Variation in Natural Durability of Seven Eucalyptus grandis \times Eucalyptus urophylla Hybrid Clones

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Abstract

Programs aimed at developing clones of hybrid trees are commonly established in Brazil to meet the demands of various forest-based industries. These programs have continually improved the quality of eucalyptus wood, which has the potential to reduce deforestation by lowering demand for other high-value species. This is particularly true in the lumber market, but little is known about the resistance of eucalyptus wood to biodegradation. This study evaluated variation in natural resistance of seven Eucalyptus grandis \times Eucalyptus urophylla hybrid clones to decay by four wood-rot fungi and feeding by subterranean termites. In addition to mass loss, the relationship between density and durability was also examined. Results showed significant differences among the various clones in density as well as in resistance to fungi and termites, although none of the clones were resistant to Trametes versicolor. Mass loss in wood specimens ranged from 9 to 61 percent in the fungal tests and from 6.9 to 20.5 percent in termite tests. Average density measurements among clone groups were calculated to be between 461 and 659 kg/m³. Among the clones, five of the seven showed resistance to fungal decay and termite feeding, which was correlated with increased wood density. Based on these results, we suggest that certain clones, particularly those with higher density values, may be considered for production of various lumber products.

 $E_{ucalyptus}$ spp. are commonly used in reforestation programs in Brazil to meet the demands of various forestbased industries, with the majority used for pulp and paper or charcoal. Species of this genus are popular because of their fast growth, with trees able to be harvested between 6 and 7 years of age (Braz et al. 2014). In Brazil, numerous cloning programs have been started to improve the quality of this material, mainly through development of hybrid species (Bassa et al. 2007, Alves et al. 2011, Brazilian Association of Planted Forests Producers 2015). In particular, hybrid trees of Eucalyptus grandis \times Eucalyptus urophylla represent the most commonly used hybrid by the pulp and cellulose industry in Brazil (Busnardo 1981).

The crossing of these two *Eucalyptus* species permits fast growth, a characteristic of E. grandis, as well as improved physical properties of the wood (i.e., increased wood density of between 460 and 650 kg/m³), which is characteristic of E. urophylla. It is also thought that this hybrid is well adapted to the ecological conditions in Brazil (Carvalho 2000). Therefore, hybrid clones of E. grandis \times E. urophylla possess ideal characteristics in terms of both growth and density, with numerous industries continuing to invest in research on genetic development of this hybrid (Brigatti et al. 1983, Ikemori and Campinhos 1983, Bertolucci et al. 1995).

Recently, fluctuations in the price of pulp and paper and the low value of charcoal have caused many of the major forestry companies in Brazil to begin considering alternative uses for eucalyptus wood. Factors such as scarcity and high cost of native woods, as well as ecological pressures from overharvesting, have contributed to the growth in utilization of eucalyptus wood materials, particularly in the flooring and lumber industry (Scanavaca and Garcia 2003). Although the major properties of wood from E. grandis \times E. urophylla hybrids have been reported in terms of pulp and

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paper, studies specifically concerning the potential use of these materials in lumber products are lacking. In particular, little is known regarding the amount of variation that can occur among the various hybrid clones and how this variation can affect susceptibility to biological deterioration. These types of studies are important as both genetics and environmental factors can influence the physical properties of the wood material (Beaudoin et al. 1992, Alzate et al. 2005). Thus, in addition to the studies related to physical and mechanical properties of E. grandis \times E. urophylla hybrids, examination of resistance to decay fungi and termites is also necessary to gain a better understanding of the possible applications of this material. The main objective of this study was to evaluate the amount of variation in hybrid E. grandis \times E. urophylla clones, with specific focus on density and durability to wood-degrading organisms, and to provide baseline data that could help identify potential clones that might be used in production of higher value products (e.g., lumber).

