**Performance of OSB Made with Soy Substituted Resin in Termite Choice Tests with** **Southern Yellow Pine**

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**Abstract**

Soy flour was evaluated as a partial substitute for resin in the manufacture of oriented strand board (OSB), a wood-based composite that often replaces solid lumber and plywood in structural applications in the construction industry. Since the presence of soy could alter OSB biodegradation properties, termite resistance of OSB panels made with 0, 10, and 20% of polymeric methylene diphenyl diisocyanate (pMDI) resin substituted with soy flour (OSB0, OSB10, and OSB20, respectively) was investigated. Single choice tests between three types of OSB and southern yellow pine (SYP) solid wood and an OSB choice test (OSB0 versus OSB10) were evaluated. Results indicated that termites always showed a preference for SYP, with the OSB becoming less palatable when soy flour was present. Percent weight losses for OSB0, OSB10, and OSB20 were 5.7x, 8.4x, and 8.6x less, respectively, compared to SYP. In the absence of SYP, termites did not differentiate OSB0 from OSB10, with OSB10 showing 1.5x less weight loss compared to OSB0. Visual rating data supported weight loss data except significantly less damage was only found when the choice paired SYP with OSB made with soy (OSB10 or OSB20). Termite consumption preference for SYP was explained by differences in water absorption kinetics. SYP reached saturation (105% moisture content) within 1 week on moist sand while moisture content of OSB composites slowly climbed to 79% over 4 weeks, never reaching a plateau. Lower moisture content was due to the presence of water-repellent resin and wax in the OSB.

**Keywords:** OSB, Performance, Termite, Southern yellow pine, Soy flour, pMDI resin

Wood is the most widely used building material with low embodied energy, low carbon impact, and sustainability characteristics. A lot of efficient, durable, and useful wood products produced from trees include members of a large family ranging from lumber to structural and nonstructural engineered wood-based composites, which encompass a range of products, from fiberboard, particleboard (PB), oriented strand board (OSB), plywood to laminated beams. As a major engineered structural-use composite, OSB is manufactured from thin wood strands bonded together with water-resistant resin and other additives, including wax. OSB is extensively used in both commercial and residential constructions, such as wall sheathing, floor underlayment, roof cover and I-joist (Falk 2010, Stark et al. 2010). Both solid wood and OSB used in buildings could be attacked by insects, for example termites, especially in high humidity and temperature areas.

Subterranean termites (Rhinotermitidae), which depend on cellulose as their main source of nutrition, are globally the most economically important structural insect pests of wood (Rust and Su 2012). Wooden structures damaged by subterranean termites are found in most of the United States (Peterson et al. 2006) but especially in the warm, humid southeastern states where the climate favors their proliferation and development (Clausen 2010). Wood-based composites, such as OSB, PB, plywood and cross laminated timber (CLT), are major replacements of solid wood in non-structural and structural applications in the construction industry. Like wood, these composites are prone to biodegradation when they are used in construction, especially in outdoor conditions and in contact with the ground (Laks 2002, Gardner et al. 2003, Kirkpatrick and Barnes 2005). Studies of cross laminated timber (CLT) have shown that it is susceptible to subterranean termite attack and would be at risk especially in the southeastern United States where subterranean termites flourish (França et al. 2018) or along the warm coastal areas where drywood termites occur. The feasibility of making CLT from dimensional lumber pressure treated with MCA-C, a copper-based wood preservative for unprotected, ground contact conditions, though, has been demonstrated with best results when the adhesive was a single component polyurethane (Lim et al. 2020).

Many factors can influence and be used to protect wood composites to ensure a long service life in interior dry/wet or exterior above ground and protected conditions. For example, wood species in untreated PB can be manipulated to increase resistance to termites, and PB with resins had better resistance than binderless PB in field tests (Suhasman et al. 2012). Untreated OSB showed lower resistance than MDF and PB samples for termite attack under field test conditions, but chemical treatment with alkaline copper quat or copper azole significantly improved the durability of the wood-based composites including OSB, MDF and PB (Kartal and Green III 2003, Tascioglu et al. 2018). Both zinc and calcium borate used in OSB also provided improved protection against termite attack. As the borate loading increased, percent sample weight loss decreased, and termite death rate increased, but termite activity was not completely eliminated (Sean et al. 1999, Wu et al. 2002, Lee et al. 2004).

As a natural and environmentally friendly material, soy flour has been used in resin formulations (Li et al. 2004, Frihart et al. 2014, Vnučec et al. 2017). It was applied as an additive in phenol formaldehyde to make wood composites (Hand et al. 2017, Hand et al. 2018). Using soy flour to partially substitute polymeric methylene diphenyl diisocyanate (pMDI) resin by 10 to 20% for the manufacture of OSB has also been found to improve mechanical and physical board properties while decreasing cost, because soy flour is about three times cheaper than pMDI (Cheng et al. 2019, Asafu-Adjaye et al. 2020). The presence of soy flour at these concentrations, however, may have changed the biodegradation properties of the wood flakes in the OSB. Therefore, the goal of this investigation was to study termite resistance of OSB panels made with 0, 10, and 20% substitution of pMDI resin with soy flour and determine if termites were able to discriminate between OSB and SYP or between OSB with and without soy flour.

