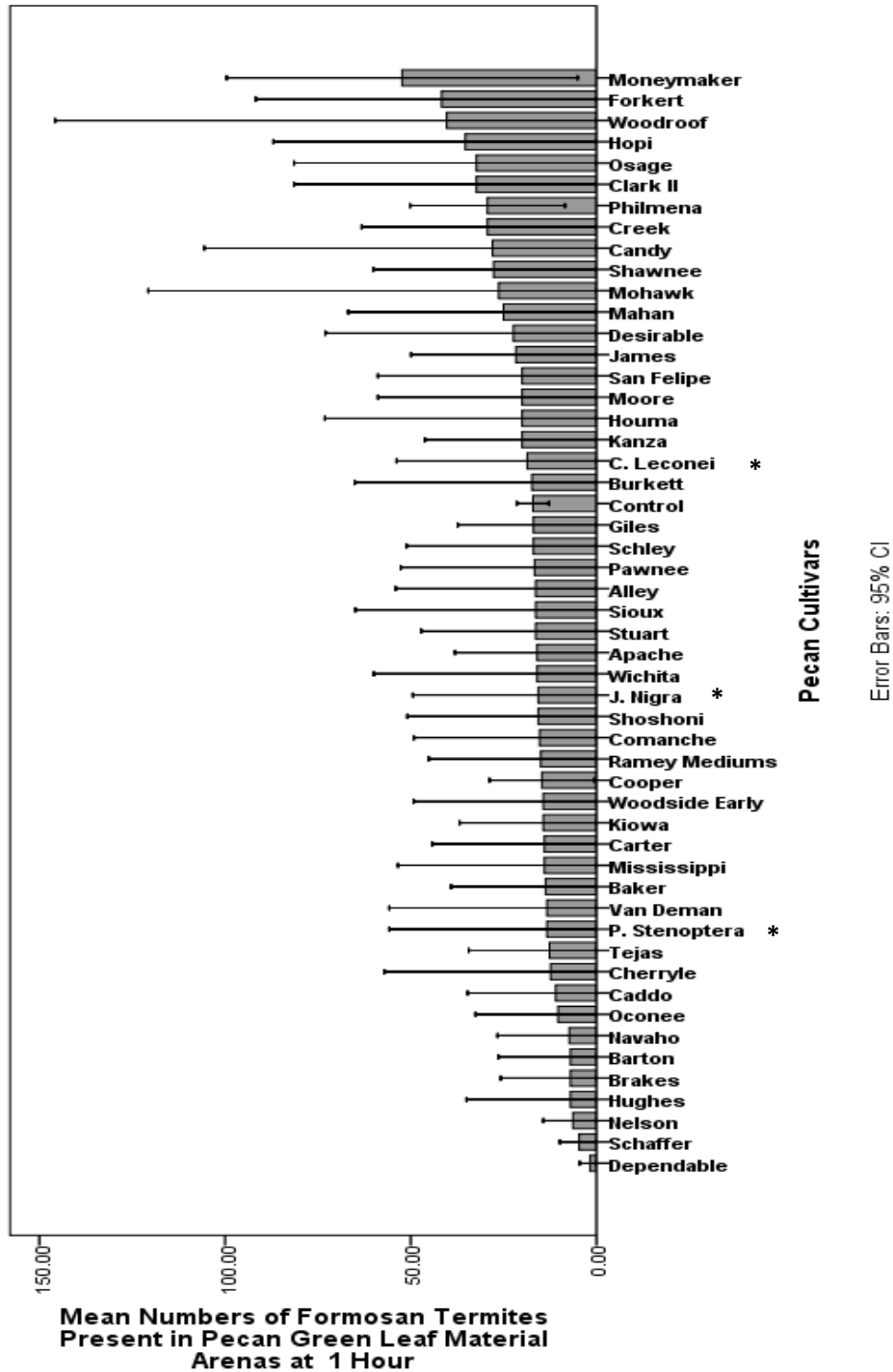
<sup>1</sup>Both Desirable and Barton were cultivars of pecan.



**Fig. 8.** Cumulative mean numbers of termites present on multiple wood types (two pecan cultivars, chinaberry and southern yellow pine) through 72 hrs. Means followed by the same letter are not significantly different using ANOVA, and Tukey HSD (Honestly Significant Difference) (SPSS v. 16.0)

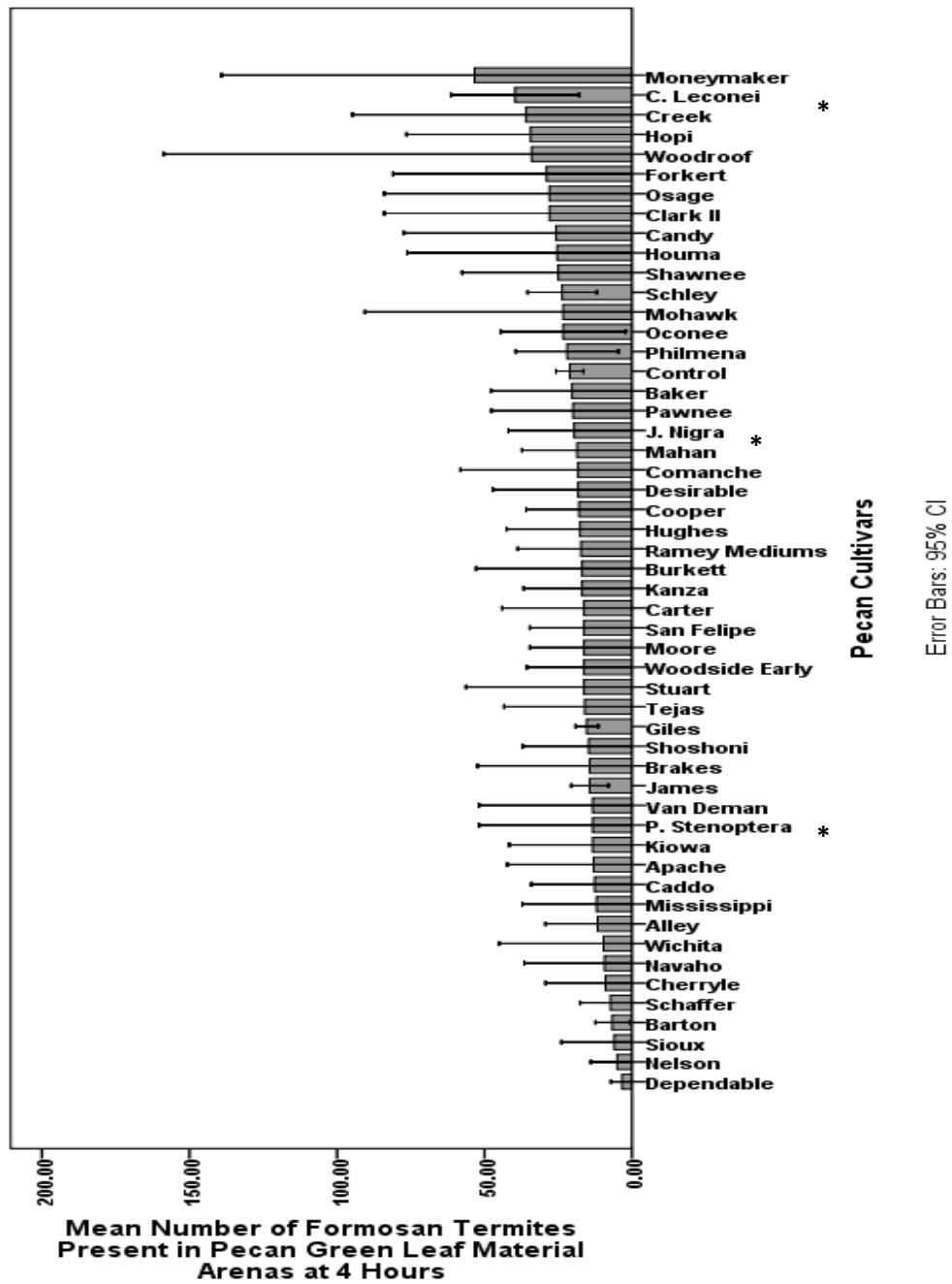
**Green Leaf Material Test.** *Coptotermes formosanus* exhibited a significantly different attraction ( $P < 0.05$ ) to green leaf material from the Creek pecan cultivar as compared to the other 50 cultivars. However, based on the results of the ANOVA with Tukey HSD, there was no significant difference in the attraction of *C. formosanus* to the remaining leaf samples as compared to the untreated controls through time (Figures 9-14 and Appendices A-Q). After a

period of 24 h, termites exhibited abnormal behaviors such as prolonged inactivity. Untreated controls had less than 10% mortality throughout the 24 h period.