Materials and Methods

Test samples were obtained from 13-year-old clones of E. grandis \times E. urophylla hybrid trees harvested from an improvement test site for pulp production in an experimental plantation located in Alcobaça, Brazil. Clone trees were planted randomly within the forest, and each tree was labeled with information relating to clone number and position within the forest for future reference. In this study, 7 of the 20 different clones were selected based on dendrometric parameters, such as diameter at breast height, crown size, and bark thickness, as identified in a previous study (França 2014).

Samples were cut from each clone in the heartwood section located directly beneath the bark of a 25-mm-thick board (Fig. 1). Samples were cut across the center of the cross section (see Fig. 1 in Choong and Barnes 1969) into specimens that were 25 by 25 by 9 mm (tangential by radial by longitudinal). For biological tests, six replicate samples were selected from each clone for exposure to each of the four fungi and for termite testing. All sample blocks were conditioned to a constant weight at 6 percent equilibrium moisture content (EMC) by maintaining wooden samples under a constant temperature and relative humidity until they reached the equilibrium moisture content. This was done so that accurate measures of density and weight could be determined before testing. Density was calculated by first determining the weight of each block at 6 percent EMC and then measuring block dimensions (cross-section size and length) with a caliper.

Decay testing followed the ASTM D2017 standard (ASTM International 2012). Specimens were exposed to a total of four decay fungi, two brown-rot fungi (Gloeophyllum trabeum and Postia placenta) and two white-rot fungi (Irpex lacteus and Trametes versicolor), obtained from the Forest Products Laboratory (Madison, Wisconsin) culture collection. Southern pine (Pinus spp.) and sweetgum (Liquidambar styraciflua) sapwood were used as controls for exposure to brown-rot and white-rot fungi, respectively. Test samples were exposed to decay fungi for a total of 14 weeks. After this period, blocks were removed from the culture bottles, carefully brushed free of mycelia, and allowed to air-dry overnight. Blocks were then conditioned at 21° C and 30 percent relative humidity (6% EMC) and weighed, and percent mass loss was calculated. Classifica-

Figure 1.—Schematic cross-section of Eucalyptus grandis \times Eucalyptus urophylla log identifying sampling locations.

tion of decay resistance based on average percent mass loss was then assigned to each clone group according to the scale presented in the ASTM D2017 standard (Table 1).

Termite testing followed the American Wood Protection Association (AWPA) E1-13 standard (AWPA 2014) using the eastern subterranean termite, Reticulitermes flavipes (Kollar). Termite species was confirmed both morphologically and genetically in previous studies (Arango 2015, Arango et al. 2015). Termite collections were done the day of test setup from dead logs and cardboard traps set out in a small wooded area in Janesville, Wisconsin. Southern pine and sweetgum samples were again used as controls. Test specimens were placed in a plastic container with 50 g of sterile, sifted sand and 8.5 mL of sterile water. One gram (ca. 300 workers and 1% soldiers) of freshly collected R. flavipes (third and fourth instar) was then added to each dish and allowed to feed for 4 weeks. After 4 weeks, samples were removed from the testing arena and cleaned using a

Table 1.—ASTM classification of decay resistance according to mass loss values.^a

| Resistance class | Average mass loss $(\%)$ |
|------------------------------------|---------------------------|
| Highly resistant | $0 - 10$ |
| Resistant | $11 - 24$ |
| Moderately resistant | $25 - 44$ |
| Slightly resistant or nonresistant | >45 |

^a Adapted from ASTM International D2017 (2012).

Table 2.—American Wood Protection Association (AWPA) classification for visual rating of test blocks exposed to subterranean termites.^a

| Visual rating classification | Rating |
|---|--------|
| Sound | 10 |
| Trace, surface nibbles permitted | 9.5 |
| Slight attack, up to 3% of cross sectional area affected | |
| Moderate attack, 3%–10% of cross-sectional area affected | |
| Moderate/severe attack, penetration, 10%–30% of cross-sectional | |
| area affected | |
| Severe attack, 30%–50% of cross-sectional area affected | |
| Very severe attack, 50%–75% of cross-sectional area affected | |
| Failure | |

 a Adapted from AWPA E1-09 (2012).

small brush to remove sand particles. The number of live termites after exposure was also recorded. Samples were oven-dried overnight and reconditioned to a 6 percent EMC to obtain a final weight, and then percent mass loss was calculated. Each sample was also rated based on the visual evaluation system provided by the AWPA E1-09 standard (AWPA 2012; Table 2).