**Materials and Methods**

**Materials**

SYP wood strands with moisture content about 7-8% were donated by Norbord (Lanett, AL). Emulsion wax was provided by Louisiana-Pacific Corporation (Hanceville, AL). Soy flour (Defatted, 7B) was provided by Archer Daniels Midland (Chicago, IL). The pMDI adhesive (polymeric methylene diphenyl diisocyanate, MONDUR 541) was donated by Huber Corporation (Commerce, GA). SYP solid wood blocks with dimensions 25.4 x 25.4 x 11 mm (radial x tangential x longitudinal) were cut from one defect-free board with 4-6 rings per inch per AWPA E1 standard (AWPA 2020).

**OSB preparation**

The mixtures of pMDI and soy flour (0, 10 or 20% of pMDI resin substitution for OSB0, OSB10, and OSB20, respectively) were prepared by adding the corresponding amounts of soy flour into pMDI in a glass beaker and then stirring two minutes using a hand immersion blender (Sensio BLA17194). In an electric cement mixer (Kobalt 4-cu ft 0.5-HP), the emulsion wax was first sprayed on wood strands at 1% loading. The pMDI or mixtures of pMDI and soy flour were then applied to achieve a 4% resin loading and was sprayed on the furnish with a paint sprayer powered by an air compressor. Mats were formed manually in a 430 x 430 mm wood frame with three layers (25 wt% for top and bottom layers and 50 wt% for the core) and all the three layers were randomly formed without orientation. Then the mats were hot pressed for 3 minutes at 213 °C and 2 MPa. The nominal thickness of the board was 11 mm with target density of 650 kg/m3 (41 lb/ft3).

**Termite collection (*Reticulitermes virginicus*)**

First, a termite infested felled tree from the Sam D Hamilton Noxubee National Wildlife Refuge in Brooksville, MS was located. Then the downed tree was sawn into sections, so that it could be placed into multiple galvanized metal cans (32 gal) and brought back to the laboratory. Finally, the termites were maintained at room temperature until they were separated from the wood and used in the choice tests.

Based on morphometric measurements of the soldiers (n = 5), the termite collection was identified as *Reticulitermes virginicus* (Banks). Table 1 shows the characteristics of the collected soldiers as compared to those published for *Reticulitermes flavipes, R. virginicus,* and *Reticulitermes hageni* (Scheffrahn and Su 1994, Hostettler et al. 1995). In addition, genetic confirmation of the species identification was obtained by amplifying the AT-rich region of mt DNA using target specific primers (Foster et al. 2004). Sequencing 4 clones from the pooled DNA of 5 workers showed highest percent sequence similarity of all 4 clones with *R. virginicus* (accession no. AY351615.1) in a standard nucleotide blast (Altschul et al. 1990) against the National Center for Biotechnology Information nr/nt database (NCBI Resource Coordinators 2018).

**Sample preparation and moisture content measurement**

All OSB samples blocks were cut to 25.4 x 25.4 mm with thickness of 11 mm. OSB and SYP blocks were then conditioned in an environmental chamber at 22.5 ± 0.5 ºC and 55 ± 0.5% relative humidity (RH) for more than two weeks or until equilibration. The equilibrium moisture content (EMC) of the conditioned SYP and OSB samples were determined by individually weighing a random selection of 10 blocks per OSB type and 10 SYP blocks. These 40 samples were then oven dried at 105 ± 0.5 ºC and weighed again. The EMC was calculated in accordance with Equation 1. The final EMC was the average of the 10 samples.

(1)

To monitor moisture content (MC) changes of the SYP and OSB samples during the 4-week termite exposure, 5 blocks per OSB treatment and 5 SYP blocks were randomly chosen from the conditioned blocks. These 20 samples were divided into 5 groups of 4 blocks (one block from each sample type) and each group was placed into separate covered containers. Each container held 150 g sand and 30 mL sterile deionized water and all containers were placed in an environmental chamber at 22.5 ± 0.5 ºC and 55 ± 0.5 % RH. There were 5 time points (1, 7, 14, 21, and 28 days) and at each time point samples were removed from containers, cleaned of sand, weighed, then returned to containers. After weights were taken for the last time point, samples were oven dried at 105 ± 0.5 ºC, and then re-weighed. The MC was calculated in accordance with Equation 2. The final MC was the average of the 5 samples for each time point and sample type.