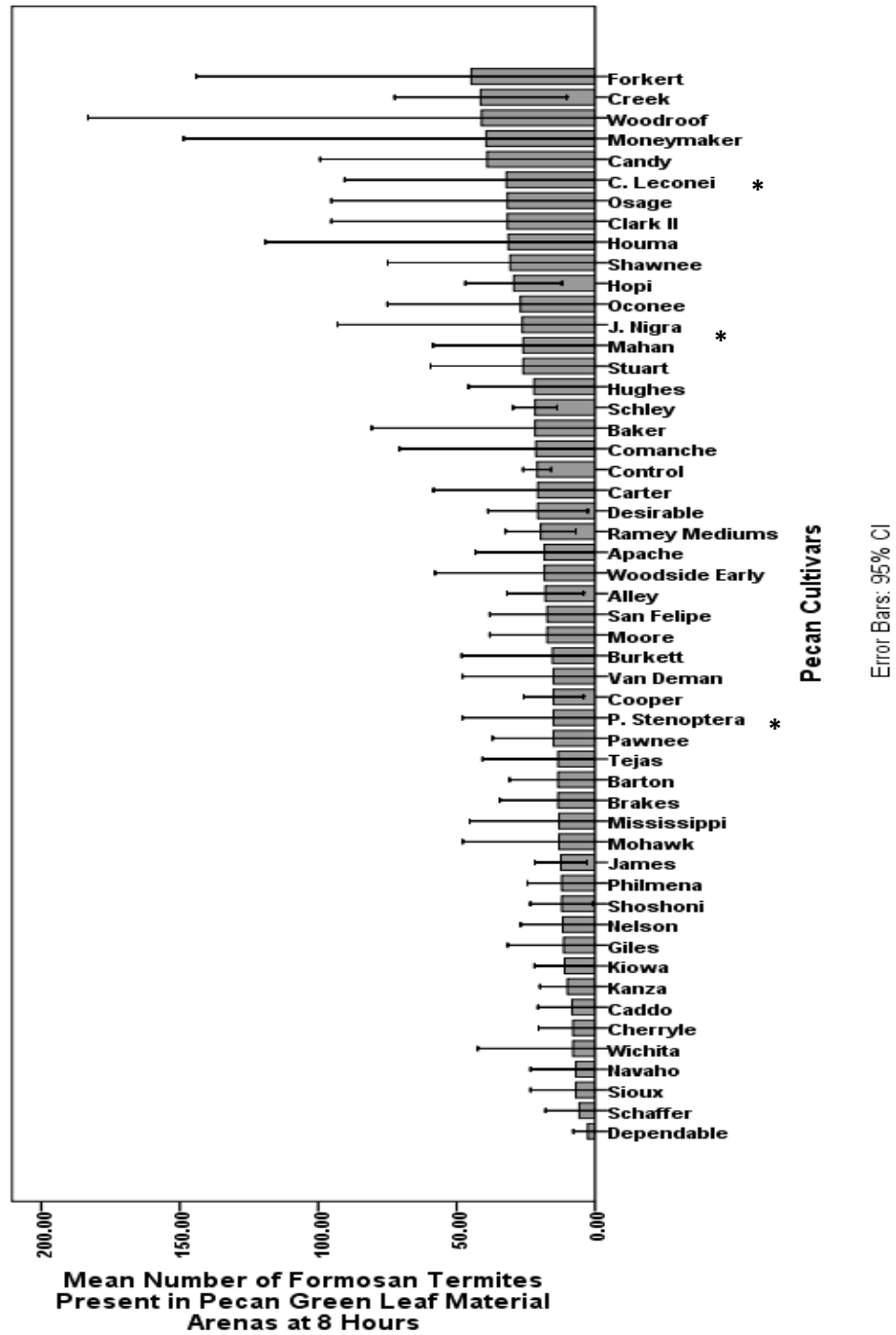


**Fig. 9.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 1 hr.

\*indicates a species not associated with illinoensis.



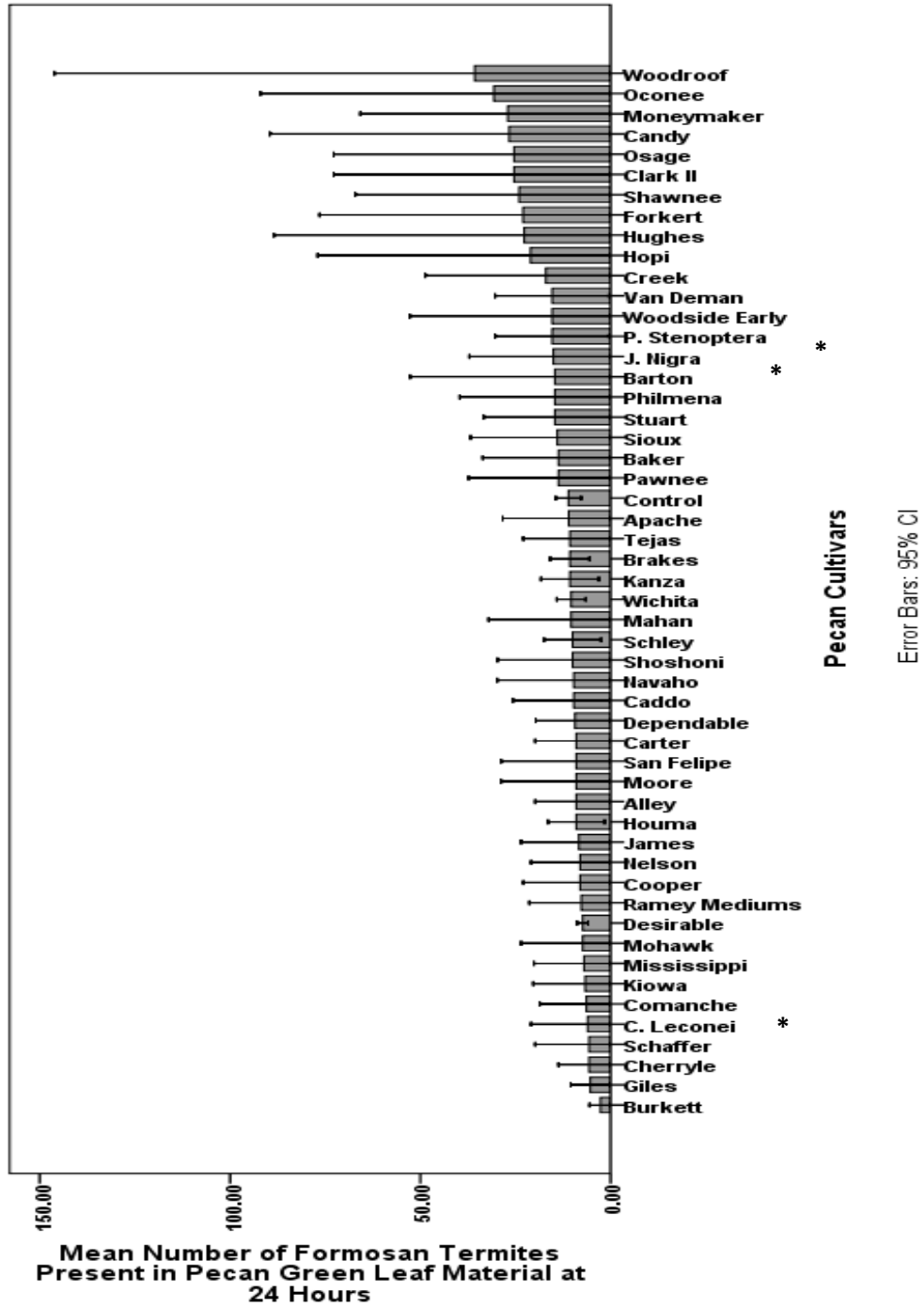
**Fig. 10.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 4 hrs.  
 \*indicates a species not associated with illinoensis



