IDENTIFICATION OF AUSTRALASIAN SPECIES OF WOOD-DECAY FUNGI — A NEW ZEALAND PERSPECTIVE

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(Received for publication 20 July 1989; revision 25 September 1989)

ABSTRACT

Identification of fruit-bodies of wood-decay fungi (mainly corticioid and polypore fungi) uses both macroscopic and microscopic characters, and the type of associated wood rot. Examination of the hyphal system of the fruit-body is particularly important. Identification of fungal cultures derived from decayed wood, in the absence of fruit-bodies of the fungus, is hindered by a lack of literature for Australasian species. Use of the Buller phenomenon may assist cultural identification. Herbaria and culture collections of wood-decay fungi are key resources for identification.

Keywords: wood decay; fungal cultures; corticioid fungi; polypore fungi; taxonomy.

INTRODUCTION

Wood-decay fungi are important natural components of indigenous forests, causing decay of fallen wood and of heartwood and sapwood of living trees. In New Zealand, with a declining harvest of wood from indigenous forests, the relative economic importance of losses due to fungal rots is decreasing, although the damage caused by wood-decay fungi in indigenous stands is generally not quantified.

Exotic plantations in New Zealand, mainly of *Pinus radiata* D. Don, are managed as short-rotation forests where wood decay in the standing tree is rarely a problem. However, on sites converted from indigenous forest, the decay fungi *Armillaria novae-zelandiae* (G. Stevenson) Herink and *A. limonea* (G. Stevenson) Boesewinkel, which survive saprophytically on dead stumps and roots, can cause a fatal root disease of young pines (van der Pas *et al.* 1983). Pines planted in an area where the decay fungus *Peniophora sacrata* G.H. Cunn. is established on native scrub species (e.g., *Leptospermum scoparium* J.R. et G. Forst.) may suffer group mortality (Gilmour 1966). Delay in shipping or processing of felled pine logs, especially during warm summer months, can lead to rapid invasion by *Phlebiopsis gigantea* (Fr.) Jülich with accompanying loss in value of logs. The wood-decay fungi on sawn timber in New Zealand are best documented for the wood of *Pinus radiata*, although in only some occurrences can the cause of the timber decay be determined (Butcher 1967, 1968). Plantings of other genera such as *Eucalyptus* and *Larix* are also susceptible to wood decay (Gadgil & Bawden 1981; Gilmour 1966; Hood 1986).

Biosystematic study of wood-decay fungi contributes to knowledge of the flora of New Zealand, and of interactions between organisms in the forest. Accurate

identification of wood-decay fungi is relevant to forest health and quarantine. Diagnosis of the causal agent(s) of wood decay is often the first step in developing strategies for control or improved management procedures. Decays which are visually similar, but which, after isolation, prove to be caused by different fungal species, may require different approaches for control.

For the development of meaningful quarantine regulations, the decay fungi already present in New Zealand must be accurately recorded and documented with authenticated herbarium collections. Species which if introduced might endanger New Zealand forest health must also be documented. An example of the importance to quarantine of taxonomic study of decay fungi was an investigation of the polypore genus *Heterobasidion* in Australasia. *Heterobasidion annosum* (Fr.) Bref. is a serious pathogen of conifers in the Northern Hemisphere. In Australasia, this species was reported (e.g., Shain & Bolland 1974) to occur as a saprophyte or weak pathogen on members of the family Araucariaceae, and occasionally on *Pinus* spp. Differences between *H. annosum* sens. str. and the Australasian fungus in characters such as fruit-body morphology, sexuality, distribution, pathogenicity, and host range supported the description of the Australasian fungus as a new species, *H. araucariae* P.K. Buchanan (Buchanan 1988). This study illustrated the importance of preventing entry to Australasia of the closely related, and potentially much more pathogenic species, *H. annosum.*

IDENTIFICATION OF THE FUNGAL FRUIT-BODY

Most wood-decay fungi belong to the Basidiomycotina. Fruit-bodies are of variable form, and the spores (basidiospores) develop naked on cylindrical to clavate reproductive cells (basidia). Basidiomycete wood-decay fungi can be divided into two large groups according to the appearance of the lower, spore-producing surface (hymenophore) of the fruit-body:

Agaricales (mushrooms) with gills;

Aphyllophorales (non-gill-bearing) without gills.

