

B.W. Held · J.A. Jurgens · S.M. Duncan
R.L. Farrell · R.A. Blanchette

Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica

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Abstract Scientists working at New Harbor, Antarctica in November 1959 used a wooden crate as a makeshift workspace and kitchen. The structure has been used intermittently over the subsequent decades and still remains at the site with various materials left in and around it. The wooden structure was assessed for deterioration and samples collected to determine the diversity of fungi at the site after 43 years in the Antarctic environment. Results from these investigations are compared to the results from research on the historic huts of Ross Island, approximately 70 km east of New Harbor that were built 48–58 years earlier. Our analysis shows the wood of the New Harbor structure is extremely weathered and soft rot decay was detected in the wood in contact with the ground. Fungal cultures isolated from wood of the structure were identified using sequences of the internal transcribed spacer region of the rDNA. Several species of *Cadophora* were identified including *C. malorum*, *C. luteo-olivacea*, *C. fastigiata* and a previously undescribed species designated *C. sp. NH*. Laboratory decay experiments using two *Cadophora* species isolated from New Harbor demonstrated extensive decay and loss of biomass in hardwood wafers after 16 weeks. Other fungi isolated from the wood included species of *Cladosporium*, *Hormonema*, *Penicillium* and *Lecythophora*. Wind erosion has also severely affected the structure's exterior wood causing deep furrowing between earlywood and latewood cells. In general, the deterioration and fungi found at the site were similar to those found at the historic expedition

huts on Ross Island, however, one species obtained is unique to the New Harbor site. This research expands our knowledge of the microbes colonizing wood brought into the polar environment and provides additional information on deterioration and decomposition processes occurring in Antarctica.

Introduction

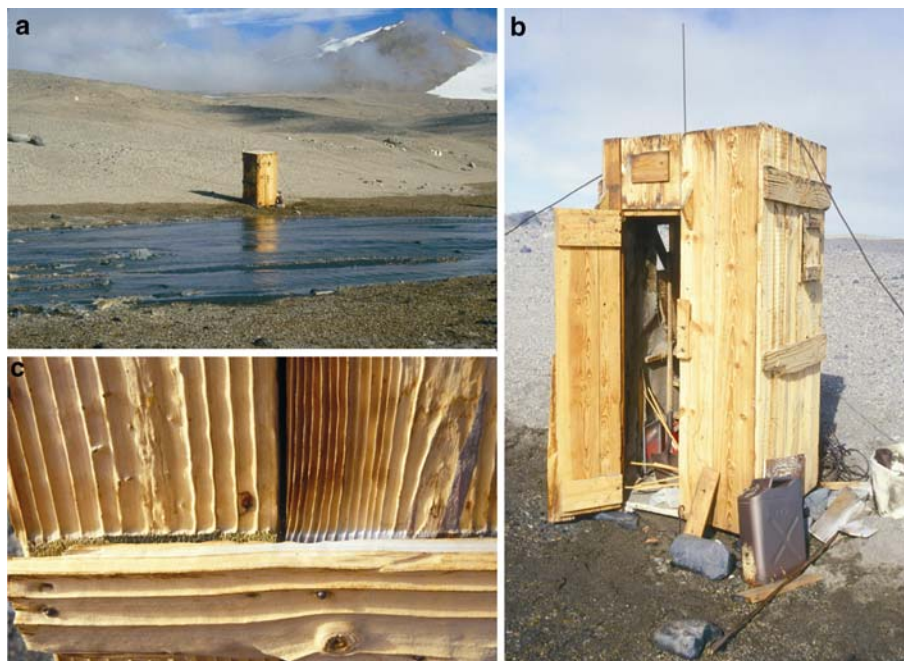
In November 1959, two scientists, J.D. McGraw and G.C. Claridge, used a large wooden crate (1×1×2.5 m) built in New Zealand to transport field gear in Antarctica. A Massey Ferguson tractor was used to pull the crate to New Harbor, Antarctica approximately 70 km west of Ross Island, with the two scientists inside the crate during the journey. The crate provided protection for the scientists from ice fragments and snow that came off of the tracks of the tractor. Once they arrived at New Harbor, the crate was converted into a makeshift cook house and work area. The structure was abandoned the following season and although evidence exists that others have used the structure during past years, no further detailed information is available about its subsequent use. The structure is presently situated approximately 300 m from the sea and less than 1 m from a seasonal stream (Fig. 1a, b). Visual observations indicated that exterior woods of the structure have suffered significant wind erosion leaving an eroded, uneven surface. In a preliminary study, fungi were isolated from wood and several species of *Cadophora* were found (Blanchette et al. 2004).