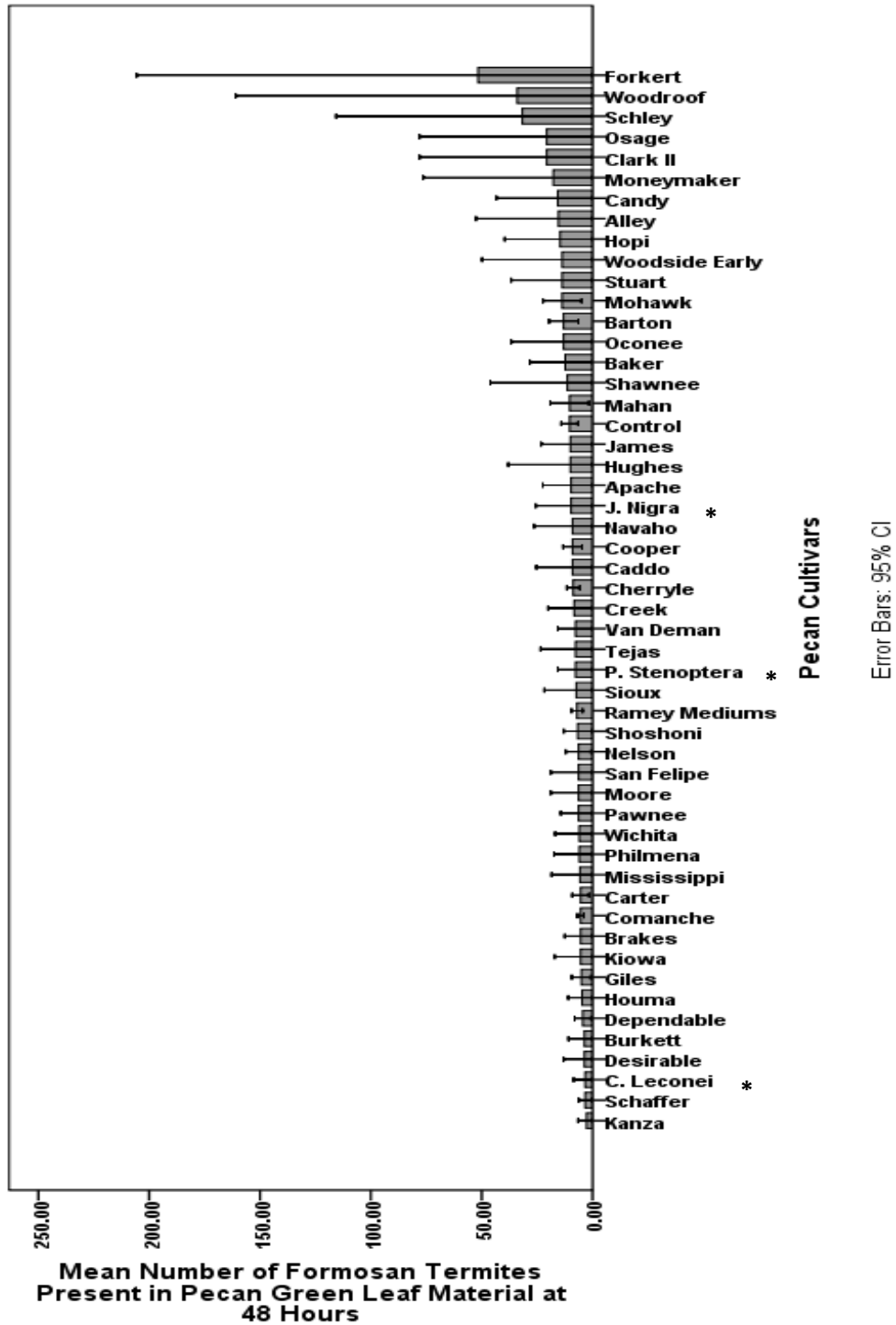
**Fig. 11.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 8 hrs.

\*indicates a species not associated with illinoensis.

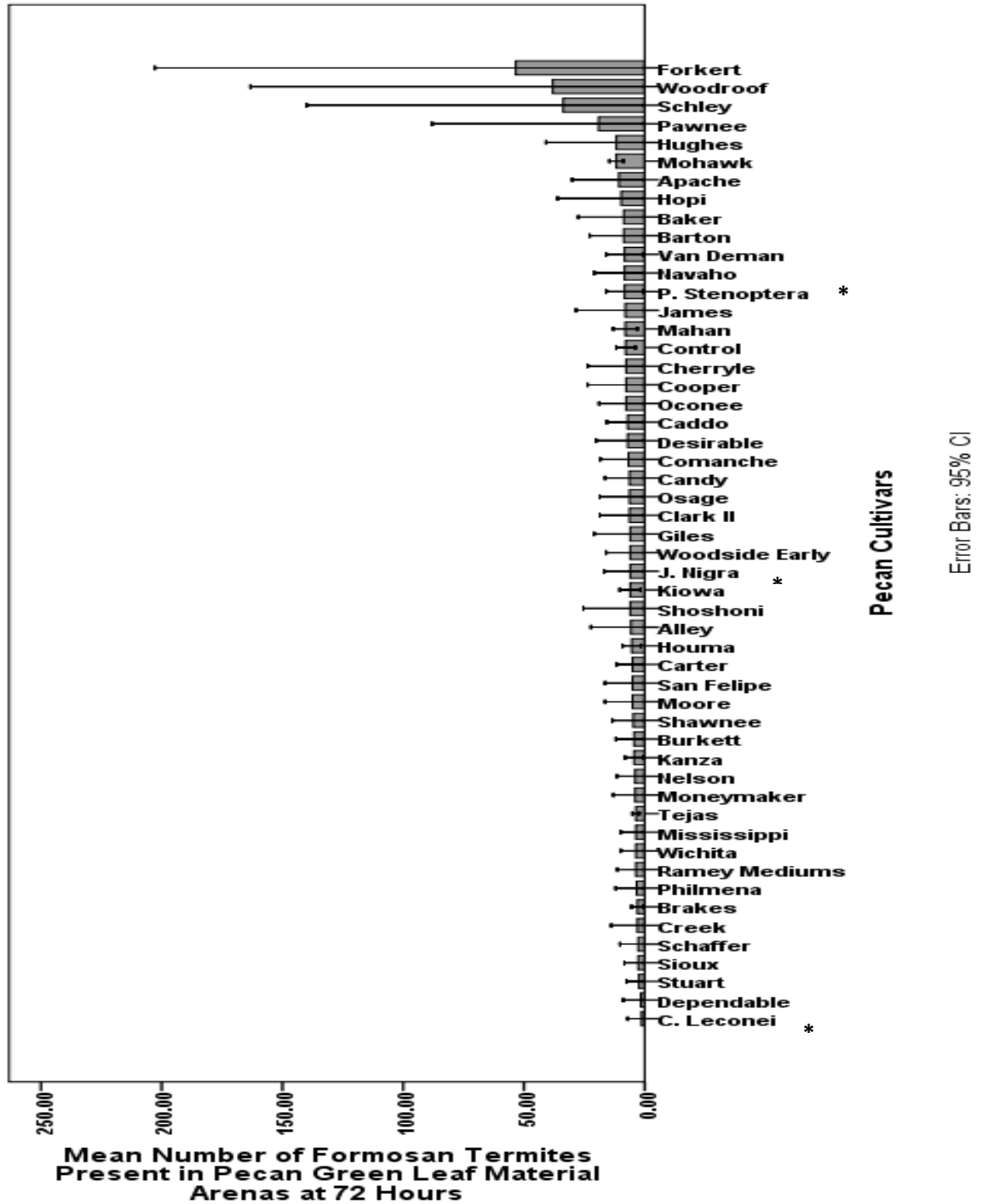




**Fig. 12.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 24 hrs.  
 \*indicates a species not associated with illinoensis.



