Lyctine (Coleoptera: Bostrichidae) pests of timber in Australia: A literature review and susceptibility testing protocol

B.C. Peters[1], J.W. Creffield[2] and R.H. Eldridge[3]

[1]Queensland Forestry Research Institute, PO Box 631, Indooroopilly, Queensland 4068, Australia
Email: Brenton.Peters@dpi.qld.gov.au
[2]CSIRO Forestry and Forest Products, Private Bag 10, Clayton South, Victoria 3169, Australia
[3]State Forests of NSW, PO Box 100, Beecroft, New South Wales 2119, Australia

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Summary

Several species of lyctine (powderpost) beetle are able to attack a range of hardwood timbers in Australia. Powderpost beetles infest only the starch-containing sapwood of certain hardwoods and do not infest softwoods. Attack by powderpost beetles on susceptible timber in Australia is almost inevitable and may continue until the food resource is completely utilised. Prevention of powderpost beetle attack is preferable to curative measures.

The Australian hardwood resource is increasingly being obtained from younger regrowth and planted forests rather than mature forests. The hardwood resource is also beginning to include species not previously used. There is no information on lyctine susceptibility of these species of eucalypts, hybrid eucalypts and some acacias. Some of these timbers are not widely utilised, yet may have unique properties for high-value niche applications on the world market. Consumer legislation places constraints on the sale and use of susceptible timber in the States of New South Wales and Queensland. Consequently, most of these timbers are regarded as provisionally susceptible in both States due to the lack of testing and historical record.

We review the biology, behaviour and management of the most common lyctine species *Lyctus brunneus* (Stephens) and discuss selected literature. A sampling and testing protocol to establish lyctine susceptibility of timber species is described for the first time in Australia, and its usefulness and limitations are discussed.

Keywords: reviews; insect pests; forest pests; control; hardwood susceptibility; testing protocol; bioassay; biology; wood borers; powderpost beetles; *Lyctus brunneus;* Australia

Introduction

Lyctine beetles belong to the sub-family Lyctinae in the family Bostrichidae. Bostrichids cannot produce cellulases and thus are restricted to sapwood, which the larvae reduce to a fine, soft powder, earning them the name powderpost beetles. The native bostrichid fauna in Australia includes several Lyctinae: *Lyctus*, *Lyctodon*, *Trogoxylon*, *Tristaria* and *Minthea acanthacollis* (Lawrence and Britton 1991). The sapwood of many Australian seasoned hardwood timbers is susceptible to attack by lyctine beetles (Bootle 1983; Brennan 1990; Creffield *et al.* 1995; Creffield 1996). The most common lyctine found attacking timber-in-service is *Lyctus brunneus* (Stephens) (Anon. 1935; Bootle 1983; Peters *et al.* 1996). The two most authoritative documents on susceptibility of the sapwood of Australian hardwoods to attack by lyctines are CSIRO Division of Forest Products (1950) and Fairey (1975). The former listed the lyctine susceptibility ratings for more than 500 species of hardwood timbers used throughout Australia, and the latter gave ratings for more than 250 commercial timbers used in New South Wales (NSW). Generally, these ratings refer to the susceptibility of sapwood to attack by *L. brunneus* under Australian conditions, and include both laboratory testing and information supplied by officers concerned with the utilisation of wood. Based on these ratings, the States of NSW (*Timber Marketing Act 1977*, TMA) and Queensland (*Timber Utilisation and Marketing Act 1987*, TUMA) place constraints on the sale and use of susceptible timber.

The Australian hardwood resource is, however, increasingly being obtained from younger regrowth and planted forests rather than mature forests. Creffield *et al.* (1995) conducted laboratory bioassays on the susceptibility of air-dried sapwood specimens, from trees of both regrowth and mature karri (*Eucalyptus diversicolor* F. Muell.) and jarrah (*E. marginata* Donn ex Sm.), to attack by *L. brunneus.* A revision of previously published ratings for both karri and jarrah was recommended. Furthermore, these authors recommended that a re-assessment of the susceptibility to *L. brunneus* of all commercially available hardwood timber species be undertaken, particularly if use of regrowth resource of these species is intended.

The hardwood resource is beginning to include species not previously utilised. There is substantial scope for inland Australian farmland to create new income streams from limited sustained harvesting and use of several naturally occurring timbers in the region. For instance, as part of the NSW Salinity Strategy, State Forests of NSW has recently initiated studies on the selection of tree species and the economic value of trees in the 500–700 mm rainfall zone of NSW. The Australian Low Rainfall Tree Improvement Group is intending to produce improved stock of a range of dryland eucalypts. Species of interest from these studies, for which there is no information on lyctine susceptibility, include *E. argophloia* Blakely, *E. cladocalyx* F. Muell., *E. occidentalis* Endl. and *E. sideroxylon* A. Cunn. ex Woolls subsp. *tricarpa* L. Johnson as well as a range of hybrid eucalypts and *Acacia mearnsii* de Willd. Some of these timbers are not widely utilised, yet may have unique properties for high-value niche applications on the world market.