Although the deterioration of wood in more temperate climates has been widely studied and understood (Eaton and Hale 1993; Eriksson et al. 1990; Zabel and Morrell 1992), degradative processes of wood in the polar environment are not as well known. Deterioration of the historic huts of Ross Island has been researched and documented and involves the degradation of wood

B.W. Held (✉) · J.A. Jurgens · R.A. Blanchette
Department of Plant Pathology, University of Minnesota,
1991 Upper Buford Circle, 495 Borlaug Hall,
St Paul, MN 55108, USA
E-mail: bheld@umn.edu
Tel.: +1-612-6250202
Fax: +1-612-62579128

S.M. Duncan · R.L. Farrell
Department of Biological Sciences, University of Waikato,
Hamilton, New Zealand

Fig. 1 The 1959 structure at New Harbor and wind erosion of exterior woods. **a** View of the structure at New Harbor. The small stream in the foreground wets the soil under the structure. **b** A close view of the structure and the remaining materials left at the site. Hazardous materials have recently been removed from the site. **c** Advanced wind erosion of the exterior woods. The wind erodes the earlywood cells more easily than the more resistant, highly lignified late wood cells resulting in deep grooves in the wood



caused by non-biological (wind, ultraviolet light and salt) and biological (soft rot decay) effects (Blanchette et al. 2002; Blanchette et al. 2004; Held et al. 2003). The soft rot fungi isolated are of particular interest since some species identified have the capacity to cause extensive wood decay as well as being indigenous to Antarctica. These fungi likely have a broader role for organic matter degradation and nutrient recycling in Antarctica. Since the wooden structure at New Harbor has been in the Antarctic environment for only about four and a half decades, compared to the historic expedition huts that have been in Antarctica for nine to ten decades, it is an excellent study site providing a different time interval and location for assessing microbial diversity and deterioration in a polar environment. The objectives of this study were to: (1) assess and document the deterioration that has occurred in the wooden structure at New Harbor, Antarctica, (2) determine the fungal diversity associated with the wood and (3) compare these findings to those obtained for other introduced wood to Antarctica, specifically, the historic Ross Island expedition huts.

Materials and methods

Samples from the structure were taken in the austral summer of 2002 under Antarctic Conservation Act Permit number 2002-001. Minute samples of wood were taken several centimeters below ground near the base as well as other locations above ground from the structure for microbiological isolations and were placed in sterile bags. Samples were kept cool during transport and frozen upon arrival to the laboratory at the University of Minnesota. In the laboratory, microorganisms were

isolated from wood samples by placing them on two types of culturing media, followed by an incubation period. The media used were 1.5% Difco malt extract agar (MEA) and acidified MEA with 2 ml of lactic acid added after autoclaving. Plates were incubated at 20 and 8°C for 4–8 days after which pure cultures were transferred to individual plates. Fungi were identified using taxonomic literature for these genera and analyses of rDNA internal transcribed spacer sequences were carried out as described by Held et al. (2005).