**Fig. 13.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 48 hrs.  
 \*indicates a species not associated with illinoisensis.



**Fig. 14.** Mean numbers of Formosan termites present in pecan green leaf material arenas at 72 hrs.  
 \*indicates a species not associated with illinoensis.

## DISCUSSION

It was determined from the research that *C. formosanus* foragers, which attack trees from below ground, had preferences for specific pecan cultivars based on wood consumption by these subterranean termites. Of the 56 cultivars examined, some, such as Creek and Barton, had only 2.71 % as much cellulose removed by Formosan termites as did the commercial cultivar MoneyMaker. The concept of acquired or selected traits through plant breeding programs that are performed by the scientists at the USDA Pecan Breeding and Genetic Program, Southern Plains Agricultural Research Center, would pertain to those cultivars with resistance or tolerance to termite feeding. It is apparent that they have successfully bred for traits such as yield, diseases and resistance to many insect groups. However, to this point, little or no attention has been given to termite feeding in orchard stocks. Based on the results of this research, there are traits that result in less termite feeding.

It is highly probable that breeding for traits that are resistant to termite feeding could be incorporated into the commercial cultivars through time. More work would need to be done to identify the specific characteristics that would have commercial value in attempting to balance nut yields to survivability of selected cultivars. Because it takes considerable time to undergo the selection process, the results of this research would indicate that there are cultivars presently available that could be moved to production as replacement stock. It is inevitable that Formosan termites will continue to move into pecan production areas, where the trees will be found, fed upon and eventually killed. In addition to loss of trees by Formosan termites feeding in commercial areas, pecans are a tree of choice in urban landscapes because of esthetics and nut production.

In these urban situations, when the pecan trees are attacked by Formosan termites, the aesthetic value is reduced and the risk of these trees falling on structures is increased,

particularly during high wind situations (Edwards and Mill 1986, Su and Tamashiro 1987, Su and Scheffrahn 1990). I have observed that Formosan termites often move from infested trees into structures increasing the amount of property damage. Therefore if a less preferred cultivar is used, even in urban landscapes, a portion of these risks can be reduced.

In multiple-choice test conducted with *C. formosanus* foragers, there were preferences shown among four wood types including two pecan cultivars (Desirable and Barton), southern yellow pine and chinaberry. The results showed that there was a significant ( $P < 0.05$ ) preference for the Desirable pecan cultivar as compared to the Barton pecan cultivar. This confirms the finding of the no-choice test and further demonstrates that Formosan termites do discriminate between pecan cultivars. Southern yellow pine was the second most preferred wood in this study. This is important because southern yellow pine is a commercial wood used in building construction throughout the United States, thus, making many structures vulnerable to Formosan termite feeding. Chinaberry was the third most fed upon wood type in this study. Chinaberry is often used in urban landscapes. While not preferred as compared to pecan, it was still consumed by Formosan termites. This offers Formosan termites a food source that is usually in close proximity to structures.

The influence of green leaf materials was evaluated using *C. formosanus* foragers on 48 pecan cultivars. There was a continuum on the level of response from non-attraction to attraction. The Creek pecan cultivar was 17 times more preferred by foragers of *C. formosanus* as compared to Dependable at 8 hrs. Apparently there is a genetic difference between the most preferred and the least preferred in terms of attraction by foragers. Moneymaker, the most fed upon in the consumption test, was also the most attractive at 4 hrs and the third most attractive at 8 hrs in this test. This correlates with the wood wafer consumption test where Moneymaker has the highest consumption rate; however, Creek was the least preferred cultivar in the wood wafer

consumption test and the most attractive in the green leaf material test. Further research is needed to understand this association.

This is the first research involving *C. formosanus* consumption of different wood types in relation to green leaf material attraction. Future work with Formosan alates, as compared to foragers, is needed to gain a more refined understanding of host-plant selection by *C. formosanus*, which is presumably an olfaction based response. This work would be seasonal because alates of *C. formosanus* usually begin to swarm in May.

This research reveals the potential impact Formosan termites might have on the pecan industry. For the commercial pecan grower, this may offer some additional knowledge when inspecting pecan cultivars for the presence of certain insect pest, but more research is needed before specific cultivar selection is considered. The consumption results may be dramatically different if *C. formosanus* is given a larger option of food sources. Human modified environmental factors (fertilizers and pesticides) at the USDA Pecan Breeding and Genetics Program, Southern Plains Agricultural Research Center, 10200 FM 50, Somerville, TX should also be considered because they could alter the feeding behavior of *C. formosanus*.

## CONCLUSIONS

The first objective of this research was to determine if there were feeding preferences of *C. formosanus* to pecan cultivars in the presence of other wood species. It was determined that *C. formosanus* have a preference to specific pecan cultivars. Formosan termites did not feed on all pecan cultivars in equal amounts. Feeding on some of the less preferred cultivars such as Barton and Creek caused high mortality in *C. formosanus* therefore; I reject the null hypothesis that *C. formosanus* would show no preference to different pecan cultivars, and accepted the alternative hypothesis that preferences were indicated. Based on these findings, I therefore; fail to reject the alternative hypothesis that *C. formosanus* will show preference to different pecan cultivars.

The second objective of this research was to determine if pecan was a preferred food choice when compared to other wood species. It was determined that a pecan cultivar was the preferred food source in a multiple-food choice test with Southern yellow pine, Chinaberry and Barton (another pecan cultivar). I reject the null hypothesis that Formosan termites would show no preferences to pecan in a multiple-choice study. I therefore fail to reject the alternative hypothesis that pecan would be preferred over Chinaberry and Southern yellow pine.

The third objective of this research was to determine if *C. formosanus* was attracted to green leaf material of pecan cultivars. It was determined that there was an apparent pecan green leaf material attraction exhibited by *C. formosanus* to the cultivar Creek. Therefore, I reject the null hypothesis that Formosan termites would show no preference to pecan green leaf material. However, Creek was the least preferred cultivar in the consumption experiment and caused high mortality when feed on by *C. formosanus*. More research is needed to better understand these results.

