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The forest, the timber industry and the microscope

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ABSTRACT: The role of the microscope as a major tool of Australian forest research and the timber industry is discussed. A history of the application of microscopy to the fields of wood anatomy, wood decay and weathering, forest pathology, entomology, soil science, and engineering is outlined, and examples detailed. Optical microscopy is compared with electron microscopy, and the various uses of each are stated. Finally, some of the Australian pioneers of microscopy in various areas of forestry and timber research are profiled.

1 INTRODUCTION

At first sight, forestry and the timber industry may seem to have little in common with microscopy but in reality this is far from the case. The microscope is, in fact, an essential tool in many aspects of these two fields. To list a few examples; wood identification, wood decay and weathering, forest pathology, entomology, taxonomy, soil science, and timber engineering are all reliant on microscopy to properly perceive the relevant living cells, organisms and structures that are often too small to be seen by the naked eye. Viewing of these micro-features is, however, usually essential for understanding the well-being or otherwise of a whole forest or of a stack of timber.

Case histories of the use microscopy in Australian forestry and the timber industry include a multitude of people and their contributions are varied. Only a few of the most outstanding names along with accounts of their efforts will be mentioned here.

The microscope itself has evolved. The demands for better resolution, higher magnifications, and ease of use have been met. Microscopes using glass lenses, and light for illumination have now been supplemented by electron microscopy and this has opened up a whole new range of applications in the field. Some of the most important changes to microscopy will be discussed.

In order to give an insight into what can be achieved in a modern well-equipped microscopy laboratory, some recent applied research is described. Since microscopy is a very pictorial science, images, rather than words will be used to give some indication of what can be achieved using modern technology.

2 EARLY HISTORY

At the time of the first European settlement of Australia in the late 18th century, the microscope was already an essential tool of the taxonomist. Pollen, floral parts and leaf morphology are usually very small and require magnification for proper identification. So the initial use of microscopy for the newly discovered forests of Australia was for taxonomy – the identification and classification of forest species.

When Sir Joseph Banks sailed with Captain Cook aboard the Endeavour in 1788 in what Bryson (2004) calls "the greatest botanical expedition in history", he probably didn't take a microscope with him on the trip but instead took specimens back to England for study by microscopy. Certainly Banks knew of microscopes. A painted portrait (by an unknown artist) that "was formerly hung at Revesby Abby, Banks' early home" (Cameron 1952) clearly shows a microscope on a table beside the young Banks (a copy of this portrait is contained in Cameron (1952) and Lysyght (1971)). Although Banks was a prolific collector of botanical specimens, according to Bryson (2004) he "left most of his specimens for his private secretary Robert Brown to make taxonomic study". Robert Brown (1773-1858) was a distinguished botanist and very competent microscopist to whom Banks bequeathed his herbarium and library. Brown also took part in the celebrated expedition of Flinders to Australia, which started in 1801 and returned with nearly 4000 new species for identification. Brown was the first to observe the cell-nucleus of plants. This was an amazing feat for Brown worked with only a simple microscope and the technique of staining cells and tissues was then unknown. Strangely, Brown was also the first person to observe, microscopically, the continual movements of minute particles (actually *Clarkia* pollen) when suspended in a liquid (Ford 1992). This 'Brownian Motion' as it is called, is now commonplace in any modern textbook of basic physics. The name of microscopist Robert Brown (abbreviated to R.Br.) is also perpetuated as the author in the full nomenclature of many Australian forest species (for example; *Callitris rhomboidea* R. Br.).

For the booming Australian timber industry of the 1800s, taxonomy continued to be the most important role for the microscope. Drawings by von Mueller (1887) show detail of pollen, flower parts such as the stigma and the style and other structures that could not have been seen by the naked eye. His work would have been carried out using a microscope similar to that shown in Fig. 1. By the beginning of the 20th century the excellent photo-micrographs of Baker and Smith (1910) show the use of microscopy for the study of cypress pine leaves important to the flourishing leaf oil industry of that time.

