

# DETERMINATION OF PERCENTAGE OF BROKEN FIBER PIECES IN MACERATED BRITTLEHEART MATERIAL

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## ABSTRACT

A method to determine the number of slides that need to be examined in order to estimate the mean percentage of broken fiber pieces (PBFP) of macerated brittleheart samples within 10% error is described. The macerated samples were prepared from brittleheart wood chips of *Eucalyptus regnans* F.v. Muell. measuring approximately 2 × 2 × 15 mm in size. Temporary wet slides were prepared from one macerated sample that contained a moderate amount of broken fiber pieces. Each slide was covered with a 22 × 22-mm cover slip on which thin lines were drawn dividing the cover slip into sixteen equal areas. There were in total 400 to 600 whole fibers and broken fiber pieces within the cover slip area for each slide. It was found that examination of one slide would be sufficient to estimate within 10% error the mean PBFP of the macerated sample from which the slide was prepared. The same result was also found for macerated samples containing high or low amounts of broken fiber pieces.

*Keywords:* Percentage of broken fiber pieces (PBFP), brittleheart, *Eucalyptus regnans* F.v. Muell.

## INTRODUCTION

The acid maceration technique has been widely adopted for qualitative and quantitative description of brittleheart. Recognition at an anatomical level has been based primarily on the characteristic occurrence of broken fibers in macerated brittleheart material (Chudnoff and Tischler 1963; Dadswell and Langlands 1934; Hillis et al. 1973; Shiokura 1981; Wardrop and Dadswell 1947; Wilkins 1989; Yang 1990). A number of techniques concerned with the preparation of slides and the counting of broken fibers have been reported (Chudnoff and Tischler 1963; Hillis et al. 1973; Wilkins 1986, 1989). However, little information appears to exist on the level of error associated with these measurement techniques. Renewed interest in the formation of brittleheart in stems of plantation-grown eucalypts necessitates that the error level associated with quantitative microscopic methods be well understood. The purpose of this paper

is to report a method used to determine the number of slides that need to be examined in order to estimate the mean percentage of broken fiber pieces (PBFP) of a macerated sample within 10% error.

## MATERIALS AND METHODS

### *Materials and slide preparation*

One *E. regnans* butt log was selected from the log yard at Duncans-VIA Pty Ltd, Victoria. This log came from a tree approximately 120 years old. Two discs were obtained, one from the butt end and the second from 3-m height. Another two *E. regnans* butt logs were chosen from the log yard of Drouin West Sawmill Pty Ltd, Victoria. These two logs were from an even-aged *E. regnans* forest, naturally regenerated after a severe fire in 1939. A disc was removed from each log at the butt end, and at 3-m and 6-m heights. Twenty wood chips measuring approximately 2 × 2 × 15 mm each were removed from near the pith of the mature and regrowth wood discs, where cell-wall deformations were previously found (Yang 1990). The wood chips were macerated using a

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modification of the technique reported by Franklin (1945). The macerating solution was a mixture of glacial acetic acid (exceeding 95% purity) and hydrogen peroxide (34.0% w/w, 120 volumes) in equal volumes. The wood chips were put into flasks containing the macerating solution, one wood chip per flask. The flasks were heated to 90 C, and the wood chips were macerated for 5 to 6 hours, at which time they became sufficiently soft to easily fall apart into individual cells when shaken.

Following maceration, the macerating solution was decanted and the chips were washed gently with tap water. The wood chips were carefully peeled and the ends were gently pulled off so that the artificially broken fibers, most likely formed during chip preparation, were effectively excluded. The wood chips were then well shaken in freshwater until they were almost completely separated into individual cells.

A macerated sample made from a wood chip from the mature log and showing a moderate amount of broken fibers was chosen after a preliminary examination of the 20 macerated samples under a light microscope. Six temporary wet slides were prepared from this sample by pipetting 2 to 3 drops of the well-mixed suspension onto each slide and covering it with a 22- × 22-mm coverslip. Based on prior laboratory experience concerning dilution, the macerated sample had been diluted to such a concentration that one drop of the sample from a pipet with an 1.4-mm orifice contained in total 400 to 600 whole fibers and broken fiber pieces. Thin ink lines had been drawn on each of the coverslips dividing it into 16 equal areas. There were about 50 whole fibers and broken fiber pieces within each area, which rarely clustered or overlapped. Eight areas were randomly chosen for each slide using a random digit table, and the total number of whole fibers and broken fiber pieces appearing in each area were counted and the numbers of each recorded.

In this study, all broken fiber pieces were counted. This included fiber pieces having one tapered end and fiber pieces without tapered ends, which presumably came from fibers that broke more than once. Because of this count-

ing method, the term "broken fiber pieces" is used here in contrast to the term "broken fibers" used by others (Chudnoff and Tischler 1963; Wilkins 1986, 1989). The methods used by those authors effectively exclude fiber pieces lacking a tapered end.

