

# Assessment of biodeterioration for the screening of new wood preservatives: Calculation of stiffness loss in rapid decay testing

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## Abstract

Demand for the development of environmentally benign wood preservatives has increased significantly. To reduce the evaluation time of prospective candidates, reliable accelerated decay methodologies are necessary for laboratory screening of potential preservatives. Ongoing research at Mississippi State University has focused upon utilizing custom built equipment to measure stiffness losses in wood wafers after 4 weeks of fungal exposure as opposed to mass losses in blocks after 12 weeks. Stiffness loss as a measure to quantify the extent of biodeterioration may allow detection of incipient decay. The resistance of untreated and treated southern yellow pine and radiata pine (*Pinus radiata*) sapwood wafers to biodeterioration by brown rot (*Neolentinus lepideus*, *Gloeophyllum trabeum* and *Postia placenta*) and white rot (*Trametes versicolor* and *Irpex lacteus*) fungi was investigated by measuring stiffness. From the data collected percentage stiffness losses were calculated based upon modulus of elasticity. It is a potentially accurate alternative to the “secant modulus” at a deformation equal to 5% of the specimen height calculation generally performed.

**Keywords:** accelerated screening methodology; biodeterioration; compression/crushing test; decay fungi; *Pinus radiata*; southern yellow pine; stiffness loss; wood preservatives.

## Introduction

Damage to timber by fungi, insects, bacteria and marine borers costs billions of dollars annually. In North America,

chromated copper arsenate (CCA), creosote and pentachlorophenol accounted for around 95% of the timber preservatives used in 2001, but regulation due to environmental concerns will likely see them account for less than 50% of the global preservative market from 2005 (Goodell et al. 2003). Existing standards for evaluating efficacy provide valid data; however, the development of novel or modified benign wood preservative systems is severely hampered by test procedures that depend upon extensive evaluation periods.

The soil block test (Australasian Wood Preservation Committee 1997; American Wood-Preservers' Association 2003a) is a laboratory screening technique currently used to evaluate candidate preservatives and ascertain if assessment in long term field trials is warranted. Comparison of the mass losses in trial samples provides sound results. A prolonged 12 week incubation period for completion of decay is used and the total time required to run the test is at least 5 months. Shorter bioassay times may be possible after examining shorter incubation periods in combination with alternate detection procedures.

Inefficiencies of the method include the time spent conditioning samples for variation in moisture content, and making adjustments for loss of wood preservative and weight gain due to fungal colonization. The test is more severe than outdoor exposure conditions, where colonization is dependent upon spore germination and decay fungi must compete against each other and other microorganisms. The virulence of fungi is dependent on the strain, which can vary with the extent of subculturing and the type of wood and soil used, and most organic biocides are susceptible to microbial or chemical degradation, an effect less apparent in the artificial short term laboratory decay test (Behr 1973; Hegerty 1987; Leithoff et al. 1999).

Numerous methods and novel approaches have potential in quantifying wood decay. Crawford (1994) used a non-destructive static bending or deflection test to progressively measure bending stiffness and monitor decay. Other procedures include the measurement of timber permeability (Carey 1983), torsional shear strength and near infrared spectroscopy (Goodell et al. 2003). Alternatively, immunodiagnosis has potential for detecting the incipient stages of decay in aboveground test samples using polyclonal and monoclonal antibodies (Goodell et al. 2003).

Brown rot fungi cause dramatic strength losses in the early or incipient stages of the degradation process. Up to 70% of modulus of elasticity (MOE) and modulus of rupture (MOR) prior to detection of losses in total wood substance have been reported (Wilcox 1978). Based upon this characteristic, researchers at Mississippi State

University (MSU) developed an accelerated test methodology, constructing a novel crushing apparatus that measured decay by applying force in the radial direction to a saturated 19 mm (rad)×19 mm (tang)×5 mm (long) wood wafer (Gui et al. 1996; Nicholas and Jin 1996; Janzen 2001).