One issue that has emerged from an effort to establish basic resource and market information on these selected native hardwoods is the determination of lyctine susceptibility. Processing and marketing of these timbers would become more expensive and complicated if there were a legal obligation under either TMA or TUMA to treat the sapwood with preservative, especially if it were not necessary. Currently, most of these timbers are regarded as provisionally susceptible due to the lack of testing and historical record, but recent testing (Peters and Fitzgerald, unpublished data) has indicated that some species may be non-susceptible. For this reason, a testing procedure to establish lyctine susceptibility is necessary.

We examine aspects of the biology, behaviour and management of *L. brunneus* in Australia, the most common lyctine species, drawing largely on reviews by Graf (1987), Brennan (1990) and Wylie and French (1991) and selected literature from elsewhere. Other species are also discussed in less detail. For the first time in Australia, a sampling and testing protocol to establish lyctine susceptibility of timber species is described.

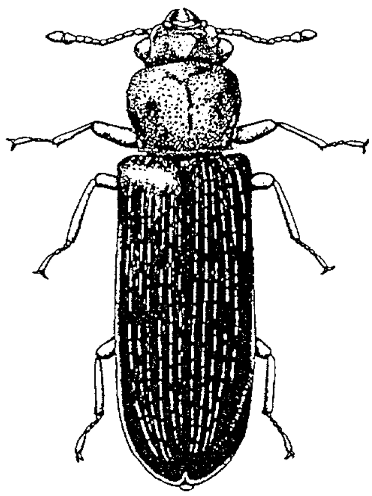

Figure 1. Adult powderpost beetle *Lyctus brunneus*

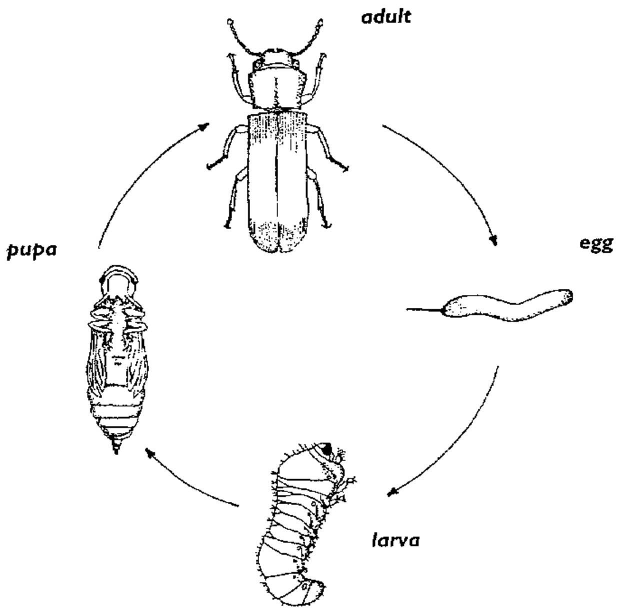

Figure 2. Life cycle of powderpost beetle. (Figures 1 and 2 reproduced from Queensland Department of Primary Industries publications.)

The powderpost beetle *Lyctus brunneus* (Stephens)

Description

The powderpost beetle is a cosmopolitan species which appears to have been first recorded from Brazil (Roughley and Welch 1929). Adults are up to 7 mm long, dark brown, shiny, flattened, elongate insects (Gerberg 1957a) (Fig. 1). They have a distinct head and the terminal segments on their antennae have a clubbed appearance. Larvae are cream coloured with brown head and jaws and three pairs of small jointed legs; on hatching, they are about 0.5 mm long and straight-bodied but later become C-shaped (Fig. 2). Iwata and Nishimoto (1981, 1982) give details of the external morphology and surface structure of *L. brunneus* eggs, larvae, pupae and adults.

Biology

Powderpost beetles are pests of the sapwood of certain hardwood timber species. Different species display minor differences in appearance, habits and longevity (Froggatt 1926).

After mating, the female beetle seeks a suitable place for egg laying and bites the wood transversely, leaving a series of grooves ('tasting marks') on the surface (Hickin 1975). These tasting marks may serve to determine whether the timber contains starch, the essential larval dietary requirement, and they also expose wood pores for subsequent egg laying (Fisher 1929; Parkin 1936; Gay 1953; Bletchly 1960a; Rosel 1969a). According to Ito and Hirose (1978) and Ito (1983), both males and females make tasting marks. Using her ovipositor the female lays into the open pores of the sapwood. In *L. planicollis* LeConte each egg takes about 45 s to be laid (Smith 1956). Each female may lay a total of 70 eggs, with a usual limit of three eggs in any pore (Gay 1953). The number of eggs laid is positively related to the number of tasting marks (Ito and Hirose 1978). Eggs are deposited at depths of 1.0–6.5 mm in the wood pores, preferentially from a transverse surface, but also through radial and tangential faces (Gay 1953).

Eggs hatch after about 14 days and larvae feed on the starch in the sapwood until fully grown (Beesley 1956). Tunnels usually follow the grain of the wood and only the larval stage destroys timber (Froggatt 1903). The development period for larvae varies from 2–18 months depending on temperature, humidity and the supply of starch in the sapwood. Under adverse conditions the life cycle may take from 30–48 months or longer in the Northern Hemisphere (Gerberg 1957b).