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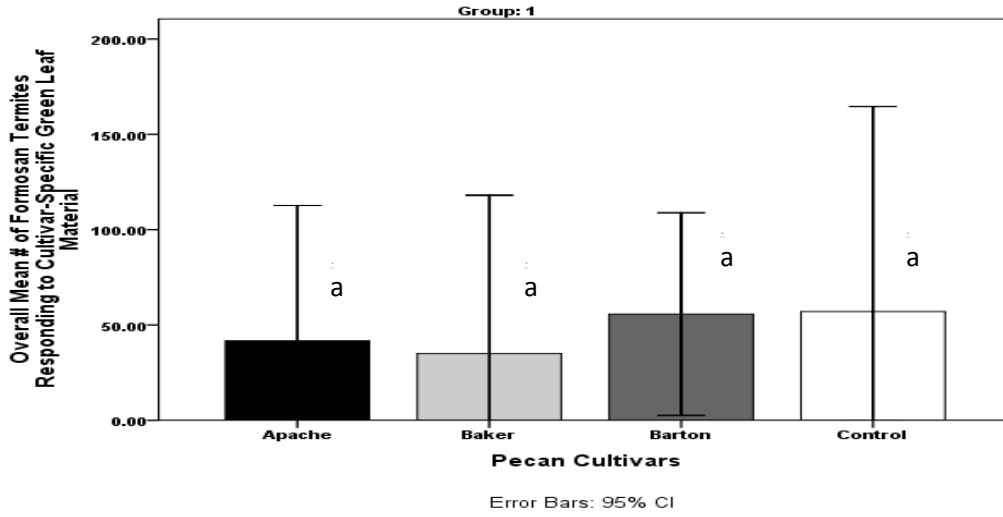
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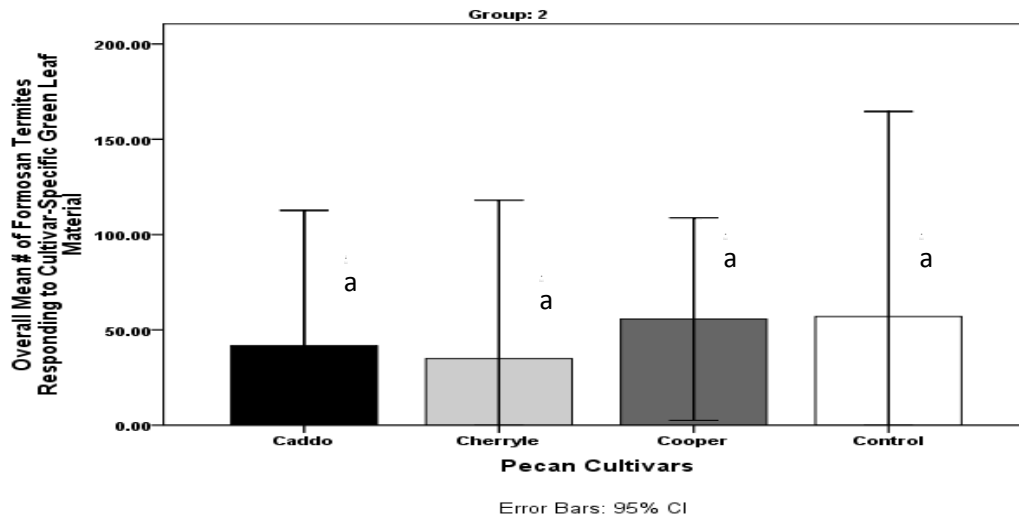
### APPENDIX A

Experiment 2. Green Leaf Material Test.