#### Data analysis

The percentage of broken fiber pieces (PBFP) for each slide was calculated from the data for 8 areas using Eq. 1.

$$\text{PBFP}_{\text{slide}} = \frac{\sum_{i=1}^8 a_i}{\sum_{i=1}^8 (a_i + b_i)} 100 \quad (1)$$

where,

$\text{PBFP}_{\text{slide}}$  = percentage of broken fiber pieces of a slide

a = the number of broken fiber pieces counted in one area on a slide

b = the number of whole fibers counted in the same area

The arithmetic mean PBFP of the 6 slides and standard deviation were calculated. The number of slides required in order to estimate the mean PBFP of the macerated sample within 10% error was calculated using Eq. 2.

$$n = \left( \frac{1.96\text{SD}}{e\bar{X}} \right)^2 \quad (2)$$

where,

n = the number of slides needed to be examined

$\bar{X}$  = the mean PBFP of 6 slides

SD = standard deviation

e = error, set at 10% in this study

#### Application to macerated samples containing high and low amounts of broken fiber pieces

Another two macerated samples were chosen after a preliminary examination of the remaining 19 macerated samples under a light microscope. One showed very high and the

TABLE 1. Mean of  $PBFP_{slides}$ , standard deviation, and the number of slides ( $n$ ) needed to estimate the mean of the macerated sample within 10% error for three samples containing high, moderate, and low amounts of broken fiber pieces.

Statistics	Macerated samples		
	High PBFP	Moderate PBFP	Low PBFP
$\bar{x}$	77.07	24.51	3.31
SD	2.47	1.17	0.11
n	0.39	0.88	0.42

other showed very low amounts of broken fiber pieces. They were respectively made from a wood chip from the mature log and a wood chip from the 1939 regrowth log. Five temporary wet slides were prepared from each macerated sample in the same fashion as previously described. On each slide, 8 areas were randomly chosen, and the total number of whole fibers and broken fiber pieces in each area were counted and the numbers of each recorded. The PBFP of each slide was calculated according to Eq. 1. For each macerated sample, the arithmetic mean PBFP of 5 slides and standard deviation were calculated. Using the mean PBFP and standard deviation values and setting the allowable error at 10%, the number of slides, "n," was calculated using Eq. 2.

#### RESULTS AND DISCUSSION

Table 1 presents the mean PBFP, standard deviation, and the number of slides needed to be examined with the error set at 10% for the three samples containing high, moderate, and low amounts of broken fiber pieces. The computed "n" equals 0.88 for the sample containing a moderate amount of broken fiber pieces. This indicates that examination of the whole fibers and broken fiber pieces in 8 areas on one slide is sufficient to estimate the mean PBFP of the macerated sample within 10% error.

The number of slides required for examination in order to estimate the mean of macerated samples within 10% error was found to be 0.39 for the sample with high PBFP and 0.42 for the sample with low PBFP. Therefore,

in both cases 1 slide should be sufficient. These results, along with the result for the moderate brittleheart sample ( $n = 0.88$ ), suggest that this methodology should be applicable for *E. regnans* samples that may cover a wide range of levels of broken fiber pieces.

The total number of whole fibers and broken fiber pieces appearing on a slide should be around 1,000, or no fewer than 50 on average in each of the 16 areas under the coverslip; otherwise the error of the estimated mean PBFP of the macerated sample is likely to increase. To achieve this, the macerated sample should be thick enough so that 2 to 3 drops of the suspension contains a sufficient number of whole fibers and broken fiber pieces. On the other hand, the sample should not be too thick; otherwise counting becomes more difficult because cell elements are clustered or overlapped. The uniformity of the cell distribution varies within slides and between slides, and such variation is associated largely with preparation of the slides.

These results apply to macerated samples made from wood chips measuring approximately  $2 \times 2 \times 15$  mm each. For samples made from larger wood chips, more slides or more areas on each slide may be required. This is because sample sizes may need to be larger for bigger populations in order to maintain the error level. In practice, we observed that the thinner a macerated sample, the better the cells were dispersed when shaken. If a larger wood chip needs to be examined, the resultant macerated sample may be divided into a few small samples of equal volume. The PBFP may then be determined for each small sample and a mean PBFP calculated. In this case, weighting may not have to be considered in the averaging process.

Using this technique, slides for macerated samples that have a PBFP less than 1% need to be prepared and examined with extreme care. This is because in such a macerated sample, if a total of 400 whole fibers and fiber pieces are examined, only about 4 of these are expected to be broken fiber pieces. With such a small number of broken fiber pieces, any

slight change in the slide preparation technique or oversights in measurement may result in a larger experimental source of error.

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