Fully-grown larvae tunnel towards the wood surface and excavate small oval cells where pupation takes place. Two to three weeks later, mature beetles begin to emerge through the surface of infested timber, making a round hole (1–2 mm diameter) as each emerges. Unlike the damage of ambrosia beetles, these exit holes have no staining around their margins (Eldridge and Fairey 1974). Small piles of frass associated with the emergence holes may collect on the surface of infested timber or fall nearby. Emerging adults push a small amount of frass out, but larvae moving within the sapwood also cause frass to continue to fall from emergence holes and from cracks in the timber. Larvae can also cause frass to fall from cracks in the timber in the absence of adult emergence holes. Adult lyctines are sexually mature upon emergence. Copulation occurs soon afterwards, often crepuscularly or nocturnally rather than diurnally (Gerberg 1957a). Egg laying begins soon thereafter. Emergence holes expose pores into which eggs can be laid (Anon. 1935). Reinfestation of timber is common and may continue until the food resource is completely used up (Veitch 1933), usually within 4–5 y of felling.

Damage

Lyctine beetles attack only the sapwood of certain hardwood (pored) timbers and do not generally attack softwood (non-pored) timbers (Boas 1947). However, the kauri pines *Agathis robusta* and *A. palmerstonii* are attacked by *L. brunneus* under restricted natural conditions: the presence of pith or bark in combination with abnormally high starch levels (Heather 1970). Attack by *L. africanus* Lesne on pine timber was induced by impregnation with soluble starch (Khalsa *et al.* 1962, 1965). Oviposition occurred in resin canals and on the surface, but larval development was limited by the small amount of starch added. Successful breeding was reported in an artificial 'biscuit' medium of wheat flour and yeast. Generally, lyctine beetles are readily cultured and methods for producing a large and continuous supply of test beetles which are freshly emerged, easily collected and free from parasites are based on Harris and Taylor (1960) in Great Britain, and Rosel (1962) in Australia. Other examples of culturing methods are Kuhne (1981) and Iwata and Nishimoto (1982).

Evidence of infestation

In Australia, attack by powderpost beetles on susceptible timber is almost inevitable. Most attack takes place at the sawmill, in logs or sawn timber that are drying (Tooke 1953). Evidence of infestation may not become apparent until the timber is in service and adults begin to emerge, usually within the first 18 mo of service life. Complaints from consumers result and the reputation of the supplier may suffer (Anon. 1959). Infested timber contains numerous galleries packed with fine powdery frass. The whole of the infested sapwood may be reduced to powder leaving only a shell of wood on the outside, perforated by emergence holes. Small piles of frass may be found where a gallery has broken the surface or where an adult beetle has emerged. The frass is smooth and floury (not gritty) when rubbed between the fingers and may continue to accumulate for many years after infestation has ceased. Infestation may occur anywhere in the structure where susceptible timber has been used (for example, in sub-floor areas, living space, roof space, or in furniture and artefacts). In new houses, emergence holes may appear in the lining materials (for example, in plasterboard and panelling) and joinery (Forests Commission, Victoria 1981). Adults emerging from the hardwood framing beneath make such holes. Borer damage to supporting timber in mines can be a major cause of timber failure (Yule and Kennedy 1978).

Wood variables affecting susceptibility to the powderpost beetle

Generally, three variables govern susceptibility to lyctine attack in sapwood: pore size, starch content and moisture content (Cymorek 1966).

Pore size

Only hardwoods have pores and lyctines attack only the sapwood of hardwood species with pores larger than the diameter of the ovipositor of the female. Susceptible hardwood species were originally thought to have pores larger than the diameter of the egg (Clarke 1928, 1929; Fisher 1929). Now, however, the pore–ovipositor relationship (Parkin 1934) is the favoured hypothesis (Abood *et al.* 1993). The average diameter of the ovipositor of *L. brunneus* is about 80 μm (Tooke and Scott 1944) with a minimum of 56 μm (Parkin 1933). No timber species with vessel diameters < 65–70 μm were susceptible to *L. brunneus* attack (Hedjazi and Soleymani 1967). In India, the oviposition of *L. africanus* on the tangential and radial faces of 52 species of timber was studied (Chowdhury 1933); eggs were laid only in those pores in which both tangential and radial diameters were more than 130 μm, the maximum diameter of the eggs. In China, Shi and Tan (1987) listed 168 tree species in 103 genera and 39 families attacked by 8 species of Lyctinae: *Lyctus brunneus*; *L. africanus*; *L. sinensis*; *L. linearis*; *Minthea rugicollis*; *Lyctoxylon japonum*; *Trogoxylon impressum* and *T. aequale*. Species with pores < 25 μm (e.g. Aquifoliaceae, Theaceae, Hamamelidaceae and Symplocaceae) were not susceptible to attack by these lyctines. The minimum pore diameter for oviposition of *L. brunneus* in Australia is about 90 μm (Cummins and Wilson 1934).

Starch content

Starch is the main food reserve substance of the plant and is present in the sapwood only (Wilson 1932). The quantity varies from tree species to tree species, from tree to tree, within the tree, from year to year, and, importantly, from season to season in all trees and particularly in deciduous species (Wilson 1935). For Northern-hemisphere hardwoods, starch is most abundant in winter (Wilson 1932). If felled timber is kept as a log for about a year, the starch is depleted completely from the sapwood, rendering it immune to lyctine infestation (Wilson 1932; Bletchly 1960b).