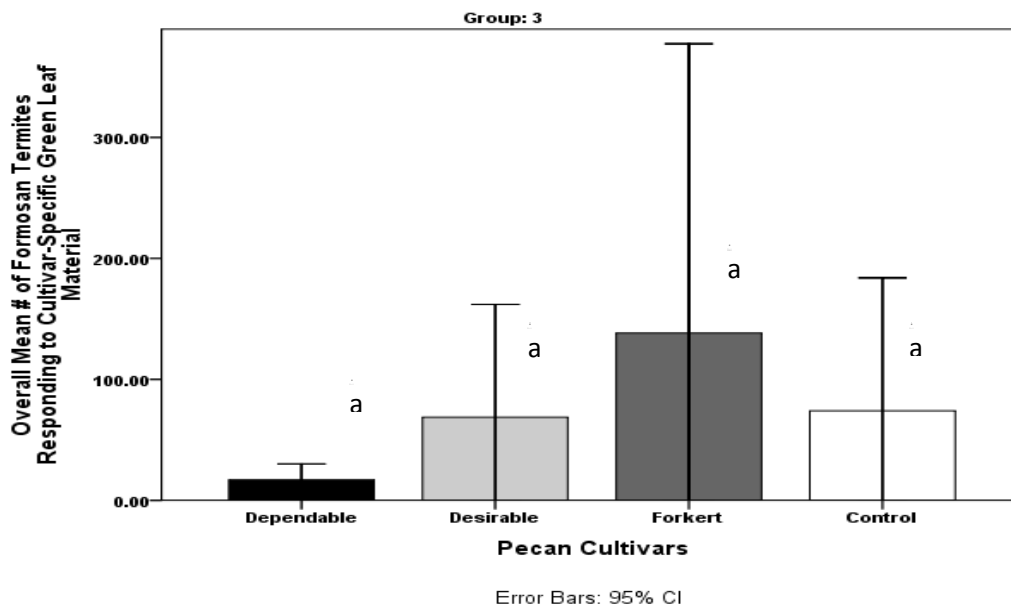
**APPENDIX B**

## Experiment 2. Green Leaf Material Test



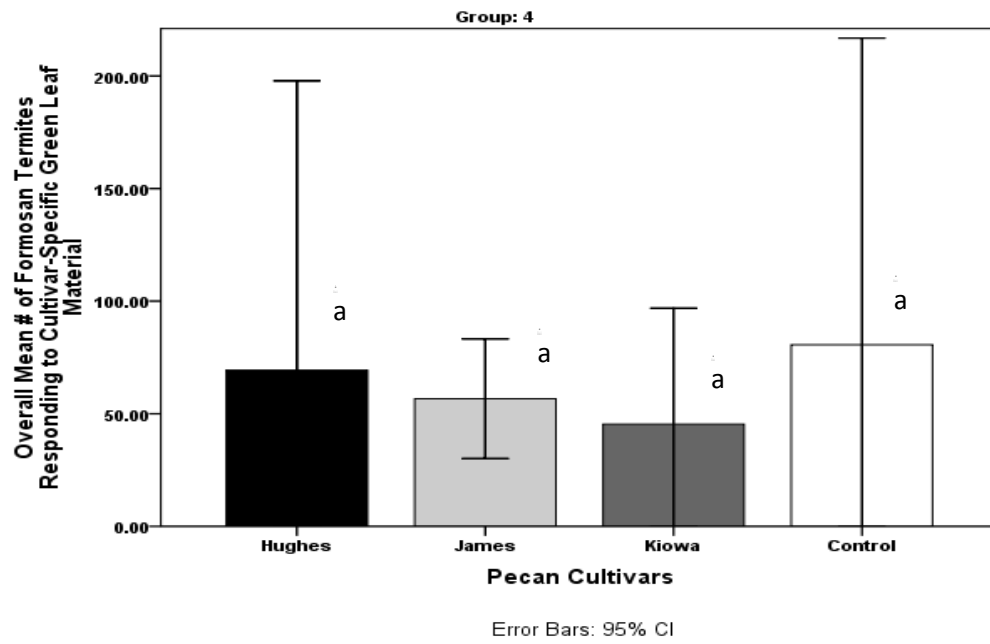
**APPENDIX C**

## Experiment 2. Green Leaf Material Test



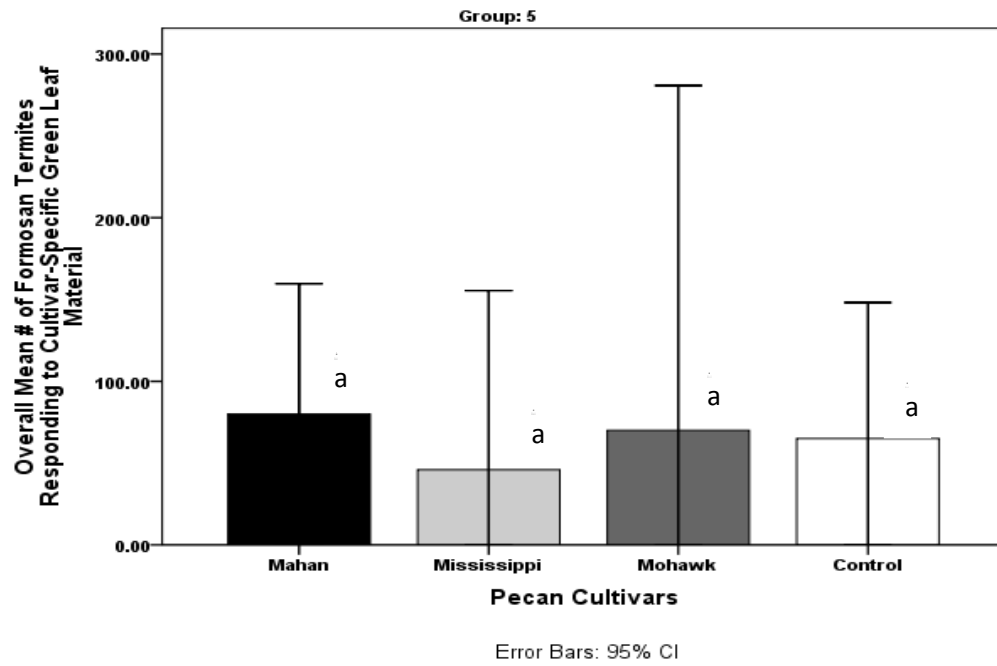
**APPENDIX D**

## Experiment 2. Green Leaf Material Test



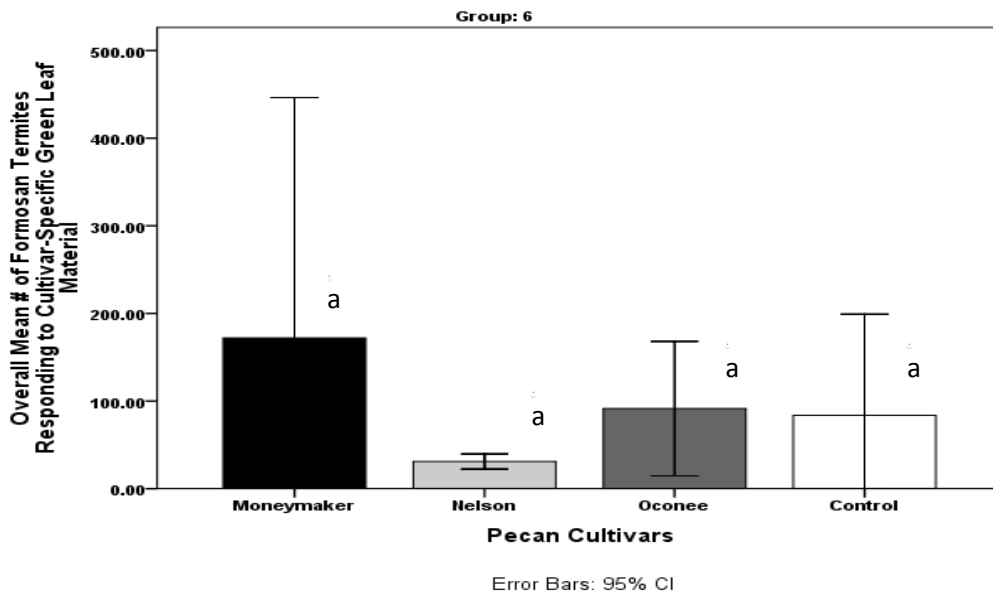
**APPENDIX E**

## Experiment 2. Green Leaf Material Test



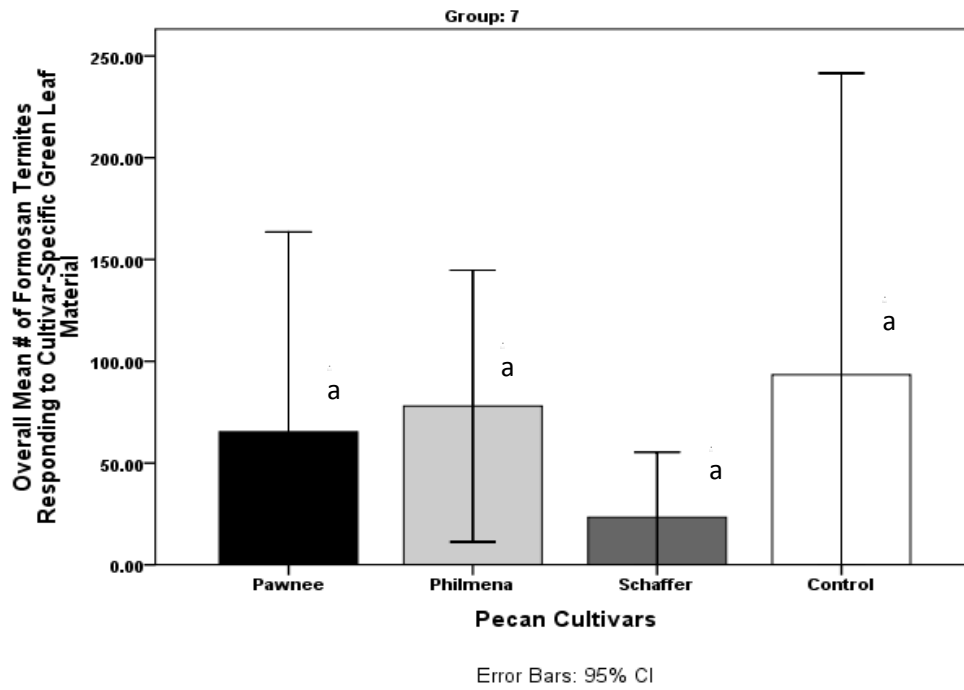
**APPENDIX F**

## Experiment 2. Green Leaf Material Test



## APPENDIX G

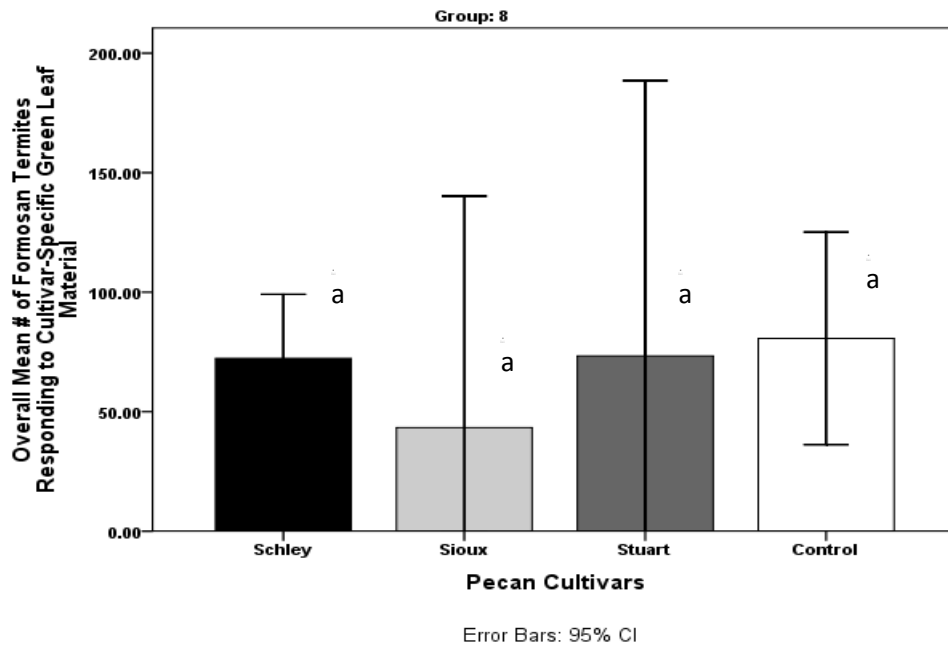
## Experiment 2. Green Leaf Material Test





