Time Dependence of Starch Levels in the Sapwood of *Eucalyptus diversicolor* (Karri) as: Standing Trees, Stored Saw-Logs, Ringbarked Trees and Trees Felled without Lopping

By Louise A. Simpson and A.F.M. Barton

School of Mathematical and Physical Sciences, Murdoch University, Murdoch, Western Australia, 6150

Keywords

Amylose
Amylopectin
Eucalyptus
Starch
Starch analysis
Karri
*Eucalyptus diversicolor*
*Lyctus brunneus*

Summary

The spread of *Lyctus brunneus* (powder post borer) throughout South Western Australia has lead to a need for information on starch levels in the sapwood of *Eucalyptus diversicolor* (Karri). Karri is one of the most commonly used structural timbers in Western Australia. Rates of starch depletion in stored Karri logs were determined, those in dry stored logs were found to be greater than those stored under an intermittent water spray regime. Starch levels in living, standing Karri were measured and found to be strongly influenced by rainfall. Starch levels in ringbarked (girdled) trees declined more rapidly than those felled and left with an intact crown. A standard colorimetric technique for starch analysis was used. While high relative precision is possible, assumptions in this and similar methods make them unsuitable for absolute estimations of the two components of starch, amylose and amylopectin. Possible alternative methods are discussed.

Introduction

Starch is normally present in the sapwood of angiosperms, occurring only rarely in heartwood, being used by the tree as an energy source for growth processes. As the starch reserves are consumed the cells die and become part of the heartwood of the tree. The width of the encircling zone of sapwood varies, though it usually consists of five growth increments. As a proportion of the total tree or log volume sapwood is therefore directly proportional to the age of the tree. With very large diameter trees such as the mature Karri of the south-west forests the sapwood could be discarded with little economic loss. Most of these very large eucalypts have now been felled. Logs now presenting to the sawmills are increasingly young regrowth trees. As a consequence of both this and also pressure from increased costs, sapwood can no longer be simply discarded.

Starch present in the sapwood is used by the larvae of *Lyctus brunneus* (powder post borer) as food. Femal beetles insert their ovipositors through a wood pore and deposit eggs in the wood. Hatched larvae eat their way through the wood digesting only the starch. Residue lignin and holocellulose are left in the form of a fine powder and as a result the wood has diminished structural integrity. Extensive attack in a load bearing beam with a large proportion of sapwood could have unfortunate consequences.

Starch levels are virtually unaffected by normal kiln conditions. Larvae present prior to kiln drying are destroyed by the high temperatures. However, reinfestations can readily occur during subsequent stages of processing and use (Creffield et al. 1988). *Lyctus brunneus* is now common throughout south-west Australia, including the Perth metropolitan area. In the eastern States of Australia (and internationally, for *Lyctus spp.* are a problem in most countries) prevention is based on treating the wood with chemical insecticides (Bootle 1983). This is a costly procedure and in the case of the polychlorinated bi-phenyls no longer acceptable to consumers.

There has been no previous research which quantifies the starch content or how it varies in Western Australian eucalypts.

Research Objectives

The principle objective was the determination of starch depletion rates in logs under two different storage conditions. If a commercially viable storage regime could be found under which starch levels decline reasonably rapidly the use of pesticide chemicals would be unnecessary.

Sawmill yards maximise profits by ensuring that checking and cracking in stored logs is minimal. In general, these defects are caused by too rapid loss of water from the exterior of the logs. Karri is a dense hardwood and is difficult to season free of defects. The stored logs sampled for starch analysis were part of a research programme into prevention of timber defects during drying, which entailed varying the conditions under which logs were stored.
A separate but related project involved measuring starch levels, over a period of time, in standing trees. These were compared to trees which had been either ringbarked (girdled) or felled and left with an intact crown.

Experimental Methods

Background

Starch in sapwood is located in parenchymatous tissue in the form of granules which are approximately 10–12 μm in diameter (Browning 1967). Levels of 0.6–7.5% (on a dry mass of wood basis) have been reported in eucalypts (Hills 1978).