Susceptible timber species must contain sufficient starch to nourish the developing larvae (Taylor 1951). The heartwood is never infested, although adults may emerge through it. Lyctine larvae cannot digest the constituents of the cell wall (Campbell 1929) and starch is the principal component of their food (Wilson 1932, 1933). Polysaccharides can be converted effectively into sugar by the gut enzymes of lyctine larvae and used for growth and development (Campbell 1935). The degree of lyctine attack is therefore related to the starch concentration (Cummins and Wilson 1935; Bamber and Erskine 1965). Natural variation in the starch content (Bamber and Humphreys 1961) may result in the starch content being below a threshold level which can support lyctine infestation, although the thresholds are undefined.

About 55% of the 116 species listed by the NSW Division of Wood Technology as resistant to *L. brunneus* had a mean pore diameter >90 µm (Bamber and Erskine 1965). The standard deviation increased with increasing mean pore diameter. Small pore size cannot therefore be a cause of resistance, and low starch content of the sapwood was thought to be a probable factor (Bamber and Erskine 1965). *Lyctus discedens* Blackburn can attack timbers with a pore size smaller than that required for *L. brunneus*. Consequently, the starch content of the wood is the predominantly important factor governing lyctine attacks in northern Queensland, where *L. discedens* occurs (Brimblecombe 1947a).

The distribution of starch along and around the bole of a spotted gum (*E. maculata* Hook.) tree was studied experimentally; samples taken at breast height from normally growing trees indicated the starch content throughout the bole at the time of sampling (Brimblecombe 1940). However, at least two samples taken on different aspects were recommended for reliable results. The starch concentration in the sapwood of eucalypts decreases above breast height (Hillis *et al*. 1962), but not in Western Australian sheoak trees *Allocasuarina fraseriana* (Miq.) L. Johnson (Creffield *et al.* 1987). Timber species in Queensland, which normally store a large amount of starch in the sapwood, do not naturally reduce the starch content to the level of *Lyctus* immunity (Brimblecombe 1945).

Brimblecombe (1961) studied eleven species of *Eucalyptus* and two of *Lophostemon* (*Tristania*) and showed that trees of the species known to be moderately or heavily attacked by *L. brunneus* contained abundant starch throughout the sapwood continuously during the year. The species liable to light attacks contained sufficient starch to support the insects only during periods of peak starch content. Generally, primary peaks in starch content occurred during the summer and secondary peaks in late winter. Most species showed a higher primary peak in alternate years. Lowest starch content occurred in late autumn or early winter and in this period some species were devoid of starch. Similarly, Humphreys and Humphreys (1966) demonstrated that the sapwood starch content of flooded gum (*E. grandis* (Hill) Maiden) followed a pattern of seasonal variation resembling that of other coastal species, reaching a peak in spring and early summer and the lowest point in late autumn. There was no relationship between starch content and flowering of eucalypts (Brimblecombe 1946a). A chemical test, using iodine, for the detection of starch in sapwood is presented in Australian Standard AS 1604.1 (2000, Appendix A). Creffield *et al.* (1995) used the intensity of colouration caused by the iodine reaction to grade the concentration of starch present in the sapwood of karri into not detected, low, medium or high; older trees were found to contain more starch than younger trees.

Moisture content

Wood with about 15% moisture content is most suitable for development of larvae, although the female beetle will lay within the range 8–25% (Parkin 1943a; Bootle 1983). Howick (1968) suggested a more restricted range of 10–20%. Eggs can be laid and larvae will feed and develop in comparatively moist timber (Fisher 1928). Beeson and Bhatia (1937) concluded that the larvae of *L. brunneus* would thrive within the range of 10–50%, the higher moisture content being the more favourable, and that wood of less than 10% moisture content would not be attacked. Higher moisture content increases total beetle emergences, but does not affect average time till emergence (Smith 1955).

Economic species

Of the species found in Australia, *L. brunneus* is the most destructive and most commonly encountered. Others include *L. discedens* Blackburn, *L. planicollis* LeConte, *L. parallelocollis* Blackburn and *Tristaria grouvellei* Reitter. Campbell (1963) recorded observations on the biology, especially pre-oviposition behaviour, oviposition, larval eclosion and duration of life cycle, of *T. grouvellei*. The Malayan powderpost beetle *Minthea rugicollis* (Walker), which was often found in rainforest hardwoods imported from South-East Asia (Froggatt 1920, 1924), is now established in Queensland. *Minthea rugicollis* is an apparently tropicopolitan species (Gerberg 1957a) of particular importance in Malaysia (Browne 1939), where it causes extensive damage to rubberwood (Norhara 1981; Abood *et al.* 1993). Exotic lyctines are frequently intercepted at the Port of Brisbane (Wylie and Yule 1977; Wylie and Peters 1987). A house in Cairns, Queensland, was fumigated in 1999 for *Minthea reticulata* Lesne, a species often mistaken for *M. rugicollis* (Ho 1993) (J. King *pers. comm.*). Similarly, a house in Bowen, Queensland was fumigated in 1998 for *L. africanus* (J. King *pers. comm.*).