The latter, containing the majority of the wood-decay fungi, is further subdivided into four main groups, also characterised by the composition of the hymenophore:

clavarioid fungi — smooth surface on erect, club-shaped fruit-body;

corticioid fungi — smooth, wrinkled, folded, or warted surface;

hydnoid fungi — toothed surface;

poroid (polypore) fungi — vertical tubes opening below to form a surface composed of pores.

Most basidiomycete wood-decay fungi are corticioid or polypore fungi, and only fungi in these groups will be discussed further.

The macroscopic characters of fruit-bodies that are used for identification include: the form, shape, colour, texture, and size of the fruit-body; characters of the upper surface such as colour banding (zonation), whether hairy (tomentose, hirsute, etc.) or hairless (glabrous); surface appearance of the hymenophore; if poroid, the shape and size of pores; and whether annual or perennial, as assessed by cutting the fruit-body vertically to show any layers formed by growth in successive years. Examples of the

variation in form of fruit-bodies of polypore species are illustrated diagrammatically in Fig. 1. Individual species may produce fruit-bodies which vary, for example, from resupinate to effused-reflexed to pileate (shelf-like), with a major influence on form being the position of fruit-body initiation on the host. Fruit-bodies of corticioid fungi are typically effused (resupinate) to effused-reflexed, less often stipitate.

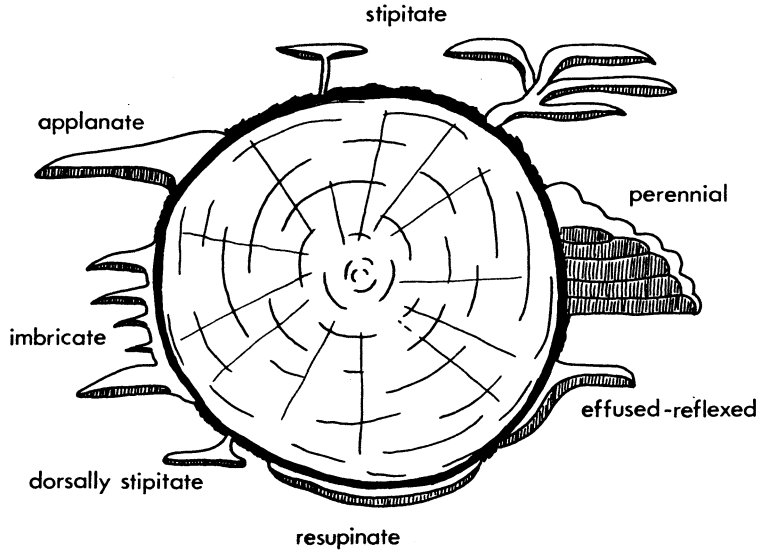

FIG. 1 — Some different types of polypore fruit-bodies — resupinate, effused-reflexed and several pileate forms.

In addition to examination of characters of the fruit-body, it is important to determine the host on which the fungus is growing (some species are host-specific), and the type of wood rot caused by the fungus, a character important taxonomically at the generic level. Of the two main types of rot, white and brown, most wood-decay fungi produce a white rot (Fig. 2a) which is characterised by a paler colour than sound wood, typically stringy appearance, a fibrous texture when crushed in the hand, and the occasional presence of black zone lines representing physical barriers dividing different populations of the same fungal species, or populations of two different species. White-rot fungi produce enzymes capable of degrading both cellulose and lignin from wood cell walls. A brown rot (Fig. 2b) is typically darker brown than sound wood, with characteristic cracks across the grain to form an irregular cubical, shrunken pattern, and in advanced stages reduces on crushing to a fine brown powder. Brown-rot fungi produce enzymes capable of degrading only cellulose and hemicellulose from wood cell walls, leaving the somewhat modified brown lignin. Only 6% of the Australasian