(2)

**Termite exposure**

*Experimental design*

The evaluation of termite resistance followed the general guidelines of the AWPA E1 single choice test (AWPA 2020), where termites were presented with two different samples per bioassay container. The choices were SYP versus soy substituted resin OSB or OSB0 versus OSB10. The reason for including SYP in the single choice test was to simulate the choice that termites would face in a typical residence where OSB is nailed to softwood framing lumber (spruce, pine, fir) as sheathing for walls, roofing, or as subflooring. The reason for the OSB0 versus OSB10 choice test was to determine if there were any effects of the soy in the resin on termite behavior. The experimental design and sample numbers are summarized in Table 2. Samples were randomly chosen from the unused conditioned blocks and conditioned weights were recorded. A total of 17 containers were set up for the OSB choice requiring 17 OSB0 and 17 OSB10 samples and 7 containers each for the SYP versus OSB choice test, requiring 21 SYP samples, and 7 OSB0, 7 OSB10, and 7 OSB20 samples. The 3 SYP vs OSB choice test identifiers were T1 = SYP paired with OSB0, T2 = SYP paired with OSB10, and T3 = SYP paired with OSB20. No termite controls were also run (1 sample block per container) and included 5 samples per OSB type and 5 SYP samples. All tests were set up on the same day.

*Container preparation*

Test containers for the termite and no termite controls each contained 150 g of heat sterilized play sand (Lowe’s Home Improvement, Starkville, MS) with 27 mL deionized water (18% MC for the sand) in a Microlite 16 oz polypropylene food grade container with lid (Anchor Packaging, Ballwin, MO) and foil cover (Figure 1). All containers and samples were sterilized by autoclaving. Placement of the samples onto the moist sand in containers was done in a biological safety cabinet using aseptic techniques. Termites were broken free of the log pieces, sieved from the coarse debris, then allowed to crawl onto slightly moistened paper towels to free them from small debris. They were then transferred to clean trays by flicking them from the paper towels. Once on the trays, they were gently aspirated and counted so that 400 workers could be used for each test container. Termites were added directly in the center of each container between the two sample blocks to avoid any initial bias (Figure 1). All containers were placed in one dark environmental chamber for 4 weeks at 25 ± 1 ºC and 70 ± 5% RH (Figure 1). A HOBO Pro v2 data logger (Onset, Pocasset, MA) was placed in the chamber to monitor temperature and relative humidity.

After 4 weeks, each container was disassembled, blocks removed and cleaned of sand, and then the sand in the container was carefully dumped onto a tray so that the number of live termites could be counted and aspirated. Termite mortality (%) was estimated using Equation 3. This was an estimate because the process of dumping the sand out of the container, even when careful, can crush and cause an unknown number of termite deaths. An unknown number of living termites can also be missed when searching through the clumps of moist sand.

*Termite mortality*  (3)

The amount of damage done to sample blocks was evaluated at the end of the testing period by assigning a visual rating score and measuring weight loss (%). Damage for SYP blocks was scored according to the AWPA E1 standard, which was based on percent area removed from the cross-sectional face for solid wood (AWPA 2020). Since the OSB blocks did not have a cross-sectional face per se, damage was based on percent area removed from the face with the most damage. Visual ratings were: 10 Sound or no visible damage, 9.5 Trace, 9 Slight up to 3% removed, 8 = Moderate 3-10% removed, 7 = Moderate/severe 10-30% removed, 6 = Severe 30-50% removed, 4 = Very severe 50-75% removed, 0 = Failure, sample broken or easily broken.

Weight loss (%) was measured by oven drying the blocks at 105 ± 0.5 ºC for 24 h then taking the posttest oven dry weight. The weight loss of each block was calculated using Equation 4. The pretest dry weight was calculated by Equation 5, which was a rearrangement of Equation 1.

*Weight loss*  (4)

*Pretest dry weight*  (5)

**Data analysis and visualization**

SAS v9.4 (SAS Institute Inc 2016)was used for all statistical analyses with alpha = 0.05 as the threshold for significance (*P* < 0.05). Mean values are reported as X̅ ± SE. One-way ANOVA was performed using Proc GLM in tests of main effects of sample type. The assumption of normality was checked by examining distribution of points in the residual versus quantile (Q-Q) plot and by results of the Kolmogorov-Smirnov test available in Proc Univariate. The assumption of homogeneity of variance was checked by examining boxplots and invoking the Bartlett’s test using a MEANS statement in Proc GLM. If assumptions were met and the main effect was significant, then the Tukey multiple comparison test was implemented for mean separation. If assumptions were not met, then the Kruskall-Wallis test, which is the non-parametric equivalent for one-way ANOVA, was run using Proc NPAR1WAY. Mean separation was then provided by the Dwass, Steel, Critchlow-Fligner Method.

Significance for percent mass loss differences and visual rating differences between the two sample types in each choice test, i.e. difference (SYP – OSB0) for T1, (SYP – OSB10) for T2, (SYP – OSB20) for T3, or difference (OSB0 – OSB10) for the OSB choice, were first checked for normality using Proc Univariate. If normal, then the paired t-test results were given by the Student’s t-test. If not normal, paired t-test results were given by the nonparametric Wilcoxon signed rank test.