Susceptibility lists

Cause *et al.* (1974, 1989), McDonald (1983) and Norton and Fett (1998) listed the lyctine susceptibility of Queensland timbers and Diehm (1987) the susceptibility of carving and turning timbers in Queensland. In NSW, Worley (1953), Fairey (1975) and the Forestry Commission of NSW (1987, 1988) published susceptibility lists. The CSIRO Division of Forest Products (1950), Kloot (1965) and Keating and Bolza (1982) have published national lists. Watson and Higgins (1950) listed the lyctine susceptibility of about 50 species of Australian timbers suitable for veneer production. Bolza and Keating (1972) produced a list for African timbers, Alston (1968) and Anon. (1969) for Fiji timbers and Calora (1973) for Philippine timbers.

Management of infestation

Overseas, four processes based on biological requirements of the pest have been developed as management strategies. Considerable reduction or complete depletion of the starch in green timber has minimised lyctine infestation and was achieved by: ringbarking ('girdling') of the standing tree; storing logs in the round with bark intact; storing logs under water; and special kiln treatment of converted stock (Parkin 1938a,b, 1943b; Phillips 1938; Parkin and Phillips 1939; Becker and Loebe 1961; Harris 1961; Esenther 1964).

In Australia, immunity from *Lyctus* infestation was easily and cheaply obtained in lemon scented gum and spotted gum trees in 6 and 8 months, respectively, by a light ringbarking at the top of the commercial bole (Brimblecombe 1947b). No trees died before depletion of the starch below the ring, and some lived for several months after depletion of starch had occurred. Wartime demands for timber necessitated the holding of large reserves of logs so that sawmills could always be kept working at full capacity. To protect the logs against *L. brunneus* attack and to minimise end splitting, bark was kept intact where possible and any exposed wood was sprayed with a hot creosote emulsion and afterwards coated with warm crude petroleum jelly (Brimblecombe 1946b). These treatments prevented serious degrade of logs for up to 22 mo in storage. Similarly, Humphreys and Humphreys (1966) showed that if logs are left with their bark on for one month or more, the chance of *E. grandis* sapwood being attacked by *L. brunneus* was very low. Starch levels in living karri are strongly influenced by rainfall, and they decline more rapidly in girdled trees than in those felled and left with an intact crown (Simpson and Barton 1991). These authors recommended that young trees should be felled during seasons when starch levels are low and storage procedures should be adopted that promote rapid loss of starch in order to reduce susceptibility to lyctine infestation. However, starch resorption induced by high ringbarking has not developed past the experimental stage as its costs and reliability compare unfavourably with immunisation by chemicals (Taylor 1955; Bamber and Erskine 1965).

Remedial treatments

Heat (French and Johnstone 1968), freezing (Peters *et al.* 1996) and fumigation (Burden and McMullen 1952) are effective remedial treatments, but do not provide protection against reinfestation. However, timber so treated, usually a manufactured article, can be protected by restoring the finish or by applying paint, varnish or wax polish to all exposed surfaces soon after treatment (Snyder 1946; Gardner *et al.* 1984). Registered residual insecticide can be sprayed, brushed or injected (Brimblecombe 1951) to the affected parts of unfinished timber surfaces (Ito *et al.* 1976; Creffield *et al.* 1983). Aqueous ammonia has also been used (Rosel 1971). Tamblyn and Rosel (1979) reported that in most timber species, spray treatment gives only negligible penetration on the side faces. They concluded that none of the insecticides tested was effective, and prevention of powderpost beetle infestation, using preservation processes, is preferable.

Preservation

The problem of marketing many rainforest timbers, often with wide sapwoods, has long been met by legislation requiring complete penetration of all susceptible wood with approved preservatives, or alternatively the limitation of susceptible wood to a defined maximum in building timbers (Tamblyn and Rosel 1979). In addition, the use of glue-line additives is the best method for protecting plywood from powderpost beetles (Tamblyn and Gordon 1950; Taylor 1968; Van Acker *et al.* 1990). Preservatives have included creosote (Froggatt 1925), arsenicals (Cummins and Wilson 1936; Rosel 1969b) and borates (Brimblecombe and Cook 1945; Cokley 1948a,b, 1951, 1960, 1995; McGregor 1958; Forestry Commission of NSW 1978; Williams and Mauldin 1985; Williams and Amburgey 1987; Cookson *et al.* 1998). Others (Moffat 1994) are now included in the relevant legislation following laboratory testing using the methods of, for example, Taylor (1961), European Standard (1992, 1993) and Australasian Wood Preservation Committee (1997).

Consumer legislation placing constraints on the sale and use of such timber was first enacted in NSW in the 1945 Timber Marketing Act, TMA, amended in 1977, and in Queensland in the 1949 Timber Users' Protection Act, amended in 1987 to the Timber Utilisation and Marketing Act, TUMA. Other States do not have similar legislation.

Generally, under TMA and TUMA,

- the sale of lyctine-susceptible timber is restricted;
- the use of lyctine-susceptible timber in the manufacture of articles is an offence;
- the sale of an article containing susceptible timber is an offence;
- the use of lyctine-susceptible timber in a building is restricted or prohibited; and
- the sale of a building containing lyctine-susceptible timber is restricted or prohibited.

In NSW, the sale of framing timber of which more than 25% of the perimeter of any cross-section comprises untreated lyctine-susceptible timber is an offence. In Queensland and NSW, all timber sold as impregnated with an approved preservative (immunised) at a timber preservation plant, must carry a registered brand. Imported timber and re-milled timber sold in these States as immunised must also carry a brand. With increasing quantities of timber being imported into Australia, both locally grown and imported stock will require assessment. Some other provisions apply to each Act, especially with regard to moisture content (Australasian Furnishing Research and Development Institute 1997).