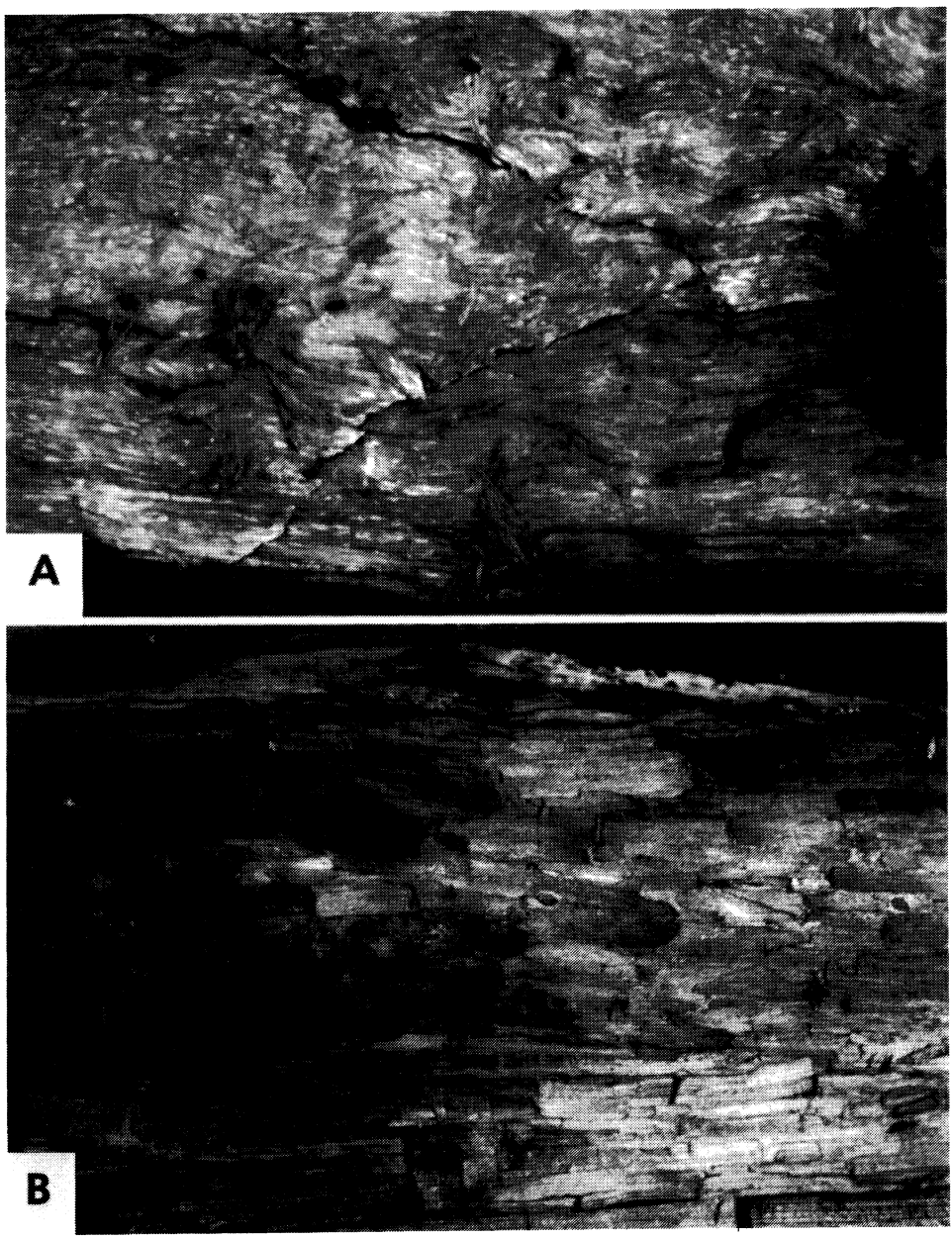

FIG. 2 — Two main types of wood rot: A, white rot with black zone lines; B, brown rot.

polypore species recorded by Cunningham (1965) cause a brown rot (Gilbertson & Ryvarden 1986–87). Only a few corticioid fungi cause a brown rot.

Macroscopic features alone are often inadequate for accurate identification, especially for corticioid species. Diagnostic microscopic features include the hyphal

construction of the fruit-body (hyphal type(s), branching, septation, colour, reactions to chemicals, dimensions, location) and the construction of the spore-producing layer or "hymenium" (shape and dimensions of basidia and number of sterigmata; presence or absence of clamps at the base of basidia; shape, dimensions, and ornamentation of spores; reaction of spore wall to Melzer's reagent and other chemicals; presence or absence of vegetative structures such as cystidia). Detailed illustrated accounts of these features have been given by Eriksson *et al.* (1973–88) for the corticioid fungi and by Gilbertson & Ryvarden (1986–87) for the polypores.