Starch is based on d-glucose molecules linked together to form the polysaccharide polymer. These polymers consist of two different molecular structures, amyllose and amylopectin. Amylose is a linear chain (unbranched) with α, 1:4 glycosidic linkages. Amylopectin has the same α, 1:4 links but the chains are branched. The branching occurs through an ether bond between carbon 6 and carbon 1 of glucose units which are separated by 20–25 intervening units (along the chain). Molecular weights of amylose and amylopectin have been estimated as (0.2–1.3) × 10^6 and (4.0–5.0) × 10^6 respectively (McCready 1970). Most starches contain 10–20% amyllose and 80–90% amylopectin.

None of the currently used methods for quantitative starch analysis could be considered to be reliably accurate. All involve some basic assumptions, which may or may not be valid, and procedures are neither simple nor rapid.

The reasons for this are:

- The particular chemical nature and gross morphology of wood makes it very difficult to extract one wood component only, and importantly, to extract that component in an unchanged form.
- The various wood components vary, both in type and proportion, and it is assumed that the ratio of amyllose and amylopectin is no exception.
- The ranges and means of the molecular weights of these two polysaccharides vary. This may influence reactivity and in spectroscopic measurements absorption/transmission may be a function of polymer size.
- The structural differences between amyllose and amylopectin result in differences in reactivity towards certain agents (McCready 1970). Thus amyllose forms a brilliant blue complex with iodine while amylopectin complexes poorly with iodine to glucose by the enzyme amylase whereas only a proportion of amylopectin reacts.

Although accuracy in absolute terms is not achievable, precision is. That is, measurements can be reliable in a relative sense and used with confidence for comparative purposes. In this study where, over a period of time, the same analytical procedure was used on the same logs and trees, changes detected were meaningful.

Methodology

Wood samples were stored chilled at approximately 4°C prior to drying. The oven temperature was held at 80°C for the first five hours then at 95°C for the remaining nineteen. This was to avoid possible starch hydrolysis while samples were water-saturated.

The analytical technique used required starch granules to be extracted from the wood substance, so grinding was necessary. Particle size was reduced to between 90–180 μm by using first a coffee grinder fitted with a specially made steel blade, and then a Tecator Cyclotec 1092 sample mill.

Dry wood is very hygroscopic, especially in a finely divided state. By keeping handling times to a minimum water uptake was found to be (1.8 ± 1.0)% w/w. Because the amount was small it was not taken into account in the starch calculations.

Starch analysis was carried out according to the method of Humphreys and Kelly (1961), viz:

- 1000 mg of starch was placed in a 100 ml beaker. Add 4.7 ml of 7.2 M perchloric acid and allow to react for 10 minutes with occasional stirring. Transfer the contents of the beaker to a 50 ml volumetric flask and bring to volume. After centrifuging, place 10 ml aliquot in a 50 ml volumetric flask together with a drop of phenolphthalein and make alkaline with 2 M sodium hydroxide. Add 2 M acetic acid until the colour is discharged and then add a further 2.5 ml followed by 0.5 ml of 10% w/v potassium iodide and 5 ml 0.01 M potassium iodate. Allow the colour to develop for 15 minutes, bring to volume and measure the absorption at 650 nm using a blank prepared without starch as zero. Repeat samples having more than 3% starch using smaller portions.

Repeat analyses on the same sample indicated that the precision range was ± ± 5.5%, the mean being ± 2.8%.

Calculations of starch content from the absorbance reading were as set out by Humphreys and Kelly (1961). The major assumption in this is the value and constancy of the amyllose/amylopectin ratio (i.e. whether this varies on a seasonal basis or if one is degraded preferentially under certain conditions).

Sampling and treelog treatments

Intermittent water spray storage

Regrowth Karri logs (21 years old) from Weld Block, Walpole District were stored with their bark intact under a water spray regime of one hour on, four hours off. Trees were felled in April 1987 and stockpiled the following day. Mean small end diameter under bark ("sedub") was 19.5 cm, length 4 m and mean sapwood width at the butt end 33 mm. Twelve logs were selected for sampling and the first sample for starch analysis was taken thirteen days after felling and thereafter every 21 days. Starch concentration is greatest at the butt end of the log (Creffield et al. 1988). Paired samples were taken 10 cm from the butt end with a Suunto increment borer.