Neither TMA nor TUMA has provision for remedial treatments. Therefore, remedial treatment will not absolve the parties concerned from any legal liability under legislation.

Discussion

Authoritative documents on susceptibility of the sapwood of Australian hardwoods to attack by lyctines use the categories of 'immune' and 'not susceptible, NS' through to 'susceptible, S' (CSIRO Division of Forest Products 1950; Fairey 1975). Within this range, categories of 'rarely susceptible, RS' and 'moderately susceptible, MS' were used. Cause *et al.* (1989) listed all species legally recognised under Schedule 2 of TUMA as being non-susceptible with the symbol 'NS'. For the purposes of TUMA, all species not so scheduled are '*legally*' (but not necessarily in fact) lyctine-susceptible. Cause *et al.* (1989) listed these species with the symbol '(s)', except where laboratory tests have confirmed susceptibility, where the symbol 'S' was used. Creffield *et al.* (1995) used four categories: 'not susceptible (NS)', 'slightly susceptible (S1)', 'moderately susceptible (S2)' and 'highly susceptible (S3)'. Two themes emerge from these works: there is a gradation of lyctine susceptibility and there is no process for testing and establishing the category of 'not susceptible (NS)'. Importantly, Fairey (1975) noted that the trees used in the earlier CSIRO work were selected at random with no attention being given to whether or not the sapwood contained starch.

Details of a sampling and testing protocol to establish lyctine susceptibility of timber species, based on this literature review, are given in the Appendix. Whilst criteria by which the lyctine susceptibility of timbers can be assessed are provided, we acknowledge that the extent of field collection in space and time must be extensive before the category NS can be assigned. Practical experience from the market-place will be difficult to obtain if preservative treatment of sapwood entering the market-place is a legal requirement.

Changing the status of species legally recognised as being non-susceptible under TMA and TUMA is not merely a technical decision, but involves commercial and regulatory consideration. For example, Tasmanian oak *E. obliqua* S, mountain ash *E. regnans* NS, and alpine ash *E. delegatensis* S are sold commercially as Tasmanian oak. Under TUMA, all are categorised as susceptible because they cannot be readily distinguished anatomically from one another and the susceptibility of alpine ash varies across its natural habitat: in Tasmania, S; NSW, S; and Victoria, NS (Fairey 1975). Additionally, preservative treatment of sapwood rated S1 may be important for high value niche applications, but not for framing timber. The sampling and testing protocol is, however, likely to provide a useful tool for establishing lyctine susceptibility of timber species generally not currently utilised or being obtained from younger regrowth forests.

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Appendix: Sampling and testing protocol to establish resistance of timber species to lyctine (powderpost) beetles

Foreword

A field sampling method and a laboratory testing method, which provide a basis for the assessment of natural susceptibility of timbers to lyctine attack, are described in this protocol. Lyctine (powderpost) beetles (Coleoptera: Bostrichidae) infest only the starch-containing sapwood of certain hardwoods. Generally, three conditions govern susceptibility in sapwood: pore size, starch content and moisture content. Only hardwood species with pores larger than the diameter of the ovipositor of the female beetle are infested. In Australia, hardwood species with pores < 90 µm appear resistant to *Lyctus brunneus* (Stephens). Susceptible timber species must contain sufficient starch to nourish the developing larvae. The quantity of starch present varies from tree species to tree species, between trees, within the tree, from year to year, and, importantly, from season to season. Variation in the starch content may result in a starch level below the threshold necessary to support lyctine infestation. The collection, handling and preparation of timber samples for a bioassay to assess the natural susceptibility of timbers to lyctine infestation are described.

The species *Lyctus brunneus* has been chosen as the test species because of its cosmopolitan occurrence as an important wood destroying pest and because it can be readily cultured in the laboratory. If, for particular reasons, other lyctines are required, for example *Lyctus discedens* Blackburn or *Minthea rugicollis* (Walker), the same test procedure can be used.

Criteria by which the lyctine susceptibility of timbers can be assessed are provided. In making this assessment, the geographic extent of field collection and temporal variation should be taken into account. Results from the bioassay should be compared with practical experience, where possible.

Section 1. Scope and general

1.1 Scope and application

A field sampling method and laboratory testing method, which provide a basis for the assessment of natural susceptibility of timbers to lyctine attack, are described in this protocol. *Lyctus brunneus* (Stephens) is used as the test powderpost beetle. This protocol is intended for use by the timber industry, assessment providers and approval authorities where the lyctine susceptibility of a timber is unknown or uncertain. Results from this protocol should be compared with practical experience, where possible. Assessments shall be undertaken by providers having the appropriate facilities, and shall be carried out by persons having relevant experience and expertise in the methodology of bioassays with insects.

1.2 Referenced documents

The following documents are referred to in this protocol.

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1.3 Definitions

For the purposes of this protocol the definitions below apply.

Experimental control**:** An element of an assessment procedure that is included to demonstrate the extent of lyctine response in a lyctine-susceptible timber test specimen.

Lyctine resistance**:** The absence of lyctine susceptibility.

Lyctine susceptibility**:** The demonstration of positive results in the laboratory bioassay, demonstrated by damage to the timber sample by lyctine feeding.