The present day test apparatus (Model 02 BC-1), developed at MSU by Nicholas and Buckner, is configured with a screw driven press head that, through monotonic loading, delivers a constant rate of deformation (strain) over the entire wafer surface (Gui et al. 1996; Nicholas and Jin 1996; Janzen 2001). A 45 kg load cell attached to the load bearing plate measures load and a linear variable displacement transducer (LVDT) measures wafer deformation under this load. A load-deformation curve is automatically generated for later analysis. Testing is terminated once the specified level of deformation is reached (Gui et al. 1996; Nicholas and Jin 1996; Janzen 2001).

The need to further develop laboratory techniques for accelerating the decay process and methods that accurately detect and quantify incipient decay and the subsequent progressive deterioration of timber structure is evident. The objective of this study was to improve the accuracy of the calculation procedure for evaluating stiffness loss. Data were generated using the equipment and procedures developed at MSU.

## Materials and methods

### Modified laboratory decay bioassay

The standard methods of the Australasian Wood Preservation Committee (1997) and the American Wood-Preservers' Association (2003a) (E10-01) for testing wood preservatives by laboratory soil block cultures were used with minor modification.

Wood wafers 19 mm (rad)×19 mm (tang)×5 mm (long) were cut from kiln dried flatsawn sapwood boards of southern yellow and radiata pine, sanded and conditioned for 3 weeks at 25°C and 65% relative humidity to constant mass. Ten replicate wafers for each fungal species and each timber species were treated by a vacuum soak schedule (-85 kPa, 5 min). The retentions achieved were above the hazard class H3 values recommended in Section 4 of Australian standard 1604.1-2000 (2000). Preservative loadings are summarized in Table 1. Wafers were conditioned for 48 h at 25°C and 65% relative humidity to slow evaporation during chemical fixation.

Following a further 3 weeks of air-drying, the wood wafers were leached according to the standard method (E11-97) of the

**Table 1** Mean (SD) preservative retention (% wt/wt oven-dried wood) of wood wafers.

Treatment	Loading
Uninoculated	0
Untreated	0
CCA <sup>a</sup>	0.54 (0.07)
ACQ <sup>a</sup>	0.60 (0.10)
Sodium octaborate <sup>b</sup>	1.04 (0.15)

<sup>a</sup> CCA and ACQ preservative retentions were above those recommended for hazard class H3 use in Section 4 of Australian standard 1604.1-2000 (2000).

<sup>b</sup> Boron treatment is only permitted under Australian standard 1604.1-2000 (2000) for H1 conditions. A preservative retention of 1.31% wt/wt was attempted, based upon results of several laboratories (B. Ahmed, personal communication).

American Wood-Preservers' Association (2003b). Following leaching, the wafers were dried in a vacuum oven at 40°C and -95 kPa for 5 days, reweighed and sterilized by  $\gamma$ -irradiation at 25 kGy (Steritech, Dandenong, Victoria, Australia). Different treatments were segregated during all procedures.

*Neolentinus lepideus*, *Gloeophyllum trabeum*, *Postia placenta* (brown rot fungi), *Trametes versicolor* and *Irpex lacteus* (white rot fungi) were chosen as the test fungi. The fungi were received from USDA, Forest Service – Forest Products Laboratory (FPL), Madison, WI, USA as isolates M534, M617, M698, M697 and M517, respectively, on malt-yeast extract agar slants.

The wood wafers were aseptically placed in the jars with the cross-sectional face centered in contact with the mycelium covered feeder strip. Each jar contained two replicate wafers. Wood wafers were also placed in the sterile control jars. With the lids slightly loosened, the culture bottles were incubated at the appropriate temperature for 4 weeks. After 4 weeks, the wafers were removed from the culture bottles, wiped free of fungal mycelium, oven-dried at 105°C for 48 h and weighed. Mass losses were calculated by comparing the dry weight of each wafer before and after incubation with the trial fungi, averaged and referred to as mean percent mass loss (Table 2).