Evaluation of the hyphal system of a fruit-body (Corner 1932) is often necessary for identification, but may be difficult to determine. Careful dissection of tissue, sometimes from different parts of the fruit-body, is required. The two main types of hyphae are generative and vegetative (Fig. 3):

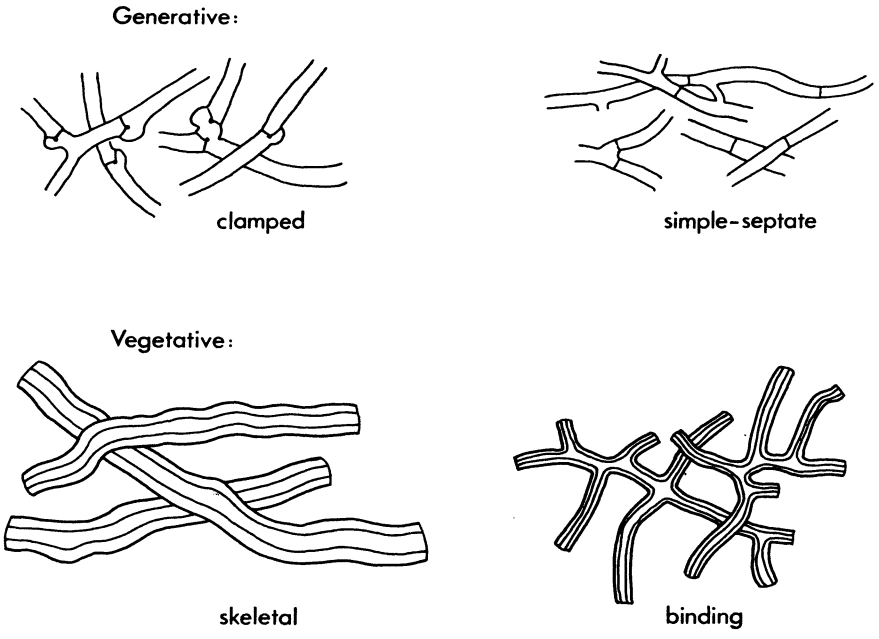

FIG. 3 — Generative and vegetative hyphae of a fruit-body.

Generative hyphae — common to fruit-bodies of all species and giving rise to the hymenium and to all other types of hyphae present; always with cross-walls (septa), either simple septa or clamp connections, or more rarely with multiple clamp connections, or both simple septa and clamp connections on the same hyphae; branched; typically thin-walled but sometimes thick-walled (sclerified); usually narrower than other hyphal types; dominant, to rare when mixed with other hyphal types and then best seen at the margin of the fruit-body, at the hymenium, or at pore mouths (in polypores). When generative hyphae are uncommon or fragmented in

preparations, staining their cytoplasm with a dye such as aqueous phloxine may help differentiate them from vegetative hyphae which have a narrow lumen sometimes lacking in cytoplasmic contents.

Vegetative hyphae — arising from generative hyphae, dominant in fruit-bodies of many polypore species, often absent in corticioid species, structural in function, typically present in fruit-bodies with a firm texture, usually differentiated from generative hyphae by a thickened wall and the absence of septa, although sometimes very thin cross-walls (adventitious septa) may result from contraction of the hyphal cytoplasm. Two main types of vegetative hyphae are skeletal and binding:

skeletal hyphae — unbranched to sparingly branched, long, thick-walled to solid, nonseptate, of more or less even diameter (not to be confused with sclerified generative hyphae);

binding hyphae — branched, thick-walled to solid, nonseptate, sometimes tapering to ends, typically narrower than skeletal hyphae.

The hyphal make-up of a fruit-body can be summarised by the following terms:

monomitic — composed of generative hyphae only (clamped or simple-septate);

dimitic — composed of generative hyphae (clamped or simple-septate) plus either skeletal or binding hyphae;

trimitic — composed of generative hyphae (always clamped) plus both skeletal and binding hyphae.

Assessment of hyphal characters is complicated by intermediate types of hyphae in some species, especially intermediates between skeletal and binding hyphae, by difficulties in achieving adequate separation of individual hyphae to determine septation and branching, and by the variation in construction that may occur in different parts of the fruit-body (e.g., in some polypore species the hyphal make-up of the context, above the tubes, may differ from that in the tubes).

The two key publications for identification of wood-decay fungi of Australasia are monographs on the corticioid fungi (Cunningham 1963) and the polypore fungi (Cunningham 1965, entitled "Polyporaceae of New Zealand" but including more species from Australia than New Zealand). A total of 261 species of corticioid fungi and 242 species of polypores are described, and dichotomous keys are provided to species. The keys rely on the use of microscopic characters, and habit illustrations are lacking for most species. Other important publications on Australasian wood-decay fungi include those by Buchanan & Ryvarden (1988), Cleland (1935), Cleland & Rodway (1929), Jülich (1978), Pegler (1964), Price (1973), Reid (1956, 1957, 1963), Rodway & Cleland (1929a, b), Stalpers (1985), and Walters (1958, 1969). Colour illustrations of fruit-bodies of Australasian fungi can be found in publications by Fuhrer (1985), Marks *et al.* (1982), Taylor (1981), and Young (1982), but only some of the common wood-decay fungi are featured. Butcher (1974) illustrated some New Zealand species common on timber and wood. Hood (1986) listed 36 of the common wood-decay fungi in New Zealand, with illustrations of 13. The wood-decay fungi recorded from Western Australia have been listed by Hilton (1982, 1988).