Boxplots were generated using R v4.0.2 (R Core Team 2020) through the RStudio v1.3.1056 interface (RStudio Team 2020).

**Results and Discussion**

**Moisture Content**

Figure 2 shows boxplots of EMC for the OSB and SYP samples after conditioning in an environmental chamber. Mean EMC values in descending order were SYP, 10.6 ± 0.02; OSB10, 9.3 ± 0.2; OSB0, 9.1 ± 0.2; and OSB20, 8.7 ± 0.2. Non-parametric analysis by the Kruskall Wallis test for the main effect of sample type on EMC was significant (P < 0.0001). Mean separation test showed that SYP has significantly higher EMC (about 1.5% higher) than the OSB samples with no difference among the OSB types (OSB0, OSB10, and OSB20). Also, note that the OSB samples showed greater variation in moisture content compared to the SYP solid wood samples. Other reports have generally shown that the MC of OSB at a given relative humidity and temperature is lower than those of solid wood (APA 2016, Boardman et al. 2017).

Figure 3 illustrates how MC increases for the SYP and OSB samples when sitting on wet sand for 4 weeks, which should model the relative changes that occurred in the termite choice tests. The lines in the graph show very different trajectories over time for SYP compared to the 3 OSB types. For SYP, MC climbed very rapidly from 8 to 86% between 0 and 1 day, reaching its plateau or saturation value of 109% by 1 week. The OSB samples, on the other hand, increased only from 8 to 31% between 0 to 1 day, then exhibited a slow but steady climb to about 82% over 4 weeks, presumably with a plateau occurring sometime after 4 weeks. For the three types of OSB samples, nearly overlapping lines indicated similar MC values and changes over time. The greatest difference between MCs of SYP and OSB (> 50% higher for SYP) occurred at the 1-day and 1-week time points, which was far greater than the only 1.5% increase observed for their respective EMCs (Figure 2). The lower rate of absorption and consistently lower values of MC at the 5 time points are probably related to the higher densities of OSB compared to solid wood (Winistorfer et al. 1996), the presence of resin binder, water-repellent waxes, and other additives (Behr 1972, Evans et al. 1997, Kartal and Green III 2003). Zhang et al. (2007), who studied SYP strands, found that 1) strands with higher density take up less total water, 2) as the resin concentration increased, the rate of water uptake decreased along with the total amount of total water absorbed, and 3) wax, like resin, had a similar inverse relationship with rate of water absorption and total water absorbed.

The slower rates of moisture absorption by the OSB was also most likely responsible for the visible changes in termite behavior that were evident in 3-week photos (Figure 4). In the OSB choice test (OSB0 versus OSB10, Figure 4 right photo), termiteshad fully encased both OSB samples with sand tubes, while in choice tests that involved SYP, minimal sand cover was found on either SYP or OSB samples and termites exhibited a clear preference for the SYP. These observed behavior differences can be interpreted in the context of the MC results of Figure 3, that is the natural amounts of water absorbed by the OSB sitting on wet sand (30% at 1 day, 50% at 1 week, and 70% at 3 weeks) was not high enough to sustain termite activity.

Although the MC of the termite exposed samples was not measured, it is known that termites alter their behavior according to their moisture requirements. In addition to relative humidity preferences (Zukowski and Su 2017), termites exhibit clear feeding preferences for wood at specific MCs. In short term (3-day) three block choice tests of wood at three different MC ranges, *Coptotermes formosanus* preferred Japanese red pine in the middle range (79-102%), while *Reticulitermes speratus* preferred the middle (79-102%), and high ranges (140-182%) (Nakayama et al. 2005). Given their taxonomic relationship, *R. virginicus* is probably like *R. speratus,* preferring wood with MC in the middle and high ranges. Due to the relatively slow increase of OSB MC, it is reasonable to infer that the wet sand encasement was a behavioral response used by the termites to raise the OSB MC. Water absorption by SYP, on the other hand, exceeded 80% MC after 1 day on wet sand, obviating the need for sand tubes.

**Weight loss**

Figure 5 shows boxplots of weight loss for the SYP and OSB samples in the SYP versus OSB choice tests (T1, T2, and T3). Within each boxplot, one-way ANOVA for the main effect of choice type found that there was no significant difference in percent weight loss of SYP or OSB blocks. Specifically, SYP samples (Figure 5, left graph) exhibited 17.2, 17.7, and 16.4% weight losses for T1, T2, and T3 choice tests, respectively, which were not significantly different, and weight losses for the OSB0, OSB10, and OSB20 samples (Figure 5, right graph) were 3.0, 2.1, and 1.9%, respectively for the T1, T2, and T3 choice tests, were also not significantly different. In addition, weight losses from no termite controls were: 0.43 ± 0.07% for SYP, 0.05 ± 0.25% for OSB0, -0.40 ± 0.31% for OSB10, and -0.05 ± 0.18% for OSB20. All means from no termite controls were within ± 0.5 of 0% indicating that leaching did not contribute to weight loss in the termite exposed samples.