### Assessment of decayed wafers for stiffness by MSU methodology

The decayed wafers were assessed for stiffness to measure bio-deterioration using the test apparatus (Model 02 BC-1) and procedures developed at MSU (Gui et al. 1996; Nicholas and Jin 1996; Janzen 2001). Analysis was carried out above fiber saturation point to eliminate variability between the decayed wafers due to moisture content (Toole 1971; Smith and Graham 1983; Nicholas and Jin 1996). The decayed wafers were immersed in a beaker filled with water, placed in a vacuum oven (Napco Model 5831) at room temperature and subjected to an initial vacuum

**Table 2** Mean (SD) percent mass loss of treated wafers following 4 weeks fungal exposure in a modified laboratory decay bioassay.

Treatment	Pine species	Mean mass loss (%)				
		<i>Neolentinus lepideus</i>	<i>Gloeophyllum trabeum</i>	<i>Postia placenta</i>	<i>Trametes versicolor</i>	<i>Irpex lacteus</i>
Untreated	Southern yellow	22.3 (4.1)	33.6 (7.9)	40.8 (8.0)	16.7 (3.6)	22.3 (6.3)
Untreated	Radiata	21.0 (6.4)	38.8 (8.8)	39.2 (9.3)	19.6 (5.3)	27.0 (6.1)
CCA	Southern yellow	0.6 (0.2)	0.7 (0.2)	0.6 (0.1)	0.7 (0.2)	0.5 (0.1)
CCA	Radiata	0.4 (0.2)	0.4 (0.1)	0.7 (0.1)	0.7 (0.1)	0.4 (0.1)
ACQ	Southern yellow	2.6 (0.5)	2.2 (0.5)	1.6 (0.3)	2.6 (0.5)	1.9 (0.4)
ACQ	Radiata	0.8 (0.1)	1.0 (0.2)	0.4 (0.1)	0.5 (0.1)	0.6 (0.1)
Sodium octaborate	Southern yellow	6.0 (1.8)	7.5 (1.0)	4.2 (0.7)	8.0 (1.6)	16.3 (3.3)
Sodium octaborate	Radiata	2.5 (0.5)	8.2 (2.7)	5.2 (0.9)	2.2 (0.8)	6.3 (1.7)

of -85 kPa for 15 min before the vacuum was isolated, and the decayed wafers were allowed to soak for an additional hour.

The saturated decayed wafers were held in position by a spring tensioned clamp with the tension set to retain the sample in place and ensure the same orientation to the loading plate for every test run. Compression of the decayed wafers was carried out by loading the radial face until 5% deformation of the sample height was achieved. The press head deformation speed was set at a continuous rate of 16.222 mm/min, in accordance with the speed of testing required in American Society for Testing and Materials (ASTM) standard D143 (1993) for compression perpendicular to the grain. The capacity of the load cell was specifically matched to the testing range. Data were generated in at least the upper third of the load cells capacity to increase accuracy. Data acquisition and control software (Force 2-02) developed at MSU was used to generate and collect data from the Model 02 BC-1 stiffness test apparatus.

### BioCompression software program

The deformation data were analyzed by software developed for this study at FPL. The BioCompression program graphically displays compression load versus load head deformation data, along with the critical data analysis information using the raw data files. An analysis generates a graphical file (Figure 1) with the wafer identification and appends a text file. Both files reside in the same directory as the experimental data in table format and the graph data collected is used to determine the compression parallel to the grain. The program calculates the compressive MOE by the FPL and MSU procedures (outlined in the Results and discussion section), determines the maximum load and the work required to achieve the maximum load.

Percentage stiffness losses were calculated by comparing the MOE of the uninoculated wafers with the preservative treated wafers after incubation with the trial fungi and are presented in Table 3.