For statistical analysis, Pearson correlation coefficient was used to determine the correlation between density and percent mass loss for the fungal data and between density, percent mass loss, and termite mortality for the termite data. Differences in wood consumption of fungi among clones and between fungi, as well as the difference in mass loss between clones for termite tests, were compared using the Tukey test (α = 0.05; SAS version 9.4, SAS Institute 2013).

Figure 2.—Correlation between density and mass loss for the Eucalyptus grandis \times Eucalyptus urophylla hybrid clones against (a) brown-rot fungi and (b) white-rot fungi.

Results

Results from decay tests showed mass losses ranging from 9 to 61 percent and from 53 to 95 percent for clone specimens and control samples, respectively. *Eucalyptus* grandis \times E. urophylla clones E6 and E7 were significantly less resistant to all fungi tested compared with the other clones, with the exception of clones exposed to T. versicolor, where all clones showed significantly lower resistance compared with the other three fungi tested. Samples exposed to the other white-rot fungus I. lacteus, however, showed analogous results with those exposed to the two brown-rot fungi (Fig. 2). Compared with clones E1 through E5, results showed nearly three to four times higher average mass loss in clones E6 and E7 (Table 3).

Test blocks exposed to feeding by subterranean termites had average mass losses ranging from 7 to 21 percent in clone specimens, compared with 23 to 30 percent mass loss in control samples. Termite mortality values ranged from 2.5 to 37 percent in hybrid clones, with 2.5 percent mortality in control samples (Fig. 3). Based on the visual rating classification of termite attack, clones E6 and E7 were classified as having very severe attack and were the clones most susceptible to termite feeding. Clones E1, E3, and E5 were classified as moderately attacked in the termite test and had lower mass loss values then the other five clones (Table 4).

Average density values for the hybrid clones ranged from 461 to 659 kg/m^3 , with significantly higher density in clone E1 and significantly lower density in E6, compared with the other hybrid clones (Table 3). The relationship between density and mass loss in decay tests suggests a negative correlation in samples exposed to G. trabeum ($P < 0.0001$), *P. placenta (P < 0.0001), and I. lacteus (P < 0.0001). Thus,* clones with higher density values had increased decay resistance compared with clones with lower densities.

Results from the termite tests were comparable to those from the decay tests. All hybrid clones were susceptible to termite feeding, with significantly higher mass losses in

Table 3.—Average density, mass loss, and decay resistance class of the seven Eucalyptus grandis × Eucalyptus urophylla hybrid clones exposed to brown-rot and white-rot fungi.^a