Within a given T1, T2, or T3 choice test, the difference in percent weight loss between the SYP and the paired OSB sample, i.e. difference (SYP – OSB), were highly significant (P < 0.0001) (Table 3). A significant difference meant that the SYP sample always exhibited a significantly higher weight loss than the partner OSB sample. Thus, the termites demonstrated a strong preference for the SYP, consuming more of the SYP than the OSB sample. This suggests that when OSB is used as sheathing for walls or roofing or as subflooring, termites might preferentially attack the softwood framing studs before they attacked the soy-substituted OSB. Furthermore, mean fold change values between SYP and OSB for T1, T2, and T3 were 5.7x, 8.4x, and 8.6x, respectively, suggesting that the soy flour substitution in the resin of OSB10 and OSB20 made the OSB less palatable compared to the OSB0 sample. Percent moisture content was most likely not involved here, given the similarity of the OSB0, OSB10, and OSB20 lines in Figure 3.

A search of the literature revealed no research on the effects of soy or other agro-based protein flours like wheat gluten as feeding deterrents for termites. There is, however, enough known from other animals and arthropod systems to speculate that the soy flour used in the adhesive had at least some antinutritional properties for termites. As described by Frihart et al. (2014), soy flour used for adhesives contains about 50% protein, 0% oil, 40% carbohydrates, and < 10% moisture. The most abundant proteins (50 - 75%) by mass in the soluble protein fraction are glycinin and β-conglycinin (Murphy and Resurreccion 1984). These two proteins are highly antigenic and known to cause many of the food allergies and associated intestinal inflammation in animals after the ingestion of soy-containing food products (Peng et al. 2018). Moreover, when glycinin and β-conglycinin were fed to juvenile Chinese mitten crabs (an arthropod like termites), results showed significant reductions in crab survival and weight gain (Han et al. 2019). These antinutritional effects were attributed to gut inflammation, digestive dysfunction, phylum-level shifts in the bacterial community, and an increase in pathogenic bacteria (Han et al. 2019). The last two responses were similarly described for the hindgut dysbiosis observed in termites fed chitosan-treated wood (Tang et al. 2018).

The paired t-test result for weight loss difference in the OSB choice test (OSB0 - OSB10) are detailed in Table 4. The difference was not significant (P = 0.07) with OSB0 and OSB10 exhibiting 8.5 and 5.6% weight loss, respectively, representing only 1.5x fold change between the two means. Thus, even though SYP was a preferred food source (Figures 4 and 5), when presented with only OSB, termites were capable of consuming the OSB, but at roughly half or less than half the consumption rate of SYP (Table 3). In addition, although the difference (OSB0 – OSB10) was not significant, it followed the same trend of decreasing palatability with the addition of soy flour, possibly due to related antinutritional effects described above. Had OSB20 been paired with OSB0, the difference may have become significant since it was nearly so with OSB10. Overall, though, OSB composites were more resistant to termite attack than SYP solid wood and substituting soy flour in the pMDI resin made the OSB composites even less attractive to termite attack. To provide some overall perspective for weight loss data, Holt et al. (2012), using similarly sized blocks (2.5 x 2.5 x 1.1 cm) in an AWPA E1 no choice test with *Reticulitermes* spp. observed 19% weight loss for SYP and 11.2% weight loss for commercial OSB, values that were roughly comparable to the values found here for single choice tests.

**Visual rating**

Figure 6 shows the no termite control samples after 4 weeks on moist sand and oven drying. SYP samples showed no visible change after 4 weeks on the moist sand, while OSB samples showed small areas of partial delamination in addition to scattered spots of dark discoloration, both of which were especially evident along the cut edges of the blocks. The no termite OSB controls were helpful in differentiating areas of delamination or other irregularities from termite damage. In general, edges and surfaces of delaminated areas were sharp, whereas areas eaten by termites were more rounded and tended to form shallow channels through the OSB. For the SYP versus OSB choice tests (Figure 7A), all SYP solid wood samples showed visible losses of early wood that extended through the entire thickness of the block. Losses from the OSB samples, on the other hand, were not always so obvious and rarely spanned the entire thickness of the block.

Boxplots of visual ratings for samples from the SYP versus OSB choice tests are presented in Figure 8. A higher visual rating number equates to less damage. Results from the non-parametric Kruskall-Wallis test showed that the choice test type (T1, T2, or T3) had no significant effect on visual ratings of the SYP samples (Figure 8, left graph). Choice test type, however, did have a significant effect on visual ratings for the OSB samples, specifically, OSB0 had a significantly lower mean rating (more damage) than OSB20, while the mean visual rating for OSB10 was intermediate and not significantly different from either OSB0 or OSB20 (Figure 8, right graph). Mean SYP damage ratings were severe (6.1 for T1, 5.7 for T2, and 6.0 for T3) or about 30-50% of cross-sectional area removed, while mean OSB damage ratings were moderate/severe (6.9), moderate (8.0), and slight (9.1) for T1, T2, and T3, respectively. Nonparametric Wilcoxon signed rank test results for difference in visual rating between paired samples in each choice test (Table 5) showed that the difference (SYP – OSB) was not significant when SYP was paired with OSB0 but was significant when SYP was paired with OSB10 or OSB20 with SYP showing lower ratings (more damage) than the OSB.