Wood is a valuable commodity. Its identification in all forms (from trees to processed timber) is important and can be of significant commercial or legal interest. Wood can be identified on the basis of its cell structure, usually by comparing the unknown with a micro-section cut from a known sample. Essential for such work are wood libraries (xylaria) – collections of known and identified wood samples and wood micro-sections. The Dadswell collection of timbers (started in 1929) is presently housed at ANU Canberra and is still being used as a resource for microscopic wood anatomy. The Dadswell wood collection was formed by H.E. (Eric) Dadswell who was an important figure the field of wood anatomy in Australia. Dr Dadswell began his pioneering studies in 1931 as a member of the Division of Forest Products, CSIRO. Much of his research involved the Eucalypts – a genus of about 500 species that are difficult to separate from one another on the basis of their wood (Dadswell 1972).

Several Eucalypts owe their common names to the external appearance of their bark. Stringybark, Ironbark and Blackbutt are some examples and in the 1870s von Mueller based a classification of the gums on the appearance of their bark. However, no one bothered about the finer detail of bark structure until Margaret Chattaway in 1953, prompted by a world shortage of tannins, realised that bark was "more than just something that had to be stripped off before the timber could be used" (Chattaway 1953). She began a series of articles on the microscopic structure of bark in Eucalypts in the Australian Journal of Botany which were illustrated by excellent artistic drawings of the microscopic features involved.

3 THE MICROSCOPES

Microscopes had their beginnings in 1675, more than two centuries before Europeans came to Australia. The technical prowess of Anton van Leeuwenhoek (1632-1723) in the grinding and polishing of lenses was outstanding. His microscopes employed single lenses which Leeuwenhoek ground himself and were able to produce magnifications of up to 270 times (Dingley 1987). Robert Hooke was the first person to use the word ‘cell’ when he examined thin sections of cork (Dingley 1987) and the term ‘cells’ was from then on used to describe the basic building blocks of plants and animals. By the end of the eighteenth century, improvements in microscope technology had identified the various types of cells and their contents, and related their form to function.

Improvements to the basic microscope design continued over time. By the close of the 19th century multiple objective lenses on a revolving nosepiece allowed rapid changes in magnification. Substage mirrors to reflect sunlight or light from oil lamps gave way to electrical illumination built into the microscope. Mechanical stages allowed free movement and rotation of the specimen.

Prior to the invention of photography and the incorporation of cameras onto microscopes in the 1830’s the only way for the microscopist to relay what was seen to others was by drawing. One way to accomplish this was by remembering what was seen through the microscope and then drawing from memory. Another method was to keep both eyes open while observing – one eye observing the specimen through the microscope and the other eye focussed onto a nearby drawing sheet. The observer then was able to draw and observe the specimen at the same time. This method had the added advantage of allowing the artist to incorporate depth into the drawing by focussing up and down. Nevertheless good microscopists in those days were necessarily good artists as well! It is also probably true to say that the correlation of photography and microscopy resulted in the first ‘impartial’ recordings of microscopic specimens.

One of the first forest publications to make use of photographs taken through the lenses of a microscope was that of Baker and Smith (1910). In their preface, the authors say: “the skill of the botanical draughtsman was not used. Plants and their parts requiring to be illustrated were too fine for pencil work so that the aid of photography was requisitioned for the illustrations and in this way nature itself has been more faithfully reproduced”.

In more recent times a further advantage to forest microscopy is the advent of digital cameras attached to the microscope. This allows a colour digital image to be displayed immediately on a computer screen - the modern microscopist no longer has to wait for photographs to be developed and printed.

