Nitrogen Fixation in Deteriorating Wood

Microfungi isolated from wood containing a meagre nitrogen content (Allison et al.[1]) have been shown to grow on an agar lacking nutrients (Sharp and Levy[2]). Cowling and Merrill[3] postulated 3 mechanisms, not necessarily mutually exclusive, by which fungi could survive conditions with a minimum of nitrogen present and still achieve some growth. They are a physiological adaptation, reuse of nitrogen already available and the utilisation of nitrogen obtained from outside of the wood. This last category could involve the translocation of nutrients along hyphae or the diffusion of soil and rain-water into the wood or the fixation of atmospheric nitrogen.

Fungi that can fix nitrogen have been reported but by using improved techniques these results have been discredited (Millbank[4]). However, it is possible that microorganisms other than fungi could penetrate wood and fix nitrogen and, indeed, such fixation has been detected in white fir trees and attributed to bacteria (Seidler et al.[5]). Since nitrogen fixation appears to be confined to procaryotic cells or tissues derived from them, and no evidence exists for fixation by free-living actinomycetes, any capacity must be ascribed to the bacteria present. *Bacillus polymyxa* and *B. macerans* have been isolated from logs immersed in water (Ellwood and Ecklund[6]) and also shown to fix nitrogen (Witz, Detroy and Wilson[7]) and likewise *Aerobacter aerogenes* (now *Klebsiella pneumoniae*) has been identified as a wood colonist (Knuth[8]) and as a nitrogen fixer (Pengra and Wilson[9]). Inadequate isolation and identification of wood bacteria hinders further nitrogen fixing species from being found, although unknown species of the genus *Clostridium* have been mentioned in isolation lists and could refer to a prominent nitrogen fixing species.

Material and method. In order to demonstrate whether nitrogen fixing microorganisms can penetrate wood and participate in its deterioration a preliminary investigation has been undertaken to detect any nitrogenase activity. Sets of 8 veneer pieces, 30 × 20 × 0.6 to 1 mm, were cut from beech, oak and Scots pine, weighed and buried in 200 g portions of soil taken from the Imperial College field station, Silwood Park, Sunninghill, Berks. The soil in some cases was augmented with glucose. Sets containing beech (*Fagus sylvatica* L.) cubes, 1 cm^3, were similarly treated with 1 set having the 2 transverse faces sealed by a silicone adhesive. The soils were moistened to 60% moisture holding capacity to encourage the growth of bacteria and then incubated for 20 days at 25°C In addition a strip of lime (*Tilia europaea* L.) of 15 × 10 mm section with one radial face exposed was sampled after completing 40 days soil burial at 28°C and a Scots pine (*Pinus sylvestris* L.) stake was extracted from a site at the field station after 3$^1/_2$ years exposure to the soil and weather.

Each of the deteriorated woods were thoroughly washed in distilled-water to remove adhering soil. The veneers were cut into slivers, the cubes were sandpapered to remove superficial growth before slicing into thin sections and a piece of the lime strip and Scots pine stake at the ground-line were cut up and the outer 2 to 3 mm wood discarded.

The wood samples were examined for microorganisms with nitrogenase activity by means of the acetylene reduction technique. Incubation was at 25°C under anaerobiosis, using bottles of 28–30 ml capacity capped with 'Suba-seal' closures (W. Freeman and Co., Suba-seal works, Staincross, Barnsley, Yorks., England). The gaseous atmosphere consisted of Ar + CO_2 (95:5) 97% and C_2H_2 3%. Ethylene was determined in a Pye 104 gas-liquid chromatograph fitted with a flame ionisation detector. Gas samples (0.5 ml) were injected into a one

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Acetylene reduction in various deteriorating woods

Wood	Length of incubation in soil	Glucose added to soil (% w/w)	Initial weight of wood, (g)	nmole C_2H_4 per h	nmole N_2 per h [a]
Beech veneers	20 days	0	2.062	0.092	0.031
Beech veneers	20 days	0.5	2.105	3.069	1.023
Beech veneers	20 days	8.0	2.056	155.0	51.6
Beech cubes	20 days	0.5	5.171	3.066	1.022
Beech cubes	20 days	8.0	5.545	0.083	0.027
Beech cubes with transverse face sealed	20 days	8.0	5.305	0.825	0.275
Scots pine veneers	20 days	0	2.432	0.086	0.029
Oak veneers	20 days	0	1.713	0.036	0.012
Oak veneers	20 days	0.5	1.527	0.781	0.26
Oak veneers	20 days	8.0	1.528	145.5	48.5
Lime strip with only radial face exposed	40 days	0	–	0.023	0.008
Scots pine stake	3.5 years	0	–	2.548	0.849

[a] The conversion of 'ethylene formed' to 'nitrogen fixed' data was assumed to be by a factor of $^1/_3$. Before assaying for ethylene formation beech cubes had their surfaces sandpapered off and the lime strip and Scots pine stake had their outer 2 to 3 mm of wood cut away.

metre column (4 mm internal diameter) of 'Poropak R' at 50 °C with nitrogen as the carrier gas flowing at 60 ml/min. With a fixed sample volume the peak height was proportional to the amount of ethylene in the sample.