The laboratory decay study to test fungal species isolated from the New Harbor structure for their ability to decay wood was conducted in the laboratory over a 16-week period. Thin wafers of a hardwood (birch, measuring 1.5×1.5×0.2 cm) and a softwood (pine, measuring 1×1.5×0.2 cm) were used that were cut from sound wood blocks. Wafers were then dried and weighed to determine their dry weight. Two different nutrient solutions were infiltrated into wafers by autoclaving them in a corresponding solution. The two solutions used were: (1) a reduced nutrient solution, RNS, containing 1.5 g NH₄NO₃, 2.5 g KH₂PO₄, 2 g K₂HPO₄, 1 g MgSO₄·7H₂O, 2.5 g glucose and 0.1 g thiamine per liter and (2) 2A (double Abrams) containing 6 g NH₄NO₃, 4 g KH₂PO₄, 5 g K₂HPO₄, 4 g MgSO₄·7H₂O, 2.5 g glucose and 0.1 g thiamine per liter (Abrams 1948; Duncan 1965; Zabel et al. 1991; Worrall et al. 1991). Petri plates with media made with the same RNS and 2A nutrients and agar were inoculated with *Cadophora* sp. NH and *C. fastigiata* and allowed to grow for several weeks. After autoclaving, excess nutrient solution was decanted from the wafers and four wafers were placed directly onto growing cultures on plates made with the corresponding RNS or 2A media. Three plates were used for each fungus and nutrient solution.

Non-inoculated wafers with nutrients were used as a control. Parafilm was placed around the plates and the plates were incubated at 21°C for 16 weeks. After the incubation period, wafers were removed from the plates and excess mycelium was carefully removed. Wafers were then oven-dried and biomass loss determined [(dry wt. before inoculation - dry wt. after inoculation)/dry wt. before inoculation]. Wood samples were prepared for scanning electron microscopy (SEM) using techniques described previously by Blanchette and Simpson (1992) and was carried out using a Hitachi S3500N SEM.

Results

Wood samples from the structure at New Harbor were identified as several different species of *Pinus* and several unattached boards as *Nothofagus* sp. The exterior woods were still intact but severe weathering was observed. The most serious deterioration was due to wind erosion. This has left the wood surface uneven where the earlywood regions eroded more than the latewood regions causing a grooved appearance (Fig. 1c). Small fragments of gravel were frequently found wedged in these grooves. Small zones of defibrated wood just above the ground were found, but this form of non-biological deterioration was not widespread. A graying or whitening of exterior woods also was apparent on some areas of exterior woods. The interior of the structure appeared sound and was free of visible mold growth and deterioration.

Wood in contact with the ground was wet and often appeared to be discolored. In addition to the exterior boards of the structure, several boards and wood frag-

ments were found lying on the ground near the hut. SEM of small samples taken from wood in ground contact indicated that soft rot decay was affecting the wood (Fig. 2). Large holes were present in secondary walls of the *Pinus* and *Nothofagus* woods typical of type 1 soft rot (Blanchette et al. 1990; Eaton and Hale 1993). Soft rot was found in several samples of wood that were in soil contact near the base of the structure. Fungal isolations from wood produced four species of *Cadophora* including *C. malorum*, *C. luteo-olivacea*, *C. fastigiata* and an undescribed species designated *C. sp. NH* (Table 1). Other fungi isolated from wood included *Cladosporium cladosporioides*, *Hormonema dematioides*, *Lecytophora hoffmanii* and *Penicillium mali*.

Microscopic examination of sections from birch wood wafers inoculated in the laboratory with *C. fastigiata* revealed extensive type 1 soft rot (Fig. 2). Although the most advanced decay was limited to the outer portions of the wafers, biomass losses with the 2R and RNS media showed a 27 and 20.3% average weight loss, respectively (Fig. 3). For the pine wood, weight losses were 1.2 and 3.1% for wafers inoculated with *C. fastigiata* on 2R and RNS media, respectively, and no decay was observed. *Cadophora sp. NH* grew slower on MEA than other *Cadophora* species and did not colonize the birch and pine wafers well. In these wood wafers a negligible weight loss of 0.6–3.2% was observed.