Regions: Differing areas in the natural, or plantation, range of a timber species.

Test specimen**:** A specimen prepared by drying and size reduction from the timber sample and on which the test is actually carried out.

Timber sample**:** A sample of timber, collected in the field, as prepared for sending to the laboratory and intended for inspection or testing. Equivalent to laboratory sample in Australian Standard AS 2929-1990.

Timber species**:** The botanical taxon of species, subspecies, cultivar, clone or hybrid used to describe a timber entity.

Section 2. Field sampling and laboratory testing

2.1 Scope of section

This section sets out the methods for determining the susceptibility of hardwood timber sapwood to lyctine attack. All tests done in accordance with this protocol shall be detailed in a report, including any variations of this protocol.

2.2 Principle

The bioassay involves exposure of test specimens to adult *Lyctus brunneus* under controlled laboratory conditions, and should include specimens prepared from a known susceptible timber as a control. *Lyctus brunneus* infests only the starch-containing sapwood of certain hardwoods with pores >90 µm. Natural variation in the starch content can inhibit lyctine infestation. Field collections of timber samples covering the species' geographic range and allowing for temporal variation may be necessary to allow for this variation. Depletion of starch content after sample collection can inhibit lyctine infestation. Accordingly, the appropriate collection, handling and preparation techniques of timber for a bioassay must be applied.

2.3 Field sampling

2.3.1 Location

Timber samples shall be collected from regions throughout the natural distribution and plantation estates of the species.

Note: Differences in lyctine susceptibility of some timber species collected from the natural distribution and plantation estates have been demonstrated by Creffield *et al.* (1995). Many tree species exhibit genetic variation in various traits between and within populations from different areas in the natural, or plantation, range (regions). Therefore, it is necessary to sample more than one region, especially in wide-ranging species with disjunct distributions. Three or four regions should be sampled, preferably near the limits and in central parts of the range.

2.3.2 Timing

Timber samples shall be collected on sampling occasions at three-monthly intervals until lyctine resistance NS or lyctine susceptibility is demonstrated in the bioassay, or for two years, whichever comes first (see Clause 2.4.6).

Note: Lyctine susceptibility can vary from season to season and from year to year. Sample collections in January, April, July and October are suggested for regions with four seasons. In other regions, different sampling times may be more useful.

2.3.3 Field starch determination

The sapwood of each intended timber sample shall be assessed for starch concentration in the field using a semi-quantitative iodine test. Timber samples should be collected only when starch is detected.

Note: A suitable semi-quantitative iodine test is described in Australian Standard AS 1604.1 (2000, Appendix A). A hand lens (×10) may be necessary for detecting starch granules in wood.

2.3.4 Number of timber samples

At least two timber samples, one from the north side and one from the south side, shall be collected from each tree. Each sample shall extend from the bark to the heartwood. The minimum longitudinal dimension of the samples shall be 100 mm. Samples may be obtained from a standing tree at breast height or from a felled tree (only within 48 hours of felling because the starch content decreases with time in a felled tree) prior to conversion of logs. Samples shall be debarked immediately in the field.

Note: The timber samples may be cut from a scarf or a disk. Scarfs or disks may be cut from the butt, middle and top of the tree trunk, consistent with milling practices and saw-log dimension.

Timber samples shall be collected from at least ten trees from one region or from at least five trees from each of three or four regions during each sampling occasion (see Clauses 2.3.1 and 2.3.2). Samples shall be stored in a cool dry environment, to avoid sweating, prior to despatch.

Note: Sweating of the timber sample may enhance fungal or mould growth, decreasing starch reserves in the sample.

2.3.5 Timber sample records

Bone fide confirmation of tree species identification shall be recorded for each tree. An audit trail shall be maintained for each timber sample.

Note: The collection data should include tree species identification, name of collector, date of collection, location description (Global Positioning System (GPS) co-ordinates may be useful), site description, width of sapwood band, results of field starch determination and relevant comments. The age of the tree may be important (Creffield *et al.* 1995).

2.4 Laboratory testing

2.4.1 Timber sample drying

The timber samples shall be kiln dried rapidly to equilibrium moisture content (EMC) as described in the NOTE, thereby minimising fungal growth and starch depletion. The time taken will depend on the dimensions of the sample.

Note: To avoid unnecessary starch depletion, timber samples should be low-temperature kiln dried. A drying schedule whereby green timber samples are initially exposed to a temperature of 25°C, rising to no more than 50°C, should be adopted. Reference to the most suitable low-temperature drying schedule for the candidate timber substrate should be sought (AFRDI 1997).

2.4.2 Test specimen preparation

Test specimens, containing the full sapwood depth, shall be cut from each timber sample using a tungsten-tipped saw. Test specimens shall be free of knots and kino gum. Test specimens should be at least 25 mm wide × the full sapwood depth × 100 mm long in the direction of the grain.

Note: The test specimens will be irregular in shape, and, owing to variable sapwood depths, contain different sapwood volumes.

2.4.3 Laboratory pore size determination

Where there is little information on the pore size of the timber species, measurements shall be made. The tangential diameter of 75 pores in a section of sapwood from a minimum of ten test specimens shall be measured and the individual measurement for each pore recorded (see Bamber and Erskine 1965). From these measurements the mean diameter and the standard deviation shall be calculated. Where the mean pore size of the timber species is <70 µm the timber species shall be deemed to be lyctine-resistant NS, for the purposes of this protocol, and there is no need for further testing.