Results and discussion. The results shown in the Table indicate that all the woods permitted the penetration of microorganisms capable of fixing nitrogen and this is, as far as we are aware, the first report of such activity in wood with ground contact. Fixation not only occurred in samples having some faces sealed that might otherwise have allowed a passive penetration via the vessels, but also in oak (*Quercus* sp.) and Scots pine which contain substances inhibitory to many microorganisms and in a stake which had undergone prolonged deterioration. The addition of glucose to the soil considerably stimulated the acetylene reduction, but this was not always consistent and was probably affected by variable soil conditions.

It has still to be shown how significant this fixed nitrogen is for fungal growth and wood decay, but the presence of such microorganisms provides another aspect to the ecology of wood colonisation.

Résumé. Divers types de bois ont été détériorés par des microorganismes du sol et leur capacité de réduction de l'acétylène mesurée. L'éthylène produit a révélé l'existence d'une activité nitrogénasique et suggéré que des microorganismes fixateurs d'azote peuvent pénétrer et vivre dans le bois.

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Veränderungen der aggressiven Handlungsbereitschaft männlicher Schwertträger, *Xiphophorus helleri*, nach sozialer Isolation (Fische, Poeciliidae)

Durch LORENZ[1] wurde in letzter Zeit eine lebhafte interdisziplinäre Diskussion zu der Frage ausgelöst, ob intraspezifisches Aggressionsverhalten als Triebverhalten aufgefasst werden kann. Nach der klassischen Vorstellung der Ethologie müsste aufgrund spontaner Akkumulation triebspezifischer Energie die Handlungsbereitschaft gegenüber einem konstanten Reiz steigen, wenn die Handlung längere Zeit lang nicht ausgelöst wird. Diese Erscheinung kann auch als Triebstauung bezeichnet werden, ohne dass damit bereits etwas über den zugrundeliegenden physiologischen Mechanismus ausgesagt werden soll.

Setzt man Schwertträger-Männchen aus verschiedenen Zuchten zusammen, so führen sie bei etwa gleicher Körpergrösse Rangordnungskämpfe aus, die mit der Unterlegenheit eines der beiden Rivalen enden. Kennzeichnend für diese aggressiven Auseinandersetzungen höchster Intensität sind die beiden Verhaltensweisen *Kreisen*, ein mehr oder minder gleichzeitiges wechselweises Beissen und Rammen der Gegner, und der *Maulkampf*. Regelmässig tritt Maulkampf erst nach dem ersten Kreisen auf,

[1] K. LORENZ, *Das sogenannte Böse* (Borotha-Schoeler, Wien 1963).

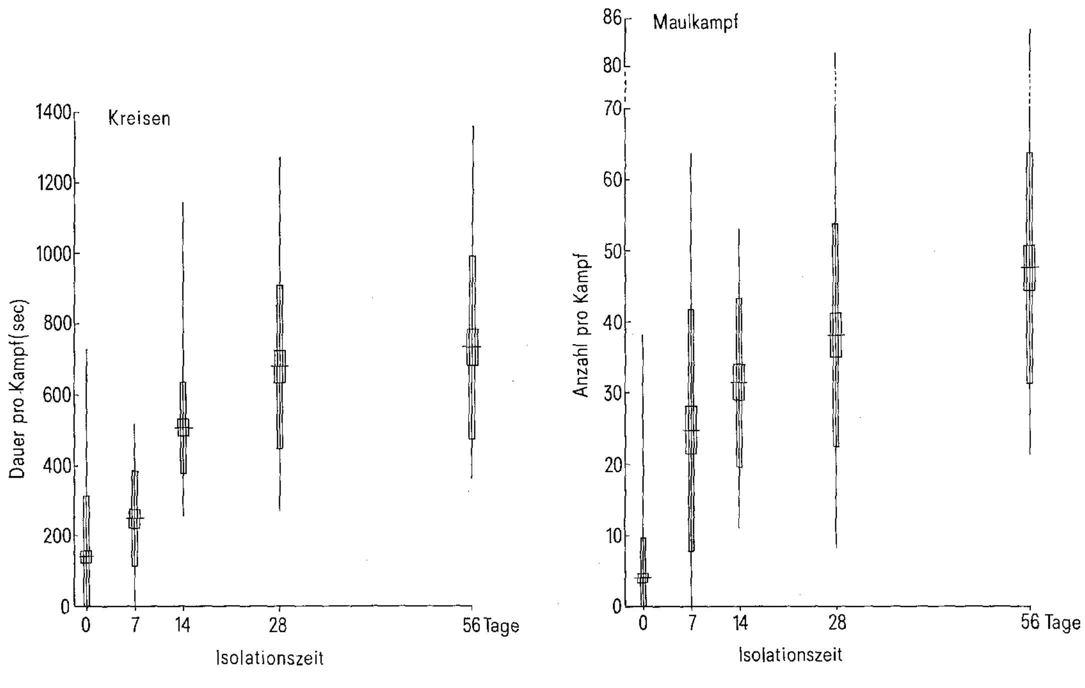

Fig. 1. Zunahme des Kreisens und der Maulkämpfe in Rangordungskämpfen nach sozialer Isolation. Die einfache vertikale Linie gibt die Variationsbreite der gefundenen Werte an, der Querstrich den Mittelwert. Die schmale vertikale Säule ist gleich der doppelten Standardabweichung (2s) und die breite Säule gleich dem doppelten Standardfehler ($2s_m$).