Discussion

Previous studies have shown that the cold, dry Antarctic environment does not completely inhibit deterioration processes and significant damage can occur over time

Fig. 2 Scanning electron micrographs showing soft rot cavities in the cell walls of wood. **a** and **b** Soft rot cavities in *Nothofagus* buried in soil at the base of the structure. Cavities coalesce to form large voids in the secondary cell walls of the wood. **c** and **d** Extensive soft rot decay in birch wafers inoculated with *Cadophora fastigiata* in the laboratory after 16 weeks. The secondary cell walls are almost completely degraded, leaving only the middle lamella which causes severe strength loss in the wood

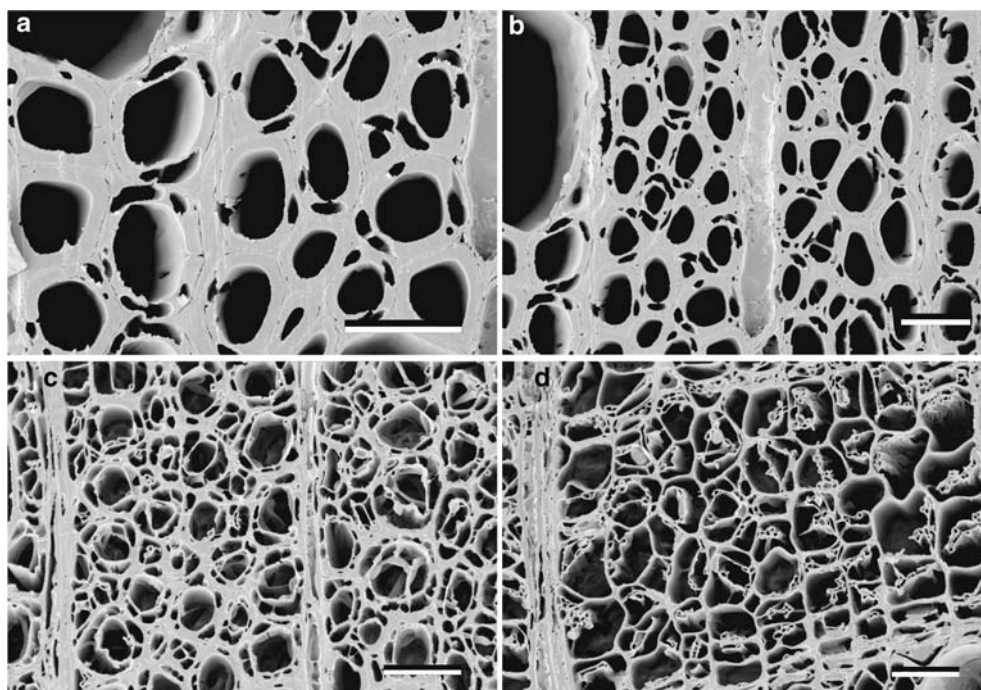
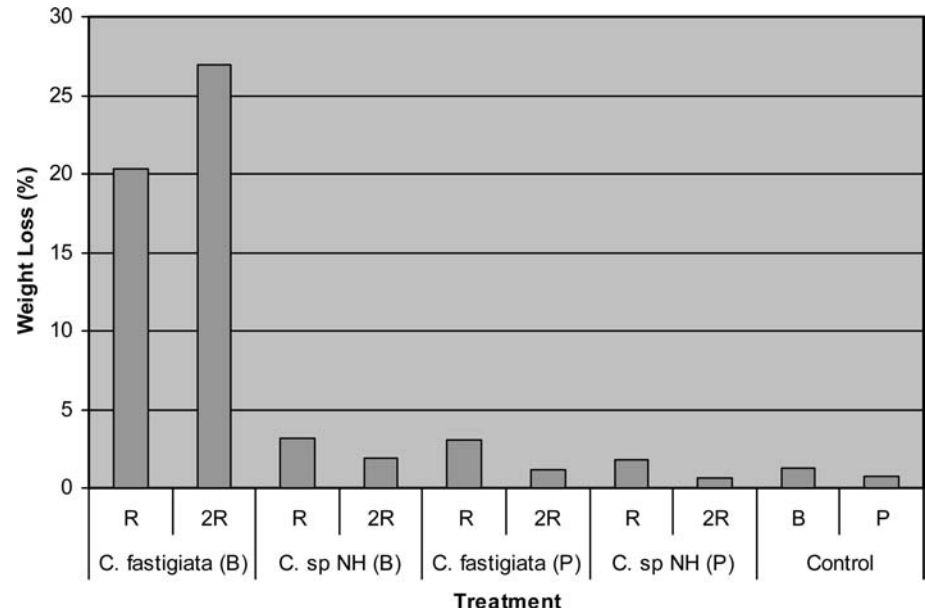