Modern overseas texts which can be used to assign collections to genus, but not always to species, because of their regional coverage and the many endemic species in Australasia, include those by Eriksson *et al.* (1973–88), Gilbertson & Ryvarden

(1986–87), Jülich (1984), Jülich & Stalpers (1980), Ryvarden (1976–78), and Ryvarden & Johansen (1980).

Authenticated herbarium collections enable identification by comparison of morphology. The principal herbaria holding wood-decay fungi of Australasia are:

Australia:

Wood Science and Technology Program, CSIRO Division of Forestry and Forest Products, Highett, Victoria — for list of holdings refer to Walters (1962).

State Herbarium of South Australia (AD), Botanic Garden, Adelaide — for collections by J.B. Cleland.

Tasmanian Herbarium (HO), University of Tasmania, Hobart — for collections by L. Rodway.

New Zealand:

Forest Research Institute (NZFRI), Rotorua.

DSIR Plant Protection (PDD), (formerly Plant Diseases Division, DSIR), Auckland — for collections by G.H. Cunningham.

Papua New Guinea:

Papua New Guinea Research Institute Herbarium, Lae.

United Kingdom:

Royal Botanic Gardens (K), Kew, Surrey, England — for list of early holdings of Australasian species, refer to Cunningham (1950, 1953).

IDENTIFICATION OF FUNGI IN CULTURE

Identification of a fungus isolated from decayed wood in the absence of a fruit-body is more difficult than identification of a fruit-body. Few species can be induced to produce fruit-bodies on agar, although some species will fruit on sawdust media or on wood blocks (Badcock 1943; Tamblyn & Da Costa 1958). The application of culture characters to diagnosis is limited by the absence of descriptions and keys for the majority of indigenous Australasian species.

Standard procedures for growing and evaluating wood-decay fungi in agar culture were developed by Nobles (1948, 1965). Characters considered are colour of the colony and reverse, growth rate, odour, development of fruit-bodies or propagules (e.g., conidia, chlamydospores), hyphal type(s) and hyphal differentiation, septation of generative hyphae, presence or absence of extracellular oxidase enzymes (which mostly correlates with the type of wood rot, white *v.* brown, respectively), host relationships, and interfertility phenomena. Descriptions were provided for 149 species (Nobles 1965) and were summarised for each species in a code composed of well-defined character states, numbered 1–60. Species were identified using a key based on the code numbers. Additional character states, in particular to describe nuclear behaviour, have been added to Nobles' code by Boidin & Lanquetin (1983). Using an expanded species code of 96 character states, Stalpers (1978) provided descriptions of cultural characters, and keys for 550 species of wood-inhabiting fungi. Eighty percent of his isolates originated from North America or Europe.

Cultural studies of some Southern Hemisphere taxa have been published. These include: from Australia, 31 species, mainly polypores (Lanquetin 1973; Matters 1953,

1955a, b, c, d; Matters *et al.* 1952, 1953; Nobles & Frew 1962; Refshauge & Proctor 1936; Stalpers 1978); from New Zealand, three corticioid and eight polypore species (Buchanan 1988; Hood *et al.* 1989; Nobles & Frew 1962; Taylor 1974, 1977); from South America, over 90 species, mainly polypores (e.g., Deschamps & Wright 1975; Rajchenberg 1982, 1983a, b, 1984; Wright & Deschamps 1976); and from South Africa, 39 species of polypores (van der Westhuizen 1958, 1971, 1972, 1973).

In the absence of keys for cultures of most Australasian species, identification may involve comparison with other cultures, either derived from identified fruit-bodies known to occur on the same host (e.g., Hood *et al.* 1989), or from fruit-bodies developing on other hosts nearby. Comparison may be made on the basis of similarity in morphological features of the colony, results of analytical methods such as electrophoresis, or results of compatibility tests.