Dry stockpile storage

Five logs were taken from the same stand and sampled in the same manner as those in the Paragraph above. Logs were stored under water spray for thirteen days before being removed to be stored dry. "Sedub" 21.0 cm and mean sapwood width at the butt end 31.0 mm.

Weld Block control trees

Weld Block is the area from where the trees for the dry and spray storage methods were harvested. Paired samples were taken 10 cm apart at a height of 1.3 m. The mean diameter at breast height over bark ("dbhob") was 23 cm and the mean top height was 13 m.

Big Brook block — crown intact trees

The foliage/crowns were left intact after felling and samples were taken after one year. Paired samples were taken 10 cm from the butt end. Trees were from a 45–50 year old regrowth stand in the Pemberton district, "dbhob" was 35 cm and mean top height was 40 m.

Big Brook block — ringbarked trees

An initial ring-barking operation was carried out in October 1986 but only bark was removed and this had no effect on tree vigour. A second treatment was carried out in March 1987; this time a ring of sapwood was removed and the crown had declined after two months. Trees 1 and 2 had some dead foliage and were sampled, 3 and 4 with no dead foliage were not sampled, 5 to 10 had a complete dead canopy and were sampled. Paired samples were taken 10 cm above the girdling. Mean "dbhob" was 37 cm and mean top height was 28 m.
Big Brook block – control trees
Five standing trees had paired samples taken at 1.3 m. Mean “dbh” was 46 cm and the mean top height was 33 m.

Results and Discussion

Starch depletion in stored logs

Results set out in Table 1 demonstrate that starch levels declined significantly more rapidly in the dry storage logs. After week 10 the rate of depletion in the dry logs decreased to practically zero. By contrast, the starch levels in the water-spray logs, became almost constant only after week nineteen. Although the dry storage logs were inadvertently destroyed after week nineteen, from the available data it appears that the starch level at which depletion slows is greater for the water-spray logs than for the dry logs.

Table 1. Mean starch (% w/w) in dry and water-spray stockpiled Karri logs from Weld Block

<table>
<thead>
<tr>
<th>Week</th>
<th>Water spray s.d.</th>
<th>Dry s.d.</th>
<th>F Value</th>
<th>2-Tail prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.72 0.60 0.81 0.48</td>
<td>1.59 0.511</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.69 0.40 0.63 0.20</td>
<td>4.02 0.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.59 0.42 0.35 0.13</td>
<td>11.05 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.64 0.39 0.27 0.10</td>
<td>14.49 0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.42 0.24 0.18 0.06</td>
<td>18.75 0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.55 0.40 0.31 0.08</td>
<td>24.96 0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.28 0.20 0.21 0.06</td>
<td>11.26 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0.31 0.22 n/a n/a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.31 0.22 n/a n/a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For starch levels to decline in wood it is necessary that it is degraded and used either by the trees or by invading organisms. Both processes entail respiration, that is, exchange or transport of the gases, oxygen and carbon dioxide. Factors affecting transport such as solubility and diffusion rates may be the reason why spray or wet storage logs had relatively slow starch depletion rates. It is not known how long the enzyme systems involved in starch metabolism remain viable and active after felling of the tree, nor what factors affect their survival.

Table 2 contains the results from the control trees from Weld Block. The mean is the same as the starch levels in the stored logs at the commencement of the trial. The between-tree variability is, however, quite marked.

Table 2. Starch content (% w/w) in standing Karri trees from Weld Block

<table>
<thead>
<tr>
<th>Tree number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.31</td>
<td>0.95</td>
<td>0.59</td>
<td>0.53</td>
<td>0.50</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.96</td>
<td>1.07</td>
<td>0.43</td>
<td>0.41</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 3. Mean starch (% w/w) in standing Karri trees from Big Brook

<table>
<thead>
<tr>
<th>Tree no.</th>
<th>1987 May</th>
<th>1987 September</th>
<th>1988 February</th>
<th>1988 May</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.22</td>
<td>0.82</td>
<td>0.43</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>0.60</td>
<td>0.45</td>
<td>0.47</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>0.36</td>
<td>0.46</td>
<td>0.25</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.58</td>
<td>0.46</td>
<td>0.36</td>
<td>0.34</td>
</tr>
<tr>
<td>5</td>
<td>0.64</td>
<td>0.78</td>
<td>0.24</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Mean s.d. 0.70 0.59 0.35 0.27 0.08

Big Brook trials

Data for control trees is contained in Table 3. Data for treated trees is in Tables 4 and 5, while interpretation of the data needs to be treated with caution, for reasons set out below in (i) and (ii), it is included on the basis that changes in levels are of interest in themselves.