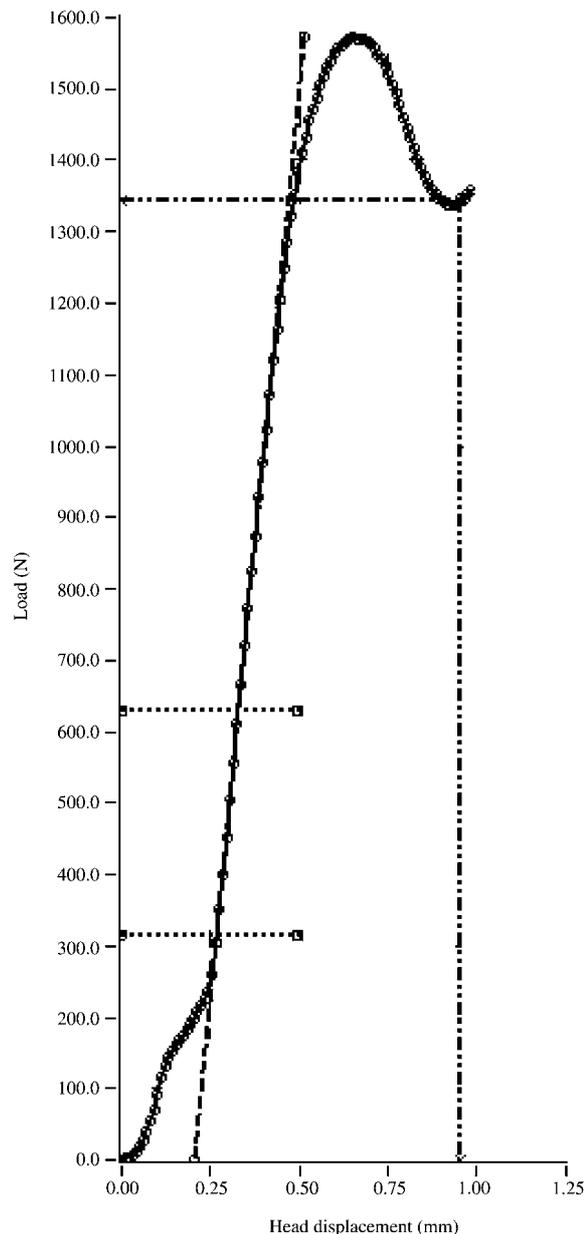
## Results and discussion

### Mass loss of wafers

Untreated southern yellow and radiata pine wafers showed mean percent mass losses of 16.7–40.8% after 4 weeks of fungal exposure (Table 2), less than the >50% values expected after a 12 week soil block trial. As anticipated, mass losses were insignificant in CCA (0.4–0.7%) and ammoniacal copper quat (ACQ) (0.4–2.6%) treated wafers. Both test species treated with sodium octaborate exhibited moderate mass losses (2.2–16.3%) indicative of partially protected wood. Significant amounts of boron were assumed to have been leached. ACQ and CCA treated wafers were included in this work as accepted fungicidal control standards and the sodium octaborate preservative treatment to confirm the severity of the leaching procedure.

### Modulus of elasticity (MOE)

The load-deformation curve of an idealized material demonstrates that below a proportional limit level, load is directly proportional to deformation. Below the limit the proportionality between axial load and deformation is related by the expression:



**Figure 1** Load deformation graph generated by the BioCompression program.

$$\Delta = \frac{PL}{AE}$$

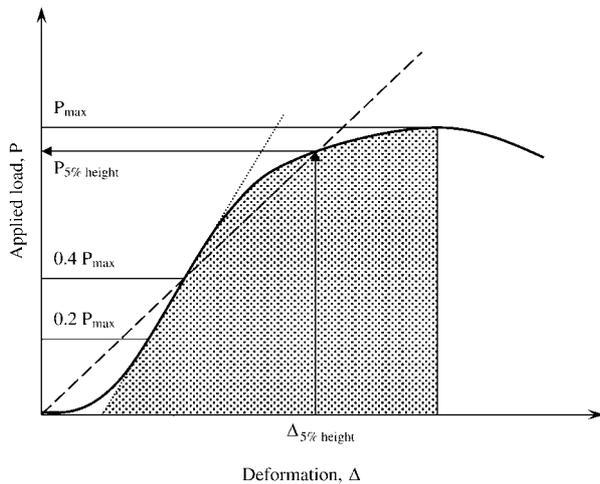
where  $\Delta$  is the deformation (mm),  $P$  is the applied concentric load (N),  $L$  is the length of the homogeneous specimen (mm),  $A$  is the uniform cross section area ( $\text{mm}^2$ ) and  $E$  is the modulus of elasticity (MOE,  $\text{N}/\text{mm}^2$ ). This expression assumes a linearly proportional relationship between load (stress) and deformation (strain) and is only valid when this condition is true.

In reality, the application of a compression load upon a wood wafer will generate a curve similar to that depicted in Figure 2. The initial portion of the curve will usually exhibit a non-linear response, predominantly attributable to the specimen settling onto the loading platform or testing apparatus seating. The level of non-linear response is variable between wafers and is predominantly due to dimensional changes (shrinking or contrac-

**Table 3** Mean (SD) MOE, % stiffness loss and R<sup>2</sup> values of treated wafers following 4 weeks fungal exposure in a modified laboratory decay bioassay.

Treatment/Pine species	<i>Neolentivirus lepideus</i>			<i>Gloeophyllum trabeum</i>			<i>Postia placenta</i>			<i>Trametes versicolor</i>			<i>Irpex lacteus</i>		
	MOE	% Loss	R <sup>2</sup>	MOE	% Loss	R <sup>2</sup>	MOE	% Loss	R <sup>2</sup>	MOE	% Loss	R <sup>2</sup>	MOE	% Loss	R <sup>2</sup>
Uninoculated															
Southern yellow	104.4 (14.2)	0	0.993	104.4 (14.2)	0	0.993	104.4 (14.2)	0	0.993	104.4 (14.2)	0	0.993	104.4 (14.2)	0	0.993
Radiata	141.8 (9.8)	0	0.997	141.8 (9.8)	0	0.997	141.8 (9.8)	0	0.997	141.8 (9.8)	0	0.997	141.8 (9.8)	0	0.997
Untreated															
Southern yellow	15.8 (2.6)	84.9	0.980	14.8 (4.0)	85.8	0.989	9.1 (1.5)	91.3	0.989	21.8 (3.5)	79.1	0.992	37.4 (5.8)	64.2	0.982
Radiata	17.6 (2.6)	87.6	0.933	19.0 (4.5)	86.6	0.981	25.7 (5.6)	81.9	0.976	18.7 (5.8)	86.9	0.990	24.4 (6.1)	82.8	0.980
CCA															
Southern yellow	109.5 (8.7)	-4.9	0.996	99.9 (7.6)	4.3	0.997	123.6 (15.1)	-18.3	0.995	120.7 (15.6)	-15.7	0.997	112.4 (11.2)	-7.7	0.996
Radiata	154.7 (18.1)	-9.1	0.994	167.0 (16.2)	-17.8	0.994	175.3 (12.6)	-23.6	0.996	164.5 (13.2)	-16.0	0.997	158.0 (9.2)	-11.4	0.996
ACQ															
Southern yellow	78.1 (16.3)	25.2	0.992	93.1 (8.7)	10.8	0.995	88.7 (17.1)	15.1	0.995	76.1 (5.9)	27.1	0.990	89.9 (6.0)	13.9	0.996
Radiata	130.4 (12.9)	8.0	0.998	158.2 (9.5)	-11.5	0.998	146.3 (11.1)	-3.2	0.998	147.7 (9.5)	-4.1	0.997	130.5 (12.1)	8.0	0.997
Sodium octaborate															
Southern yellow	72.5 (6.4)	30.6	0.988	59.4 (7.6)	43.1	0.994	84.5 (12.8)	19.1	0.997	55.5 (12.9)	46.9	0.989	60.8 (8.9)	41.8	0.989
Radiata	120.2 (14.5)	15.2	0.997	127.9 (12.7)	9.8	0.998	93.2 (19.7)	34.3	0.987	128.5 (14.1)	9.4	0.997	113.8 (19.7)	19.8	0.995