In the "Buller phenomenon" compatibility test (Buller 1931), a colony of an unknown isolate derived from wood (i.e., typically comprising binucleate (dikaryotic) mycelia with clamp connections at septa) is confronted with a colony of a known strain derived from a single basidiospore (i.e., typically comprising uninucleate (monokaryotic) mycelia with simple septa). The known strain is inoculated in a curved band surrounding the unknown (Fig. 4), so that the growing margins of the two colonies meet. The absence of a line of dense mycelial growth at the zone of confrontation may suggest a compatible reaction. Compatibility can be accurately assessed, however, only by microscopic observation of the hyphal septa of the test strain, at a distance of about 2 cm outside the curved inoculum band, c. 4–5 weeks after initial confrontation. If the unknown is conspecific with the single-spore test strain, nuclei will move from the dikaryotic unknown to the monokaryotic test strain, with the result that the now dikaryotised test strain will develop septa with clamp connections. If the unknown is dikaryotic but lacks clamps at hyphal septa, determination of compatibility may require nuclear staining to count the number of nuclei in hyphal cells.

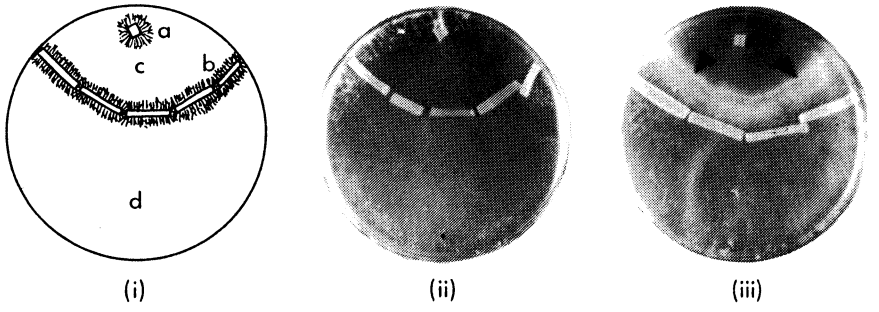

FIG. 4 — Buller phenomenon compatibility test. (i) Diagram of compatibility test plate at an early stage: a, unknown dikaryotic isolate from decayed wood; b, known monokaryotic test strain from a single basidiospore; c, future zone of confrontation between a and b; d, position for microscopic examination of hyphal septa to determine compatibility. (ii) A compatible confrontation. (iii) An incompatible confrontation, often indicated by the development of a line of dense mycelial growth (arrowheads) at the zone of confrontation.

Application of the Buller phenomenon depends on the availability of potentially compatible single-spore strains. Few are as yet available from culture collections in Australasia, although single-spore strains of New Zealand polypores are being accumulated by the author for incorporation into ICMP culture collection (DSIR Plant Protection, Auckland). For investigations into the cause of serious decay in a single host species, it would be useful to acquire a set of identified single-spore strains, derived from fruit-bodies commonly seen on that host, for compatibility studies.

The major collection of cultures of wood-decay fungi in Australasia is:

Culture Collection, CSIRO Division of Forestry and Forest Products, Highett, Victoria, Australia; no catalogue, but holdings have been listed by McGowan & Skerman (1982); refer also to Da Costa *et al.* (1952).

For information on other, smaller, culture collections in Australia, refer to Walker (1980).

In New Zealand, collections are maintained by:

Forest Research Institute (NZFS), Rotorua; no catalogue.

DSIR Plant Protection (ICMP), Auckland; catalogue available; most strains of wood-decay fungi not yet accessioned.

Major international collections which hold cultures of wood-decay fungi include:

Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands — catalogue available.

Canadian Collection of Fungus Cultures (CCFC), Biosystematics Research Institute, Ottawa, Canada — no catalogue, but holdings listed by McGowan & Skerman (1982).

Culture Collection of Wood-inhabiting Fungi (EFPL, WFPL), Forintek Canada Corp., Ottawa, Ontario, Canada — catalogue available.

Culture Collection of the Department of Systematic Botany, Gothenburg University, Sweden — catalogue available, mainly corticioid fungi.

The National Collection of Wood-Rotting Macro-Fungi (NCWRF, formerly FPRL), Building Research Establishment, Garston, Watford, England — catalogue available.

For information on other culture collections, refer to Hawksworth (1985) and McGowan & Skerman (1982).

ACKNOWLEDGMENTS

I thank John Allen (Christchurch), Gary Johnson (Highett), and Ian Hood (Rotorua) for their helpful comments as referees.

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