While light microscopy has remained the mainstay of the forest microscopist because of its simplicity and cheapness, it is limited to a magnification of approximately 1200 times and a resolution of about one micron (0.001 mm). This is because the resolving power of light microscopy (the ability to make very small and closely spaced details visually distinct) is a function of the wavelength of light. This makes light microscopy no use for observing very small things such as viruses that

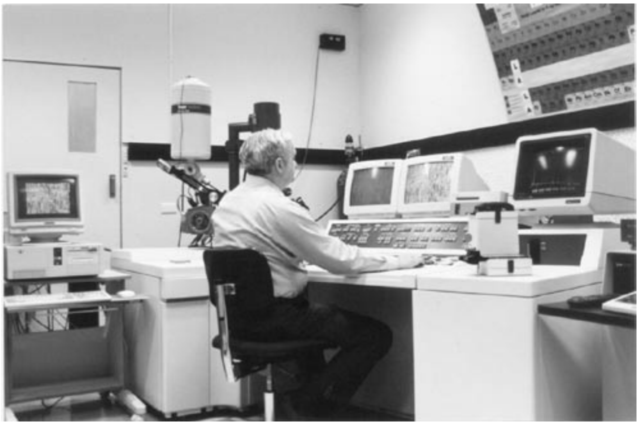

Figure. 1. A microscope manufactured in 1885 (left) and modern scanning electron microscope (right).

are smaller than a micron. In 1965 scanning electron microscopes (Figure 5) came onto the commercial markets for the first time and revolutionised the range of useful magnifications available to microscopists. For scanning electron microscopy (SEM) high-speed electrons rather than light are used to ‘illuminate’ the specimen and a resolution of 5 nanometres (five thousandths of a millimetre) at magnifications of 50,000 or more is possible.

SEM images are formed by scanning a narrow beam of electrons over the specimen. Since it is not possible to form an electron beam in air (because electrons are scattered by air molecules) the samples for SEM must be kept in a strong vacuum in order to view them. There is no difficulty with robust and dry specimens such a wood, pollen grains, or insects with hard exoskeletons that are able to withstand vacuum. However, soft and wet samples such as leaves, soft-bodied insects or gut contents must be dehydrated before use. Such dehydration usually causes cellular distortion and reduction of size. The modern technique used to overcome this problem is cryo-preservation. In this technique the specimen is first plunged into liquid nitrogen and then transferred onto a special stage inside the SEM where it can be kept at –190 ^{o}C. At this temperature, the specimen can be viewed in its ‘natural’ state over long periods of time with no deterioration due to loss of moisture.

4 SOME MODERN APPLICATIONS OF FOREST MICROSCOPY

4.1 *Taxonomy*

Scanning electron microscope images of the wood anatomy of Wollemi pine (*Wollemia nobilis*) was recently used to show that, taxonomically, *Wollemia nobilis* belongs to the Araucariaceae family (Heady, Banks and Evans 2000). This was made evident by the ‘alternate biseriate pitting’ within tracheids (Figure 6) a form of pitting arrangement that is exclusive to the Aracariaceae.

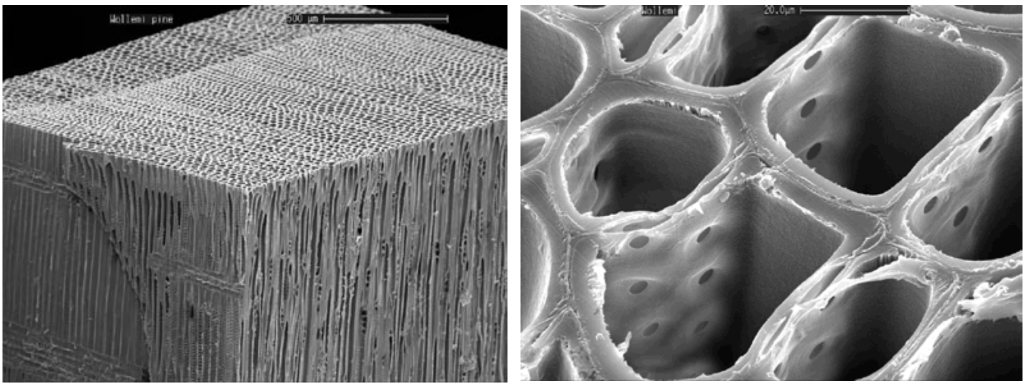

Figure 2. A small block of the wood of Wollemi pine (left) showing ‘alternate’ bordered pitting (right).