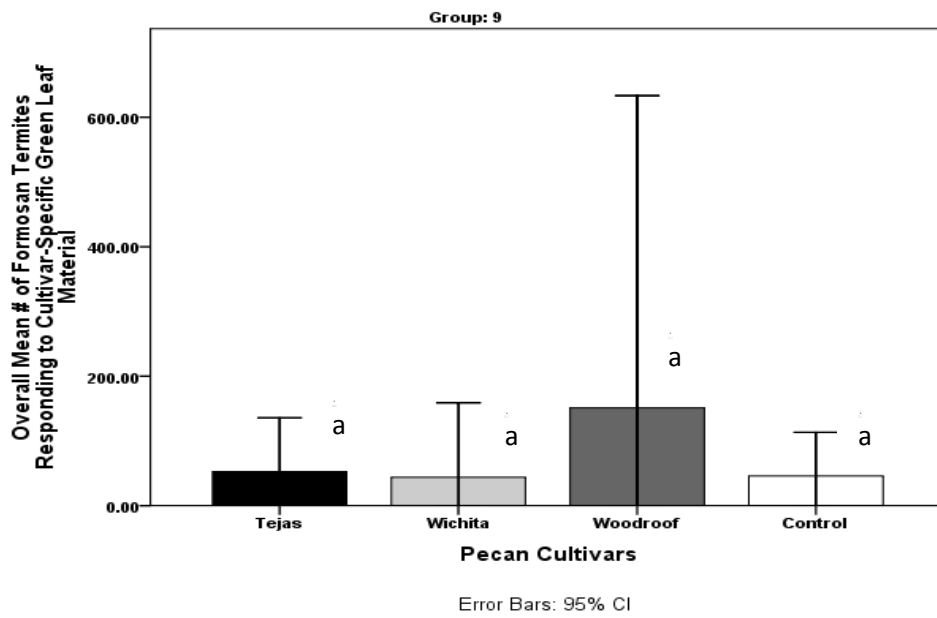
**APPENDIX H**

## Experiment 2. Green Leaf Material Test



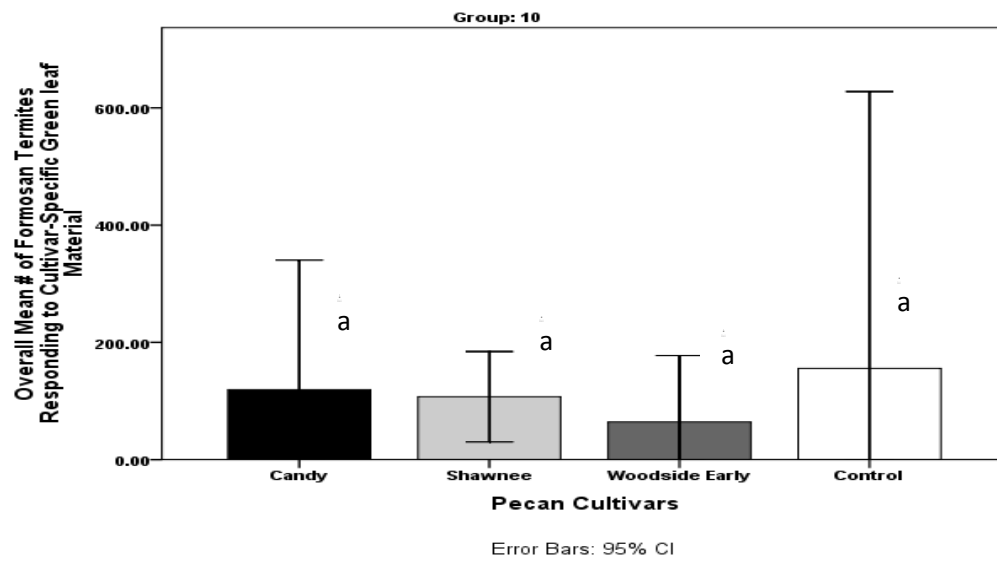
**APPENDIX I**

## Experiment 2. Green Leaf Material Test



**APPENDIX J**

## Experiment 2. Green Leaf Material Test



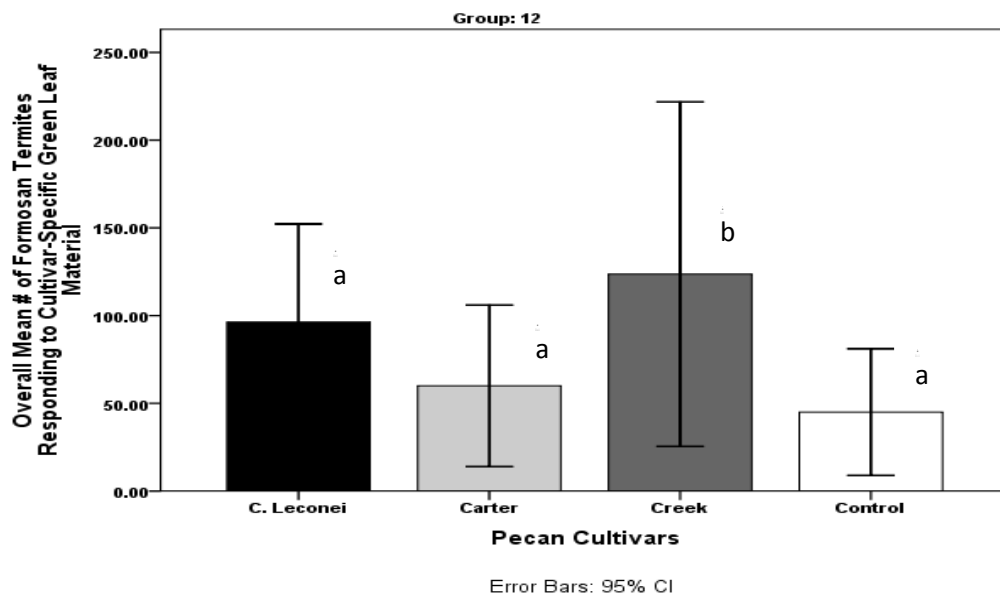
**APPENDIX K**

## Experiment 2. Green Leaf Material Test



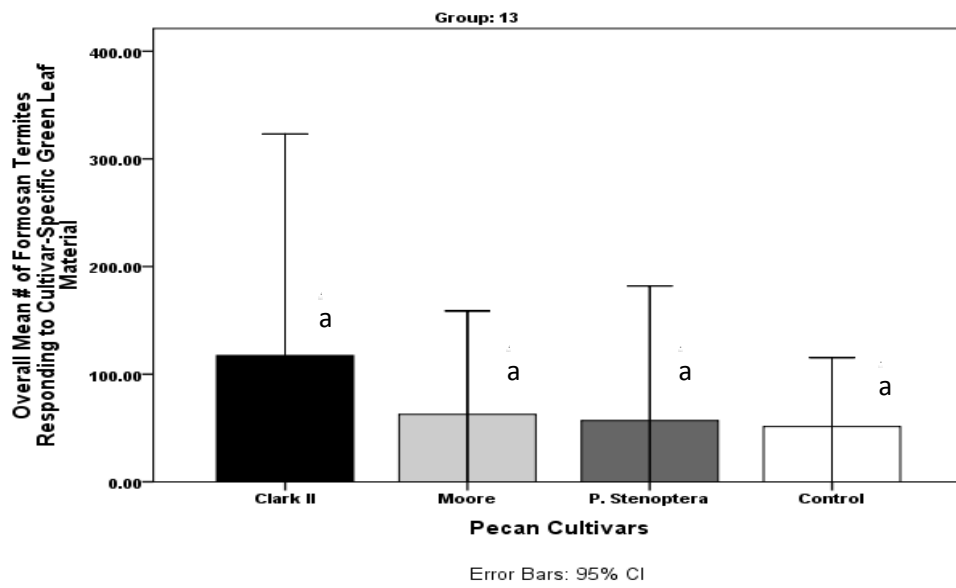
**APPENDIX L**

## Experiment 2. Green Leaf Material Test



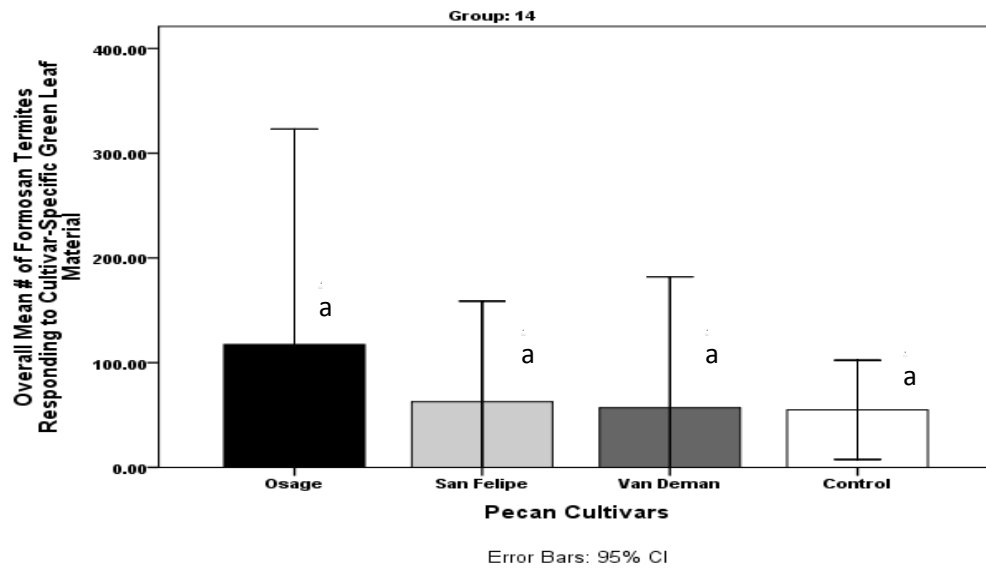
**APPENDIX M**

## Experiment 2. Green Leaf Material Test



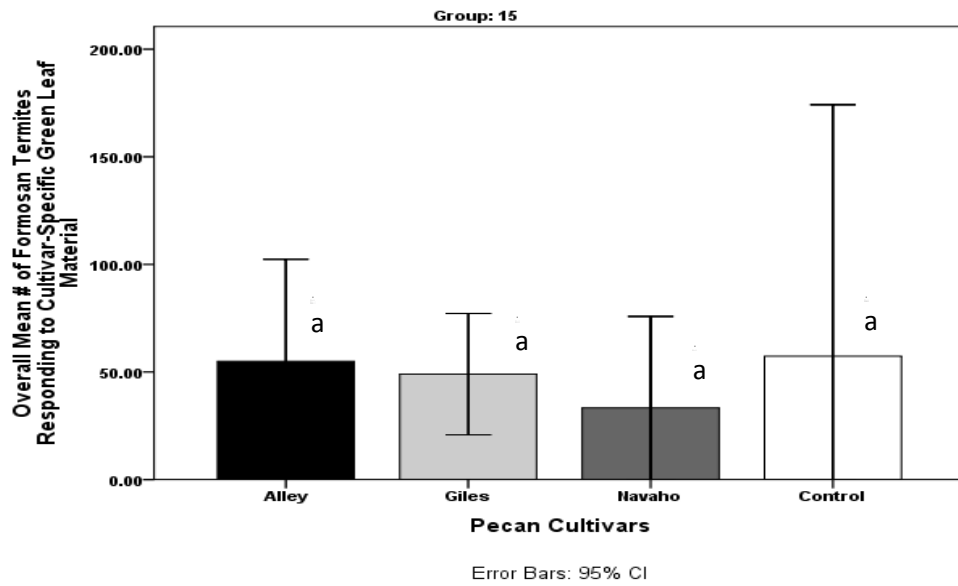
**APPENDIX N**

## Experiment 2. Green Leaf Material Test



**APPENDIX O**

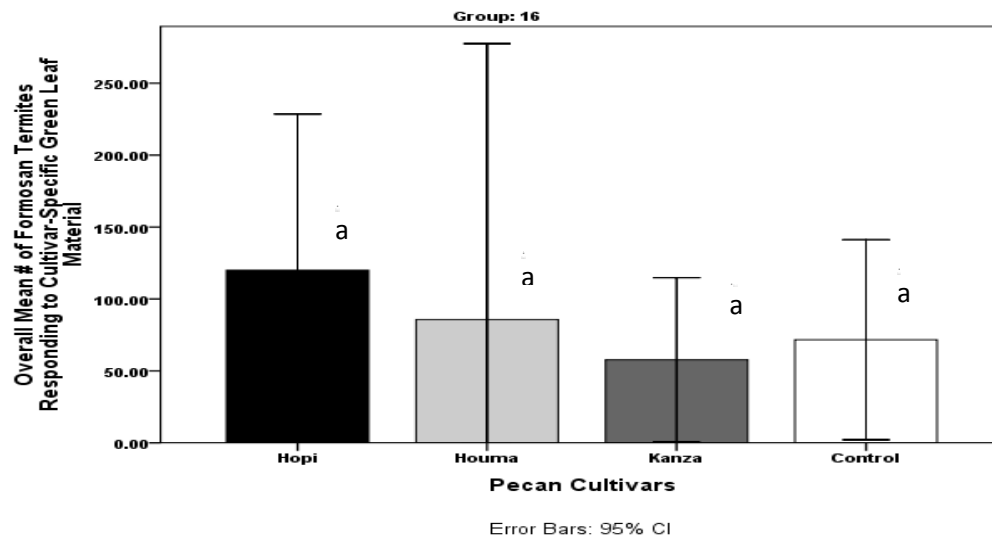
## Experiment 2. Green Leaf Material Test





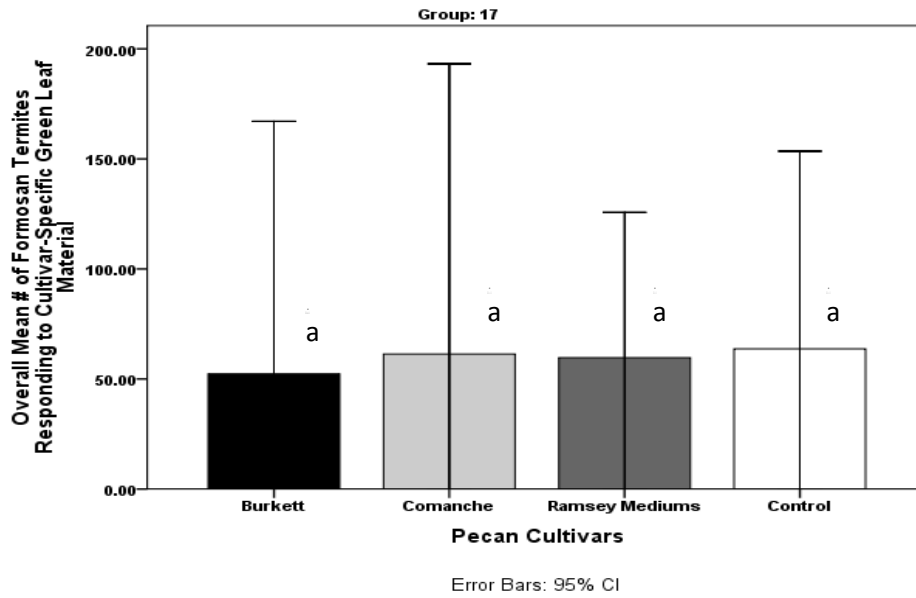
**APPENDIX P**

## Experiment 2. Green Leaf Material Test



**APPENDIX Q**

## Experiment 2. Green Leaf Material Test



**VITA**

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Chris Swain was born in Brenham, Texas in 1972. Chris Swain is a veteran of the United States Navy. He worked as a roughneck, truck driver, certified applicator and store manager while pursuing a bachelor's degree. He began taking classes at Blinn College in 1997. Chris received his bachelor's degree in entomology from Texas A&M University in 2007. He is the owner/operator of Premier Pest Control in College Station Texas and works full-time for the Center for Urban Entomology as a Research Assistant. His responsibilities include maintaining insect populations, assisting and performing bioassays which determine the efficacy and effectiveness of certain insecticides and baits.

He is married to Shavon Swain and they are the parents of Chelsea, Courtney, CJ, Jalen and Jordan. He and his wife are both Aggies and graduate students at Texas A&M University.