Table 1 Fungal species isolated from various wood samples from the 1959 structure at New Harbor, Antarctica

Species	Collection data and isolate number ^a
<i>Cadophora malorum</i>	Wood in contact with soil; NH8-1; NH15-3
<i>Cadophora luteo-olivacea</i>	Wood, E sideboard below soil; NH6-1
<i>Cadophora fastigiata</i>	East sideboard, below soil; NH5-1
<i>Cadophora</i> sp. NH	Wood, below soil, SW corner; NH1-2, NH9-1
<i>Cladosporium cladosporioides</i>	East sideboard, below soil; NH6-3
<i>Hormonema dematioides</i>	Southwest corner sideboard, below soil; NH7-3
<i>Lecythophora hoffmanii</i>	Wood, below soil, SW corner; NH1-1
<i>Penicillium mali</i>	Wood, below soil, SW corner; NH1-3

^aCultures with two isolate numbers were found in two separate locations

Fig. 3 Comparisons of percent weight losses of wood wafers, birch (*B*) and pine (*P*) when infiltrated with reduced nutrient (*R*) and double Abrams (*2A*) solution followed by inoculation with *Cadophora fastigiata* and *C. sp. NH*



(Blanchette et al. 2002; Blanchette et al. 2004; Held et al. 2003; Hughes 2000). The structure at New Harbor provided a good opportunity to study wood that has been in the Antarctic environment for a shorter period of time than the historic huts on Ross Island (43 years instead of 90). After only 43 years, severe damage from non-biological and biological agents has taken place.

Three types of non-biological deterioration are affecting the structure: wind abrasion, salt defibration and UV light degradation. Wind abrasion, which is commonly found in wood exposed to the Antarctic environment (Blanchette et al. 2002; Held et al. 2003; Harrowfield 1996; Hughes 2000), is affecting the wood at New Harbor in a similar way as the historic expedition huts in other locations (Fig. 1c). Exposure to powerful winds and particulate matter are the main factors responsible and currently no acceptable treatment exists to protect wood that is subjected to this type of deterioration. The second type of non-biological deterioration is in the form of defibration of the exterior wood caused by high concentrations of salts. This type of damage has been documented in the historic huts on Ross Island and in other environments where wood is in contact with high salt concentrations. It consists of a chemical degradation of the middle lamella that results in the separa-

tion of wood fibers (Blanchette et al. 2002). The structure at New Harbor is located near a seasonal stream that provides continuous moisture to the wood in ground contact. As moisture is absorbed by the wood it is evaporated above the ground and high concentrations of salt precipitate. Although the extent of exterior wood defibration is not severe, some damage is occurring to the structure. The third form of deterioration is from UV light causing a graying or whitening of wood surfaces, most easily seen on exterior, horizontal cross member woods of the structure. This type of deterioration involves the degradation of lignin in the wood surface caused by UV light absorption (Hon 1981; Gellerstedt and Petterson 1977). Although this damage is limited on the structure at New Harbor more significant effects can be seen on the huts on Ross Island which can be attributed to Antarctica's intense UV light levels (Booth et al. 1994).

Wood decay caused by soft rot fungi is a newly described phenomenon occurring in Antarctica (Blanchette et al. 2004; Held et al. 2003). Advanced stages of wood decay have been reported in wood in ground contact from Shackleton's 1909 hut and in various woods near Scott's Cape Evans hut on Ross Island (Blanchette et al. 2004). Species of *Cadophora*