(i) The trees were treated at different times and the initial starch levels are unknown. From the data on the control (standing) trees it is apparent that starch levels vary widely according to season, and, probably more importantly, rainfall. This makes it difficult to estimate with any accuracy what the initial starch levels would have been.

(ii) As the trees were treated at different times they were exposed to different climatic conditions in the post-treatment period. As was shown in the stockpile trials, humidity appears to be an important factor in the rate of starch depletion. Temperature could also be an important factor. Therefore the conditions experienced in the post-treatment period need to be the same in order to make valid comparisons between treatments.

The changes in starch levels in the control trees over time are interesting, especially the difference between

Table 4. Mean starch (% w/w) in felled, crown intact Karri from Big Brook

<table>
<thead>
<tr>
<th>Tree no.</th>
<th>1987 May</th>
<th>1987 September</th>
<th>1988 February</th>
<th>1988 May</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>0.35</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>0.34</td>
<td>0.23</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>0.35</td>
<td>0.35</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>0.36</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>0.32</td>
<td>0.32</td>
<td>0.16</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>0.53</td>
<td>0.23</td>
<td>0.27</td>
<td>0.24</td>
</tr>
<tr>
<td>7</td>
<td>n/a</td>
<td>0.18</td>
<td>0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>n/a</td>
<td>0.17</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>9</td>
<td>n/a</td>
<td>0.17</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>10</td>
<td>n/a</td>
<td>0.10</td>
<td>0.15</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Mean s.d. 0.39 0.23 0.21 0.19 0.06
Table 5. Mean starch (% w/w) in ringbarked (girdled) Karri from Big Brook

<table>
<thead>
<tr>
<th>Tree No.</th>
<th>1987</th>
<th>1988</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>August</td>
<td>February</td>
</tr>
<tr>
<td>1</td>
<td>0.45</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>0.59</td>
<td>0.18</td>
</tr>
<tr>
<td>6</td>
<td>0.60</td>
<td>0.22</td>
</tr>
<tr>
<td>7</td>
<td>0.57</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>0.45</td>
<td>0.14</td>
</tr>
<tr>
<td>9</td>
<td>0.56</td>
<td>0.13</td>
</tr>
<tr>
<td>10</td>
<td>1.01</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Mean | 0.60     | 0.17     | 0.19    |

s.d. | 0.19     | 0.05     | 0.04    |

the two month of May estimations (i.e. 1987 and 1988). In an attempt to find a simple and reasonable explanation for this, rainfall data for the area were collected. From Figure 1, it can be seen that rainfall for May 1988 was twice the average value and approximately 60% more than May 1987. The majority of the May 1988 rain fell prior to sample collection. There is obviously a relationship between rainfall and the levels of stored starch. Starch is used as a source of stored energy particularly during periods of rapid growth (Bamber 1965). What may have occurred here was that the recent, unusually large rainfall initiated a growth spurt which depleted the starch stores. Variation in starch levels over time suggests a strong interaction between climatic variables and the quantity of stored starch. Other studies have also described this phenomenon. Brimblecomb (1945, 1961) found that starch levels in Queensland eucalypts had a fairly consistent primary peak in summer and a secondary peak in late winter. These studies were qualitative rather than quantitative as the starch content was estimated by applying an iodine solution to the wood surface and judging content by observing the intensity and pattern of the resulting blue colour. Humphreys (1966) and Bamber (1965) found that eucalypts in New South Wales had a peak starch level in spring/early summer with lowest values in late autumn. Both these studies used the Humphreys and Kelly method. Humphreys (1966) also found that starch in felled trees declined from 0.7% to 0.1% within five weeks of felling (mean values). In Japan, Hayashi et al. (1988) found that starch levels, determined by the Humphreys and Kelly method, decreased from 0.2% to 0.03% three months after Sugi trees were felled without lopping. Control trees were cut down and then sampled, rather than left standing, and this was done at the commencement of the trial in July, again in September and finally in November. Starch rose from 0.23% in September to 0.56% in November. A prominent feature in all of these studies was the large between-tree variability in starch estimations.