4.2 *Wood Engineering*

The timber of Turpentine (*Syncarpia glomulifera* - family *Myrtaceae)* which is endemic to coastal districts of NSW and Queensland, has a reputation for being resistant to attack by marine borers such as *Bankia* spp. and *Teredo*. For this reason it is used for marine work, shipbuilding, wharf decking, piling and poles. It has long been recognised that silica particles in the wood and bark of *Syncarpia* impart the resistance to borers. The same silica particles cause rapid wear of saws used to cut the wood. However, de Silva and Hillis (1980) were unable to determine whether the impediment was due to toxicity of the silica, or provided a physical barrier affecting the boring mechanism, or interfered with the digestive system of the borer.

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Figure 3 shows examples of the silicon particles in *Syncarpia* taken on an SEM using back-scattered electron detection. The silicon shows as light-coloured particles within the rays of the wood. At higher magnification the silicon particles are shown to be crystalline conglomerates, each about 20 µm (0.02 mm) in diameter.

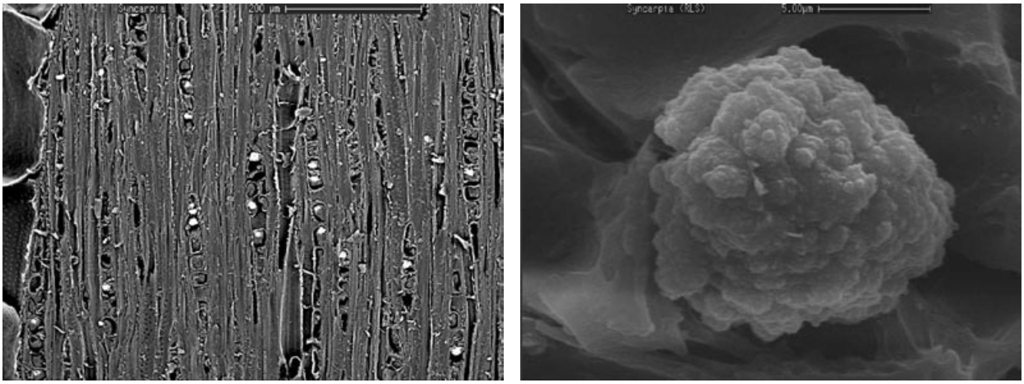

Figure 3. Silicon particles (white spots) in wood of Syncarpia and a close-up view of one of the particles.

4.3 *Entomology*

To the forest entomologist, accurate insect identification depends on good reference collections. Insect pathology may involve species identification of the organism itself by light microscopy, or the detection of viruses or bacteria within organism body fluids by means of electron microscopy. There are 300 or so species of termites in Australia (Greaves 1959). Those that cause economic losses for the timber industry need to be properly identified. Identification of the specimen may require a range of different views taken at different magnifications (Fig. 4).

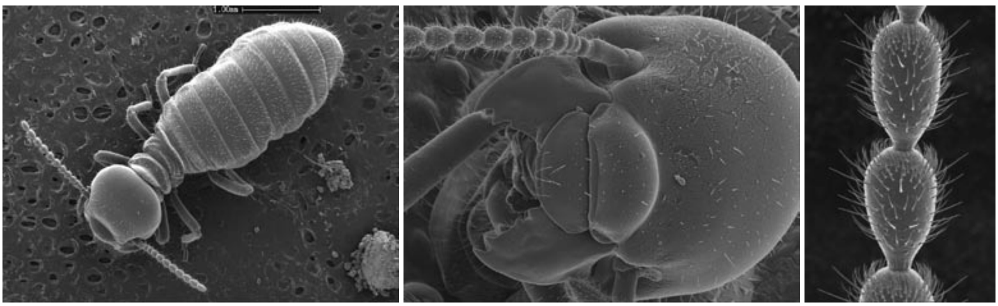

Figure 4. Sequence of three SEM images showing a whole termite, its head, and part of its antenna