| | | Gloeophyllum trabeum | | Postia placenta | | Irpex lacteus | | Trametes versicolor | |
|----------------|-------------------|----------------------|-------|-------------------|-------|-------------------|-----------|---------------------|-------|
| Clone | Density $(kg/m3)$ | Mass loss $(\%)$ | Class | Mass loss $(\%)$ | Class | Mass loss $(\%)$ | Class | Mass loss $(\%)$ | Class |
| E1 | 659.6 A (28.2) | 16.6 B c (6.8) | R | 20.7 C b (2.5) | R | 24.1 B b (7.2) | R | 50.1 A a (2.7) | NR. |
| E2 | 551.4 CD (50.2) | 9.5 B c (4.1) | HR | 22.9 C b (8.4) | R | 28.9 B b (14.2) | MR | 51.4 A a (6.0) | NR. |
| E3 | 619.4 D (20.2) | 22.5 B c (11.0) | R | 24.3 BC c (8.4) | R | 31.4 B b (6.3) | MR | 55.2 A a (4.3) | NR. |
| E4 | 542.5 CD (18.2) | 14.3 B c (10.3) | R | 28.4 C b (6.5) | MR. | 30.9 B b (7.4) | MR | 52.6 A a (2.5) | NR. |
| E ₅ | 568.2 C (6.5) | 11.3 B c (6.1) | R | 17.5 C b (7.8) | R | 19.5 B b (5.4) | R | 49.9 A a (5.1) | NR. |
| E6 | 461.5 E (26.0) | 51.8 A b (11.4) | NR. | 55.0 A ab (8.9) | NR. | 60.9 A a (12.3) | NR. | 52.3 A b (4.6) | NR. |
| E7 | 521.0 D (26.0) | 40.0 C b (11.4) | NR | 38.6 B b (8.9) | NR | 60.9 A a (12.3) | NR | 52.3 A b (4.6) | NR. |
| Avg. | 560.6 | 23.7 | | 29.6 | | 36.7 | | 52.0 | |
| SP | 747.8 (9.4) | 53.1(1.1) | NR. | 58.6 (1.9) | NR | | | | |
| SG | 655.9 (17.5) | | | | | 94.7(1.3) | NR. | 66.8 (1.5) | NR. |

^a Values are the means of six samples (standard deviations in parentheses). Means with the same letter designation are not significantly different ($P > 0.05$), where capital letters indicate comparisons between clones and lowercase letters indicate comparisons between fungi. Classes of decay resistance are based on ASTM D2017 categories (ASTM International 2012). HR = highly resistant; R = resistant; MR = moderately resistant; NR = not resistant; SP = southern pine; $SG =$ sweetgum.

Figure 3.—Correlation between density, mass loss, and termite mortality in Eucalyptus grandis \times Eucalyptus urophylla hybrid clones exposed to feeding by subterranean termites.

Table 4.—Mass loss, termite mortality, and density of the seven Eucalyptus grandis \times Eucalyptus urophylla hybrid clones exposed to feeding by subterranean termites.^a

| Clone | Mass loss (%) | Termite mortality $(\%)$ | Visual rating | Density (kg/m^3) |
|----------------|------------------|-----------------------------|------------------|-----------------------|
| E1 | 8.1 B(1.8) | 29.6 A (38.0) | 8 | 655.4(25.7) |
| E2 | 9.6 B(2.1) | 8.8 A(6.9) | 7 | 541.6 (42.9) |
| E ₃ | 7.5 $B(1.5)$ | 36.7 A (36.7) | 8 | 612.7(23.7) |
| E ₄ | 8.4 B(1.8) | 36.7 A (46.4) | 7 | 544.3 (16.1) |
| E ₅ | 6.9 B (1.3) | 22.5 A (32.1) | 8 | 567.4(4.7) |
| E6 | 20.5 A(4.4) | 2.5 A(0.0) | 4 | 457.8 (24.0) |
| E7 | 16.7 A(3.0) | 2.5 A(0.0) | 4 | 523.6(24.1) |
| Avg. | 11.1 | 19.9 | 7 | 557.5 |
| SP | 29.2(0.04) | 2.5(0.0) | Ω | 463.2(20.4) |
| SG | 23.0(0.03) | 2.5(0.0) | θ | 653.04 (11.4) |

^a Values are means (standard deviations in parentheses). Means with the same letter are not significantly different ($P > 0.05$). SP = southern pine; $SG =$ sweetgum.

clones E6 and E7. Statistical analysis indicated a negative correlation between density and mass loss ($P < 0.0001$). However, no statistical difference was found between clones in terms of termite mortality, nor was a significant correlation found between density and termite mortality (Table 4).

Discussion

Density is often one of the major properties of wood associated with natural durability (Arango et al. 2006 and references therein). Because E. grandis \times E. urophylla clones are commonly used in pulp and cellulose production, numerous researchers have examined density values, as well as other characteristics associated with durability, specifically for this hybrid. In this study, clone density ranged from 462 to 660 kg/m^3 , which is comparable to values reported from published literature (e.g., Bassa et al. 2007, Gonçalves et al. 2009). However, in a study on longitudinal variability in wood density of different E. grandis \times E. urophylla clones, Alzate et al. (2005) reported slightly lower density values (Table 5).