Provided the wood pore size is appropriate, the quantity of starch is the most important factor in \textit{Lyctus brunneus} infestation. It seems reasonable then that the large variations in starch levels with local climatic conditions could be exploited. Thus trees should be felled when starch levels are low.

**Variability in starch levels**

**Within-tree variability**

Starch levels vary considerably within the same tree. The starch is contained in distinct structured granules which vary in shape and size (Browning 1967; Phillips 1938). The observed variability in starch levels indicates that distribution of the starch granules is not uniform within the sapwood and/or the mean size of the starch granules varies. In terms of susceptibility to \textit{Lyctus brunneus} infestation this means that there may be areas where larval growth is possible and other areas where starch levels are not adequate for this. It also means that sample size is very important. Large sample size will yield starch levels tending toward the mean whereas small sample size will reflect greater apparent variability.

**Between-tree variability**

Starch level vary more widely between trees than within trees. This is as expected as many factors influence starch levels, including genetic characteristics, the external environment (both site and climate) and fruiting/flowering processes.

It follows that multiple sampling would be required in order to obtain a representative starch level value for a given stockpile of logs and thus predicting their likely susceptibility to \textit{Lyctis} infestation.

**Starch analysis**

An accurate method of starch analysis should be established. It need not be used as a routine matter but
The major difficulty in this proposal is in the 'quantitation problem, though the ratio itself could be determined by HPLC using a column designed for high molecular weight molecules (Kobayashi et al. 1985). The major difficulty in this proposal is in the 'quantitatively extracting and hydrolysing' step.

The simpler, routine method could be achieved by using Fourier transform infra-red (FTIR) spectroscopy. The ground, whole wood samples absorption spectrum could be measured directly as the KBr disc or indirectly with a diffuse reflectance (DRIFT) cell. Thin wood sections would not be suitable due to the variability of starch within the sapwood. However, with the assistance of an FTIR microscope attachment, wood sections could be used to study the distribution of the starch granules.

A normal coordinate analysis of V-amylose by Cael et al. (1975) using FTIR and Raman spectroscopy, established band assignments for the vibrational modes. These assignments, together with the results of the comprehensive studies carried out by Barker et al. (1954 part I and II) on differentiating between $\alpha$ and $\beta$ forms of various pyranoses, should enable selection of useful absorption band which could be used to quantify the starch content in sapwood. Procedures similar to this have been developed for estimating proportions of the various wood components in samples of pulps and wood. For example, Schultz et al. (1985) was able to predict lignin, glucose (cellulose) and xylose (hemicellulose) contents of wood samples which had been pre-treated to produce a wide range of compositions. Absorbance ratios of selected bands between 1600–700 cm$^{-1}$ were used and these were measured using a DRIFT cell. There are many other examples in the literature. The major foreseeable problem would be the low absorption of the assigned, absorption bands coupled with the relatively small proportion of starch in the sapwood.

Conclusions

Dry storage in terms of starch depletion is preferable to water-spray storage of logs. This is thought to be due to restrictions in the transport of respiration gases necessary for starch utilisation by enzyme systems within sapwood cells.

Treating trees by ringbarking or felling with an intact crown does result in a decrease in starch levels. Ringbarking appears to cause a greater rate of decrease. This conclusion is based on the data presented here and cannot confidently be extrapolated to other conditions and tree species.

Standing Karri trees have starch levels which vary both within and between trees. There are marked seasonal variations with the major climatic factor appearing to be rainfall volume.

While acknowledging that timber mills need to work throughout the year, it should be possible to time the felling of young (high proportion of sapwood) trees during those seasons when starch levels are low. This would decrease the susceptibility of the timer to attack by Lycus brunnneus.

Acknowledgements

This study was funded by the Department of Conservation and Land Management (CALM), Western Australia. Grateful thanks to Gary Brennan (CALM, Bunbury) who supervised sample collection.

References