2.4.4 Laboratory starch determination

The sapwood of each test specimen shall be assessed for starch concentration in the laboratory using a semi-quantitative iodine test and recorded. The concentration of starch present shall be assessed with the aid of a light microscope. Using the intensity of colouration caused by the iodine reaction, the starch concentration should be graded as not detected, low, medium or high.

Note: A suitable semi-quantitative iodine test is described in Australian Standard AS 1604.1 (2000, Appendix A). The grading of low, medium or high is subjective.

2.4.5 Bioassay materials

(a) *Bioassay chamber*: Glass jars shall be used as bioassay chambers. Suitable material to act as a beetle foothold shall be added to the bioassay chambers. One test specimen shall be placed into each jar and allowed to condition to an EMC (11–16%) in an insectary before being inoculated with *L. brunneus* adult beetles. Controls shall be in separate jars.

Notes:

1. Glass jars (1.2 L vol., 100 mm × 100 mm × 150 mm high) are suitable.
2. A thin layer of forest loam is a suitable beetle foothold.
3. Jars containing soil should be heat sterilised.
4. Test specimens can be conditioned to an EMC of 14% in an insectary (26°C, 70% relative humidity) for at least 7 days. (Source: Creffield *et al.* 1995).

(b) *Bioassay chamber lid*: The bioassay chambers shall have lids with a mite-proof seal. The lids shall contain a hole fitted with a mite-proof membrane, for example filter paper, which allows aeration and permits the test specimens to be maintained at EMC.

Notes:

1. A screw-top metal lid fitted with a paraffin-wax-impregnated-cardboard wad is suitable.
2. A 30 mm-diameter hole punched through the lid and the cardboard wad is suitable. (Source: Creffield *et al.* 1995).

(c) *Lyctine collection:* Recently emerged adult *Lyctus brunneus* adults that are robust and active shall be taken from culture at random and used in the bioassay.

Note: Culturing of *Lyctus brunneus* to obtain a regular supply of adults that have not already laid eggs requires care. Rosel (1962) and European Standard (1993) describe culturing techniques which experience has shown to be suitable.

(d) *Insectary*: An incubator (or room) shall be used with air circulation, and controlled at 26°C ± 1°C and 70% relative humidity (RH) ± 5% RH.

(e) *Experimental control test specimens*: At least one test specimen of known lyctine-susceptible sapwood shall be used as an experimental control during the bioassay to demonstrate continuing viability of the lyctine culture under the conditions of the bioassay. The experimental control test specimen should be at least 25 mm × 25 mm × 100 mm long in the direction of the grain.

2.4.6 Bioassay procedure

(a) *Inoculations with beetles*: Twenty recently-emerged *L. brunneus* beetles of unknown sex shall be placed onto each test and control specimen. This is the first inoculation. Three weeks after the first inoculation, a second inoculation shall be made.

Note: Replication of the inoculation provides an additional lyctine 'pressure' including, perhaps, more viable females. Additional control test specimen(s) (i.e. known susceptible species) should be included.

(b) *Bioassay duration*: The bioassay shall continue until adult emergence, or for at least 16 weeks, whichever occurs first.

(c) *Assessment*: At the termination of the bioassay, each test and control specimen shall be examined for adult emergence holes.

Note: Mature beetles emerge through the surface of infested timber, making a round hole (1–2 mm diameter) as each emerges. Small piles of frass associated with the emergence holes may collect on the surface of infested timber or fall nearby. Emerging adults push a small amount of frass out, but larvae moving within the sapwood also cause frass to continue to fall from emergence holes and from cracks in the timber. Larvae can also cause frass to fall from cracks in the timber in the absence of adult emergence holes.

In the absence of adult emergence holes, evidence of larval activity shall be examined by splitting each test specimen longitudinally and tangentially. Any larval channelling shall be recorded as number and length of tunnels. The extent of larval channelling shall be used as a semi-quantitative assessment.

(d) *Assessment criteria*: The assessment criteria shall be as follows.

1. The bioassay shall be deemed invalid if emergence holes are not present in the experimental control.

2. A rating of susceptible, S1 to S3, shall be given to test specimens when:
   (i) emergence holes are present, S3; or
   (ii) larval channelling is present, S1–S3 and further differentiated by the amount or intensity of channelling as:
   - slightly susceptible, S1, attack confined to a small amount of larval channelling, with channels at least 10 mm in length along the vessel;
   - moderately susceptible, S2, moderate amount of larval channelling; and
   - highly susceptible, S3, complete destruction of the sapwood with broad, frass-packed larval galleries.
3. A tentative non-susceptible rating (ns) shall be given when a test specimen has no larval channelling.

2.5 Assessment report

An assessment report shall be prepared in accordance with AS 2929, and shall include the following:

(a) Name of assessment provider
(b) Name of client
(c) Dates of initiation and conclusion of assessment
(d) Name of timber species assessed
(e) Details of field sampling and laboratory testing methods
(f) Results
(g) Discussion of results, conclusions and recommendation with respect to lyctine susceptibility of the timber species assessed
(h) Date of report
(i) Number of pages; e.g. page — of — .