4.4 *Wood decay and weathering*

Wood is a very durable material when kept under certain conditions. For example when kept very dry, wooden objects survived in the pyramids since the time of the pharaohs. Similarly waterlogged wooden ships can survive intact for centuries. However, wood that is damp but not saturated will quickly suffer fungal decay. Moulds and fungi colonise the wood and live on the sugars and carbohydrates therein. Changes in the colour of wood, its loss of toughness, and musty odour are certainly of concern to the timber industry. The shape and size of the fungal fruiting body, visible only to high-magnification microscopy (Fig. 5) is essential knowledge for those concerned.

4.5 *Soil science*

Knowledge, familiarity and control of soil micro-organisms such as mycorrhiza, nemotodes and root diseases are an important aspect of both native forest and exotic mono-culture plantations. The fungus-like plant pathogen '*Phytopththora*' which causes 'dieback' in Jarrah is a well-known example. Identification depends upon an examination of samples taken either from the diseased plant directly or from cultures made from the diseased tissue. The image shown in Figure 5 is that of *Phytophthora nicotianae* which infects the roots and lower stem of host plants. It is closely related to the 'jarrah dieback' form of *Phytophthora.*

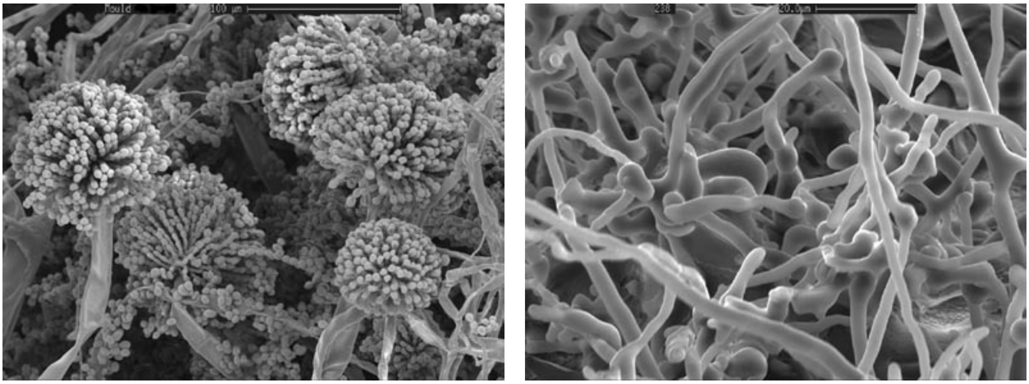

Figure 5. (left) Mould of type that colonises wood and (right) fungus of *Phytophthora nicotianae.*

4.6 *Forest Pathology*

In the field of forest pathology, disease detection and control is an important aspect of both native forest and exotic mono-cultures. Identification of some pathogens depends upon an examination of the features of fungal fruiting bodies taken either from the diseased plant directly or from cultures made from the diseased tissue. An example of this in Poplar mono-cultures lies in the disease poplar-rust *(Melampsora)*. Poplars were introduced into Australia during the mid-19th century. Apart from ornamental purposes, poplars are grown commercially as a fast growing timber, for matchsticks, veneer, light boxing materials and paper. Poplars were free from rust until 1972 when a severe epidemic broke out across NSW. Two species of rust were involved: *Melampsora larici-populina* (an American species) and *Melampsora medus*a (a European species). The rust infects the lower surface of the poplar leaf – penetrates – then pustules of yellow spores erupt that give the underside of the leaf a distinct yellow colour (Wilkinson & Spiers 1976). Identification of the fungus and its effect on the leaf (Figure 6) is vital information for the control of this disease.