R<sup>2</sup> represents the relative predictive power of a model and is a descriptive measure between 0 and 1. The closer the R<sup>2</sup> value to 1, the greater the ability of the quadratic regression to provide predictions.



**Figure 2** Realistic load-deformation curve and determination of MOE by two methods.

tion of the wafer during conditioning and after fabrication) or uneven decay of the wafer. These distortions inhibit the wafer surface from sitting parallel with the loading plate.

Following the initial non-linearity, sound wood typically displays a region of linearly proportional load-deformation response before becoming non-linear after the proportional limit. For compression loading, the maximum load occurs within the second larger non-linear region. Of the two non-linear areas, normally the initial region is small compared to the post-yield area for sound wood, but this can be affected by the orientation of the loading. Load applied parallel to the longitudinal axis of the wood fiber should display a less significant initial non-linearity than a loading applied perpendicular to the longitudinal axis of the wood fiber.

To determine the linearly proportional response region of the load-deformation curve, a linear regression was performed on all load-deformation data between 20% and 40% of the maximum load to determine the linear slope of that region (Figure 2, dotted line). This 20–40% maximum load region is comparable with the historical data of the FPL. The MOE,  $E$ , is then calculated using the linear slope as determined by the linear regression analysis ( $P/\Delta$ ) in the following expression, where  $L$  is the original length of the wafer (mm) and  $A$  is the original cross-sectional area of the wafer ( $\text{mm}^2$ ).

$$E = \frac{PL}{\Delta A}$$

The strength of this procedure is that it accurately identifies the section of linear response between the two non-linear regions. If the data within the 20–40% range exhibit some non-linear response, the percent values are adjusted accordingly. A suitable procedure to adopt would be to establish the percent ranges for the most severe conditions and maintain this range for all tested wafers.

Similar to the secant method, where the linear response is defined by a point on the curve and the origin (Figure 2, dashed line), MOE is determined at MSU by using the load at a given deformation equal to 5% of the

wafer height in the above equation. This approach is valid if the initial non-linear response region is negligible or constant across wafer conditions and deformation at 5% of the wafer height is below the proportional limit of the given wafer. Unfortunately for wafers exhibiting various decay stages, the initial non-linear response region may not be negligible and can increase with the severity of the decay.

Further, as the decay level increases, the probability that the evaluation point is below the proportional limit decreases. These concerns may lead to inconsistent and more variable results. Such a calculation method does not determine a true MOE value, because the linear response region below the proportional limit is not identified. The value would be more accurately quoted as a “secant modulus” at a deformation equal to 5% of the specimen height.

### Comparison with the MSU calculation procedure

Some debate exists between Mississippi State University (MSU) and Forest Products Laboratory (FPL) as to which calculation procedure is superior. The data analysis method described previously was examined in this study using the BioCompression software due to the MSU calculation having difficulty estimating MOE in heavily decayed wafers.

MSU outline their procedure as the calculation of the slope between adjacent data points. After marking the lower significant point of the curve (the point at which the curve becomes relevant post alignment), MSU identify the next data point at which the slope first falls lower than 50% of the maximum achieved slope. This is described by MSU as the proportional limit and the upper point of the significant curve. This definition of proportional limit is different to that normally understood (the point on the curve at which deformation ceases to be proportional to load) and results in a higher value. MSU then determines the regression line of the load-deformation curve between the lower and the upper significant points. A MOE value based upon the derived regression analysis can then be calculated and the maximum load determined.