Comparing Tables 3 and 5, it is apparent that between the T1, T2, and T3 choice tests, there was within test correspondence and between test consistency for the SYP percent weight loss and visual rating data. Percent weight losses were all around 17% while visual ratings were all around 6.0 for severe damage. Within test correspondence and between test consistency for the OSB percent weight loss and visual rating, however, was lacking. Even though weight losses were 2-3%, visual ratings ranged from 6.9 for moderate/severe damage (OSB0) to 8.0 for moderate damage (OSB10), and 9.1 for slight damage (OSB20). The most likely explanation for this discrepancy was the OSB was more difficult to visually rate, even when no termite controls were run concurrently as a visual reference. Areas of delamination and other gaps due to manufacturing irregularities could have inflated damage ratings, resulting in a lower score than that suggested by the corresponding weight loss value. These problems were especially evident when rating OSB0 and OSB10. Either way, there appeared to be better correspondence between weight loss and visual rating data for OSB20, suggesting that the soy flour, in addition, to decreasing palatability to termites, also improved adhesion between the wood and pMDI resin (Hand et al. 2017, Hand et al. 2018, Cheng et al. 2019). Differences in feeding behavior were also apparent between SYP and OSB. On SYP, termites typically consumed through the entire thickness of the block, which was rarely true for the OSB (Figure 7A). Consequently, an SYP sample scored as 7 (10-30% of the cross-sectional face affected) would have a much higher percent mass loss than an OSB block with the same score.

Correspondence and consistency between weight loss and visual rating were better for the OSB choice test. Like the difference in percent weight loss (Table 4), the difference in visual rating (OSB0 - OSB10) was not significant (Table 6 and Figure 7B) and weight losses of 6-9% corresponded to visual ratings of 6.9 or moderate/severe damage.

**Termite mortality**

Boxplots of termite mortality for the four types of choice tests are shown in Figure 9. One-way ANOVA for the main effect of choice type detected no significant differences suggesting that mortality did not differentially affect moisture content, weight loss, or visual rating data. Mean percent mortality values for T1, T2, and T3 SYP versus OSB choice tests were 64.3 ± 4.2, 45.0 ± 12.2, and 51.0 ± 10.3, respectively, and 48.6 ± 4.9 for the OSB choice test. Overall, mortality, was highly variable from container to container and had higher mean values than similar tests run with other species of subterranean termites. Higher mortality for *R. virginicus,* however, is not uncommon. Haverty (1979) found that *R. virginicus* survival in 8 week laboratory tests was considerably less compared to *R. flavipes* and *Coptotermes formosanus* with *R. virginicus* exhibiting 12% and 21% more mortality, respectively, than the other two species*.* Telmadarrehei et al. (2020) reported that the AWPA E1 bioassay duration with *R. virginicus* had to be shortened to 18 days in order to maintain about 25% mortality in water-treated controls (water-treated wood), compared to 90% mortality in starvation controls.

Moisture content of the food substrate can also directly influence termite mortality. McManamy et al. (2008) showed that under conditions of saturated humidity, ST50 (time to reach 50% survival) for *R. flavipes* on SYP wood at 30% and 28% MC was 35.8 and 19.1 days, respectively, with 100% mortality on wood at 20% MC by day 17. It should be noted that McManamy et al. (2008) oven-dried their wood at 60 °C for MC determinations, which means MC values were underestimated. Instead, the correct method of estimating MC requires oven-drying at 100 - 105 °C since lower temperatures fail remove all bound water from the wood cell walls (Eckelman 1997, Glass and Zelinka 2010).

Another factor that may have contributed to mortality is the aspiration method of counting used. The AWPA E1 standard (AWPA 2020) recommends aspiration over weighing to get an estimate of 400 termites, but it is possible that *R. virginicus* with its smaller body size is more prone to damage during aspiration compared to other termite species. Since our tests did not include a SYP versus SYP control, it was difficult to isolate the cause of the mortality, which may have been due to previously mentioned factors, i.e. anti-nutritional effects of soy, moisture content requirements, starvation, or the aspiration method of counting termites. It can be said, however, that mold was not detected in any of the containers, nor was there any evidence of red-colored strains of the soil-borne opportunistic bacterial pathogen, *Serratia marcescens*, which can infect subterranean termites (Osbrink et al. 2001, Tang et al. 2018).