Results from both fungal and termite testing in this study were also comparable to those given by other researchers, although published literature on decay and termite resistance is limited. Mass loss values reported by Silva et al. (2014) and Dytz (2014) for G. trabeum and P. placenta are comparable to those shown here. However, mass loss values reported by both Silva et al. (2014) and Dytz (2014) after exposure to T. versicolor were lower than those from the present study, with specimens classified as moderately resistant. In one of the only studies on termite resistance of E. grandis \times E. urophylla clones, Lopes (2014) reported similar mass loss values, but higher levels of termite mortality, using the termite species Nasutitermes corniger. A summary comparing values from published studies with the results presented here is shown in Table 5.

Natural resistance to termites and decay has often been correlated to levels of extractives in wood, which has the potential to explain some of the variation in durability. Low levels of extractives are ideal for pulp and cellulose production but likely result in increased susceptibility to biological degradation (e.g., decay and insect feeding). Overall, hybrid E. grandis \times E. urophylla clones are thought to be relatively low in extractives (average total extractives of \sim 2.5% in Bassa et al. 2007 and \sim 3.5% in dos Santos and Sansígolo 2007), with higher levels of extractives in heartwood compared with sapwood (7.6% vs. 3.7%, respectively; Gominho et al. 2001). Although extractive content was not measured in this study, Boa (2014) reported total extractive content of E. grandis \times E. urophylla hybrid clones to range from approximately 2.08 to 4.82 percent, with clones E1, E3, and E5 having significantly higher extractive content compared with the other clones. These higher levels of extractives might explain the increased resistance to decay and termite feeding observed in our study.

Overall, results show that E. grandis \times E. urophylla hybrid clones vary in terms of biological resistance and density, suggesting that clone variation is an important consideration in choosing materials for new markets. In the United States, natural resistance was determined to be a major consideration of end users before purchasing lumber products (Nicholls and Roos 2006). Based on statistical analyses from this study, it might be possible to estimate natural durability properties against fungal and termite attack based on wood density values and then select for clones with higher densities as potential alternative materials to be used in the lumber products. Future studies should focus on the correlation between density and extractive content to determine if one or both of these factors significantly improve resistance to biological deterioration.

Conclusions

This study demonstrated one possible method for selecting the best clones for lumber production based on natural resistance to biological deterioration. Although E. grandis \times E. urophylla hybrids showed variation between

Table 5.—Summary of termite and decay data as well as density values from published literature on hybrid Eucalyptus grandis \times Eucalyptus urophylla clones.

| Reference | | Average mass loss $(\%)$ | | | | | |
|-------------------------|------------------------------|---------------------------|--------------------|-------------------------|-------------------------------|---------|-------------------------------------|
| | Average density $(kg/m3)$ | Gloeophyllum trabeum | Postia placenta | <i>Irpex</i> lacteus | Trametes versicolor | Termite | Average termite mortality $(\%)$ |
| Present study | 560.1 | 23.7 | 29.6 | 36.7 | 52.0 | 11.1 | 19.9 |
| Alzate et al. (2005) | 490.0 | | | | | | |
| Bassa et al. (2007) | 505.0 | | | | | | |
| Gonçalves et al. (2009) | 580.0 | | | | | | |
| Silva et al. (2014) | | 26.1 | 26.8 | | 28.6 | | |
| Dytz (2014) | | 37.0 | | | 25.7 | | |
| Lopes (2014) | | | | | | 15.7 | 55 |

clones in terms of resistance to fungi and subterranean termite feeding, two of seven clones did show higher levels of natural resistance. These results suggest that certain E. *grandis* \times *E. urophylla* clones might have a future potential use in lumber production, which has more added value than pulp and paper products.

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