have been found associated with this decay. Different environmental conditions between the two sites may be responsible for more decay present in some of the New Harbor samples despite being exposed for a shorter time period. Since the soil around the wooden structure at New Harbor is continuously wet during the austral summer, conditions for soft rot fungi are likely to be more conducive for decay. The fungal species causing the decay also may be more aggressive than those found affecting the woods on Ross Island. *C. fastigiata*, which this study shows to be capable of causing advanced stages of decay in laboratory studies, has not been isolated from wood associated with the Ross Sea huts. Cultures of *C. malorum*, *C. luteo-olivacea*, *C. fastigiata*, *H. dematioides* and *C. cladosporioides* have been isolated from samples of wood collected from the New Harbor structure (Table 1). Similar fungi, isolated from other materials have been previously shown to cause soft rot (Blanchette et al. 2004; Morrell and Smith 1988; Morrell and Zabel 1985; Zabel et al. 1982). The frequency that *Cadophora* species were isolated from the wood at New Harbor and the extensive decay that species of this genus produced in laboratory decay experiments (Fig. 2) indicates it is likely the major cause of the decay observed. Results from the laboratory wood decay studies also showed *C. fastigiata* caused considerable weight loss in birch but not pine wood (Fig. 3). In a previous investigation using a different isolate of *C. fastigiata*, Morrell and Zabel (1985) showed both type 1 and 2 soft rot in southern yellow pine and beech. Nilsson (1973) also showed that a strain of this fungus produced soft rot cavities in hardwood, pine and spruce. Additional research is needed on the physiology of these species to fully understand what governs their capacity to cause decay. It is well known that nutrients influence soft rot activity (Duncan 1965; Worrall and Wang 1991; Worrall et al. 1991) and increased concentrations of some nutrients are needed to cause soft rot unlike other forms of wood decay. The presence of nitrogen and other sources of nutrients from food stores left at the historic huts as well as penguin guano may influence the extent and rate of degradation in Antarctica.

Environmental conditions within the historic huts of Ross Island have been monitored using data loggers and high relative humidity and temperatures above 0°C have been reported (Held et al. 2005). The high relative humidity and excess moisture in areas of the huts provided conditions that allowed extensive mold growth to occur, especially in areas of restricted air movement. Unlike the historic huts, environmental monitoring at the New Harbor structure has not been carried out. Since surface molds were not observed on the interior of the structure it is a good indication that relative humidity is not high enough in this structure to support surface mold growth.

Apart from the Ross Sea huts, *Cadophora* species have been previously reported in Antarctica on a range of different substrates and locations. They have been

found in soils in the Vestfold Hills and near Davis Station (Kerry 1990), in contaminated soils in the Ross Sea Region (Aislabie et al. 2001), and isolated from feathers and mosses from Victoria Land (Azmi and Seppelt 1998; Frate and Caretta 1990; Tosi et al. 2002). These reports indicate a wide polar distribution of the genera and suggest a role for this fungus in organic matter decomposition and nutrient cycling. A previously undescribed species of *Cadophora*, designated *C. sp. NH* isolated from the New Harbor site, has not been reported at any other location in Antarctica or the world. This species did not cause soft rot in the laboratory wood decay study and did not grow well on the pine and birch wood wafers, unlike *C. sp. E* an undescribed species isolated from Cape Evans which caused soft rot in birch and thought to be endemic to Antarctica (Blanchette et al. 2004). Additional studies that vary the substrate, nutrients and environmental conditions are needed to determine if *C. sp. NH* has the capacity to cause wood decay, and to elucidate its role in nutrient recycling in the Antarctic environment.

These studies have provided new information on the microbial diversity found on wood introduced into the Antarctic environment, the fungi causing wood decay and other factors responsible for deterioration taking place. The occurrence and role of the *Cadophora* species isolated from different locations in Antarctica demonstrate their widespread distribution and diversity. The previously undescribed species, *C. sp. NH*, discovered at this site suggests this fungus may be endemic to Antarctica (Blanchette et al. 2004). New studies are warranted to better understand this fascinating group of microbes and their biology and ecology in the polar environment.

Results presented here provide an assessment of deterioration and fungal diversity associated with the 43-year-old wooden structure at New Harbor. Environmental staff from both the US and New Zealand Antarctic programs have documented and inventoried items in and around the structure, and items that posed environmental concerns have been removed. At the present time, the structure's historic value and whether action will be taken for its conservation are still being determined. The scientific value of the structure, if left in place, appears to be significant since it provides an excellent study site to monitor microbial diversity and evaluate deterioration processes in the Antarctic environment. The presence of an undescribed fungal species suggests it may have unique microflora and further investigations are warranted.

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