**Conclusions**

The results of this investigation clearly showed that OSB panels made with pMDI resin substituted with soy flour were more resistant to termite attack than SYP. This conclusion was supported by both weight loss and visual rating data. The consumption preference of SYP over OSB0, OSB10, or OSB20 in single choice tests was most likely related to the high MC requirements that termites have for their food source. Due to the presence of resin and wax, the three types of OSB tested exhibited much slower rates of water absorption compared to SYP. In addition, when the single choice only involved OSB, termites constructed and coated both OSB blocks with sand tubes, a behavior modification that was probably triggered by the low moisture content of the OSB. Significant differences and trends observed in the weight loss data for both SYP versus OSB and OSB versus OSB single choice tests suggest that the soy flour made the OSB less palatable. Antinutritional effects of soy flour, although not yet studied in insects, are based on the antigenic effects of its major constituent proteins, glycinin and β-conglycinin, which, can lead to inflammation of the gut and dysbiosis. The biological resistance of soy-substituted OSB to *R. virginicus* adds to the growing list of advantages being discovered for this new forest product, which include the potential of decreasing manufacturing costs without compromising the physical and mechanical board properties and reduced environmental impact since some of the resin is replaced with a bio-based material. Future research will be needed, however, to determine its resistance to other species of subterranean termites, like *C. formosanus*, which has different moisture content requirements than *Reticulitermes* spp., and to other biologically degradative organisms such as mold and decay fungi.

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**Figure captions**

*Figure 1. – Termite containers in the environmental chamber. The inset (lower left) shows the arrangement of the samples in one container and how termites were initially congregated in the center after being added.*

*Figure 2. – Boxplots of EMC for OSB and SYP samples (unshared lowercase letters above each box indicate a significant difference between means). Boxplot symbols are: box length, the distance between the 25th and 75th percentiles; open diamond, the mean; horizontal line within the box, the median; whiskers, minimum to maximum values; points beyond the whiskers, outliers (greater than 3 times the interquartile range Q3-Q1).*

*Figure 3. – Moisture content changes of SYP and OSB samples when sitting on wet sand.*

*Figure 4. – Three-week photos showing termite preference for SYP in SYP versus OSB20 choice test (left) and no obvious preference in the OSB choice test, OSB0 versus OSB10 (right, samples also encased in moist sand).*

*Figure 5. – Weight loss for SYP (left) and OSB (right) samples in choice tests (shared lowercase letters above each box indicate no significant difference for within graph mean comparisons). See Figure 2 for boxplot symbol definitions.*

*Figure 6. – No termite control samples after 4 weeks on moist sand and oven drying.*

*Figure 7. – SYP versus OSB samples (a) and OSB0 versus OSB10 (b) after termite exposure for 4 weeks in choice tests and oven drying.*

*Figure 8. – SYP vs OSB choice test boxplots of visual rating for SYP (left graph) and OSB (right graph) samples (unshared lowercase letters above each box indicate a significant difference for within graph mean comparisons). See Figure 2 for boxplot symbol definitions.*

*Figure 9. – Boxplots of termite mortality in choice tests (shared letters above each box indicate no significant difference between means).* *See Figure 2 for boxplot symbol definitions.*

*Table 1. – Average morphometric measurements of the collected termite soldiers compared to other Reticulitermes species found in MS.\**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Structure | MS Collection | *Rfla* | *Rvir* | *Rhag* |
| Head length + mandibles mm  Pronotum width mm  L mandible curvature  R mandible curvature | 2.68 ± 0.05  0.80 ± 0.01  70-90⁰  gradual | > 2.8  0.88 ± 1.03  70-90⁰  70-90⁰ | < 2.7  0.73 ± 0.83  70-90⁰  gradual | --  0.58 ± 0.67  45⁰  straighter |

\* Measurements for *R. flavipes (Rfla), R. virginicus (Rvir),* and *R. hageni* (*Rhag*)were compiled from Scheffrahn and Su (1994) and Hostettler et al. (1995).

*Table 2. – Experimental design of the termite choice test and associated sample numbers.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Test | SYP | OSB0 | OSB10 | | OSB20 |
| OSB choice  SYP vs OSB choice  No termite control | 21  5 | 17  7  5 | | 17  7  5 | 7  5 |

*Table 3.**– Paired t-test results for percent weight loss difference in the SYP versus OSB choice tests.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Choice test | SYP  Weight loss (%)  X̅ ± SE | OSB  Weight loss (%)  X̅ ± SE | Difference (SYP - OSB) *P* > |t| | Fold change (SYP/OSB) |
| T1 = SYP versus OSB0 | 17.2 ± 1.0 | 3.0 ± 0.8 | < 0.0001\* | 5.7x |
| T2 = SYP versus OSB10 | 17.7 ± 0.7 | 2.1 ± 0.5 | < 0.0001\*\* | 8.4x |
| T3 = SYP versus OSB20 | 16.4 ± 1.0 | 1.9 ± 0.3 | < 0.0001\*\* | 8.6x |

\* Significant by Wilcoxon signed rank test; \*\* Significant by Student’s t-test.