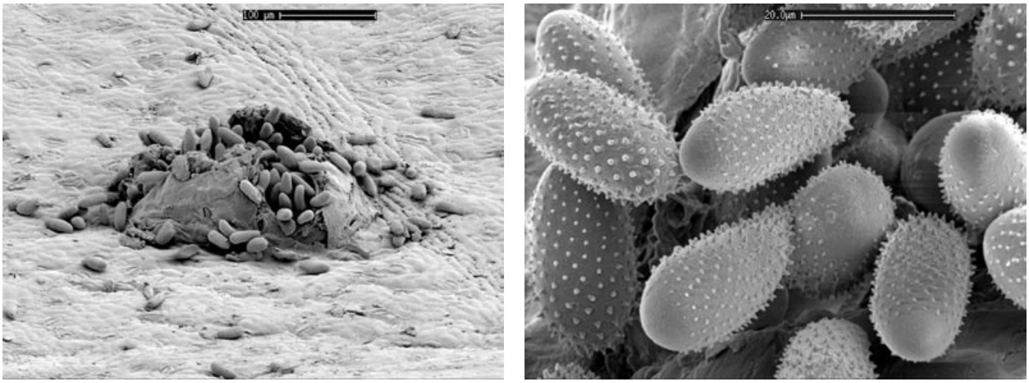

Figure 6. (left) Spores of *Melampsora* erupting from the surface of a poplar leaf and (right) close-up view.

In summary, the modern forester or timber industry researcher can view microscopic things with clarity and magnification unheard of previously and, as a consequence, their respective fields have progressed into far more precise sciences. As a final example, consider the pollen of *Acacia* as it was able to be distinguished in 1887 and as it can be viewed today (Fig. 7).

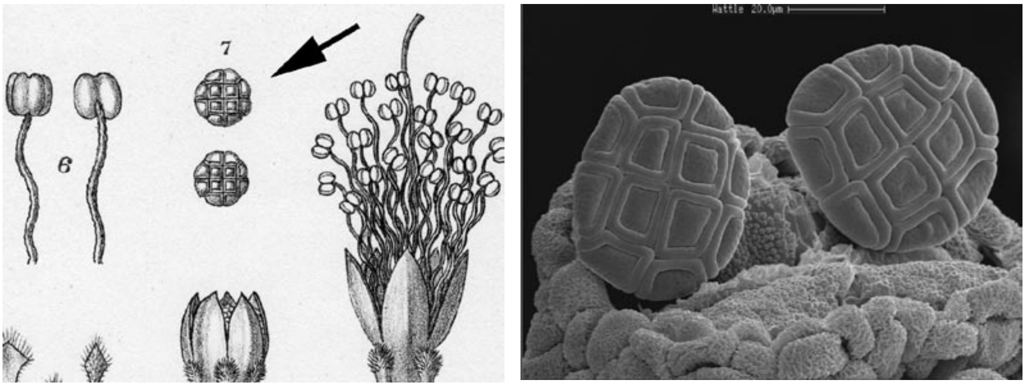

Figure 7. (left) Acacia pollen (arrowed) as drawn by Baron Ferdinand von Mueller (1825-1896) and as seen by a modern scanning electron microscope.

5 CONCLUSIONS

The work of modern microscopists is certainly far removed from those of earlier times who persevered with instruments of low power and poor resolution, used oil lamps to illuminate their specimens and had to draw pictures in order to record what they saw. One thing has not changed in more than two centuries, however. The motive for using microscopes in order to understand the workings of the forest and in timber research remains as it has always been. That is the usefulness and delight in being able to see, and show to others, those small but vital parts of nature that are otherwise invisible to the eye.

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