MSU considers the procedure outlined in this study as a static method examining a region of the curve and argues that they use a more dynamic method of determining MOE. MSU considers the objective to be to identify the longest and straightest portion of the curve, while the 20–40% region described in this work may or may not be representative of the true characteristic of the curve.

### Stiffness loss of wafers

Failure of the test specimens when loading is applied in the radial direction (growth ring orientation is perpendicular to the direction of load) occurs when earlywood cells are crushed as their proportional limit is exceeded (Janzen 2001). Kunesh (1968) described this failure as earlywood collapse. Buckling originates in the weakest ray of the entire ray unit that acts as spaced columns to bear the load. After initial ray failure, tangential shear action is attributed to the failure of the tracheids acting as lateral

support to the rays (Kunesh 1968). Radial compression failure is described as progressive collapse. Following the initial maximum stress point, a sequence of failures ensues as more earlywood layers collapse under sustained compression. No latewood ray failure is apparent after assessment, even though all the rays in every earlywood layer fail (Kunesh 1968).

Untreated southern yellow and radiata pine wafers displayed extensive structural failure after 4 weeks of fungal exposure, but with one exception (64.2%), stiffness losses in excess of 80% were recorded (Table 3). The extensive stiffness losses in the untreated wafers support previous work (Wilcox 1978; Gui et al. 1996; Nicholas and Jin 1996; Janzen 2001) that postulated stiffness loss after 4 weeks fungal exposure was a valid alternative to mass loss after 12 weeks exposure for assessing decay. Although similar conclusions based upon mass loss after 4 weeks fungal exposure (Table 2) may be drawn, the values are less than the >50% losses necessary for validity according to the standard method (E10-01) of the American Wood-Preservers' Association (2003a).

Given CCA's efficacy under extreme hazard conditions [Australian standard 1604.1-2000 (2000)], wafers treated with CCA exhibited no significant stiffness loss (Table 3) in either pine species, as expected with losses between -23.6% and 4.3% observed. CCA treated wafers demonstrated an increase in stiffness relative to the uninoculated samples. This increase cannot be attributed entirely to differences in the initial properties of the wafers compared. It has been reported that preservative treatment can increase stiffness by a process known as embrittlement (Barnes et al. 1990; Winandy and Lebow 1997). Southern yellow pine wafers containing ACQ unexpectedly demonstrated stiffness losses of between 10.8% and 27.1% (Table 3). It is doubtful whether this result was due to inadequate preservative penetration during treatment, given the wafer size. ACQ treated radiata pine wafers showed no losses in stiffness when exposed to *G. trabeum*, *P. placenta* and *T. versicolor*. Stiffness losses of 8.0% were noted for ACQ treated radiata pine wafers exposed to *N. lepidus* and *I. lacteus* (Table 3). Sodium octaborate treated wafers demonstrated major structural failure due to the active boron component of the formulation being removed from the substrate during leaching. Stiffness loss ranges of 19.1–46.9% and 9.4–34.3% were recorded for southern yellow and radiata pine wafers, respectively (Table 3).

### Work to maximum load

Wood decay affects the MOE and compressive strength at different levels and therefore in future studies, a parameter that evaluates both responses should be considered. One such parameter is the energy to maximum load, represented as the area under the load-deformation curve to the maximum load (Figure 2, shaded area). For such a calculation, the initial non-linear response region, attributed to settling and seating effects, would be ignored.

## Conclusions

The technique developed by MSU offers significant time savings in the screening of new preservatives. Stiffness analysis of thin wafers reduces the 16 weeks necessary for the standard soil block test, based upon mass loss, to approximately 6 weeks. The reconditioning period required in a standard soil block test to equilibrate the samples to specific moisture content is eliminated, as wafers are assessed for stiffness above fiber saturation point. The test apparatus developed at MSU is portable, precise and economical to install. The calculation procedure outlined in this study offers an alternative for quantifying MOE in heavily decayed wafers.

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