*Table 4.**– Student’s t-test result for percent weight loss difference in the OSB choice test.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Choice test | OSB0  Weight loss (%)  X̅ ± SE | OSB10  Weight loss (%)  X̅ ± SE | Difference  (OSB0 – OSB10)  *P* > |S| | Fold change (OSB0/OSB10) |
| OSB versus OSB | 8.5 ± 0.8 | 5.6 ± 0.8 | 0.07 | 1.5x |

*Table 5.**– Wilcoxon signed rank test results for visual rating differences in the SYP versus OSB choice tests.*

|  |  |  |  |
| --- | --- | --- | --- |
| Choice test | SYP  Visual rating  X̅ | OSB  Visual rating  X̅ | Difference (SYP – OSB) *P* > |S| |
| T1 = SYP versus OSB0 | 6.1 severe | 6.9 moderate/severe | 0.30 |
| T2 = SYP versus OSB10 | 5.7 severe | 8.0 moderate | 0.03\* |
| T3 = SYP versus OSB20 | 6.0 severe | 9.1 slight | 0.02\* |

\* Significant

*Table 6.**– Wilcoxon signed rank test result for visual rating difference in the OSB choice test.*

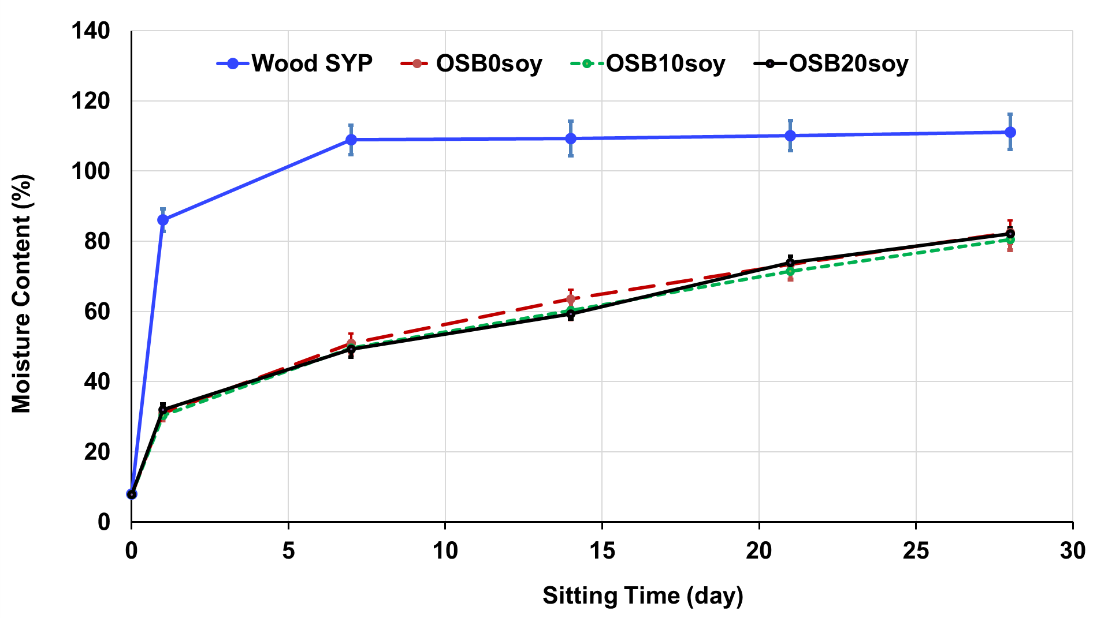
|  |  |  |  |
| --- | --- | --- | --- |
| Choice test | OSB0  Visual rating  X̅ | OSB10  Visual rating  X̅ | Difference  (OSB0 – OSB10)  *P* > |S| |
| OSB versus OSB | 6.9 moderate/severe | 6.9 moderate/severe | 0.67 |



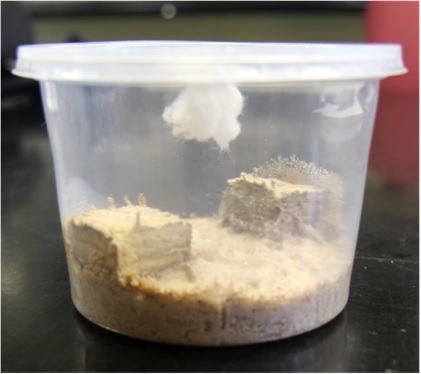
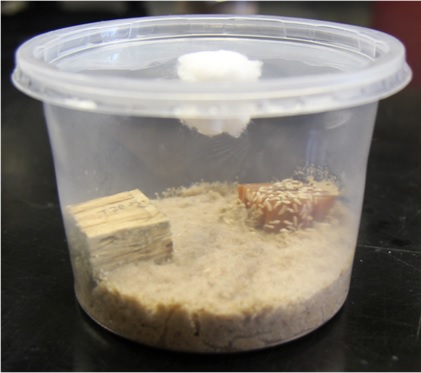
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