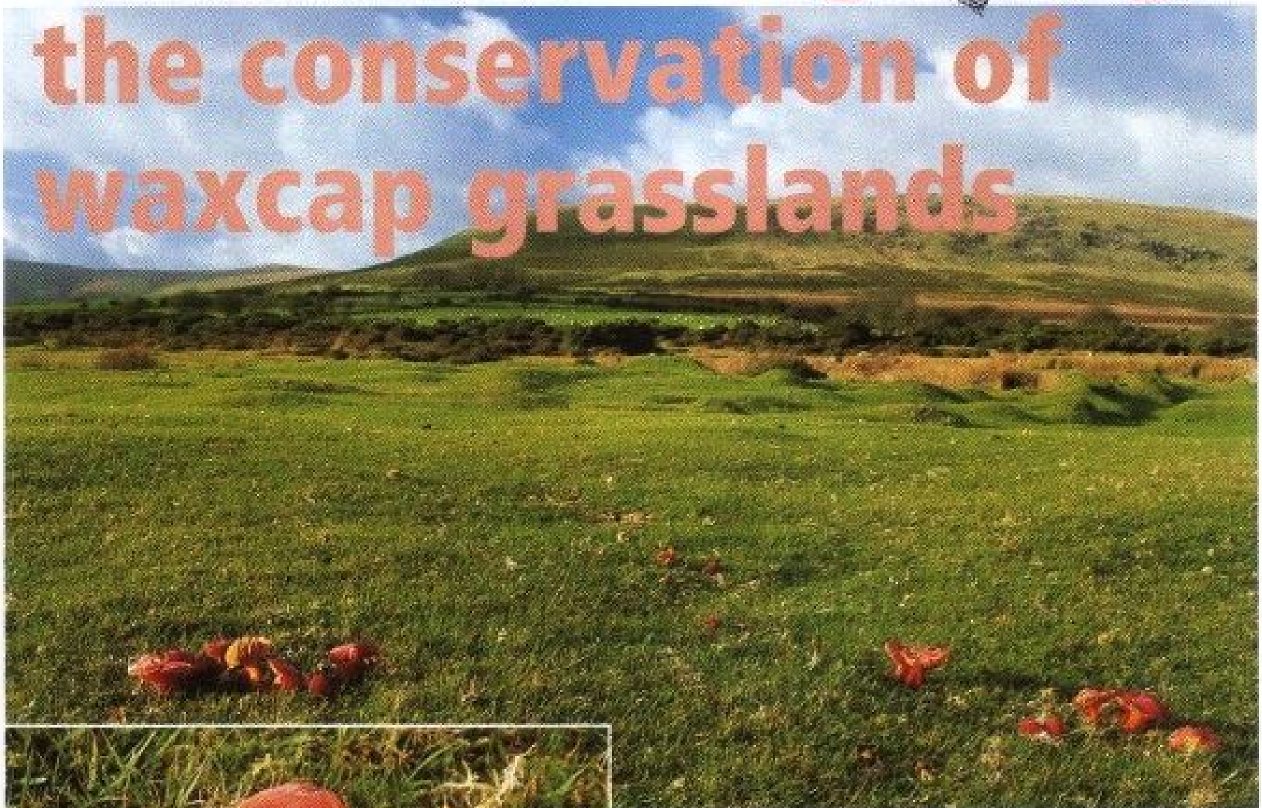
Charismatic megafungi the conservation of waxcap grasslands



Waxcap grassland in the Preseli Hills, Pembrokeshire. Inset ***Hygrocybe splendidissima*****.** Bob Gibbons

Gareth W Griffith, John H Bratton and Gary Easton

When walking over your favourite grassland, have you considered that the largest inhabitants might not be the sheep that are eyeing you with suspicion but some of the fungi beneath your feet? These fungi reveal themselves for only a brief period of each year, often in huge fairy rings, but, rather like icebergs, the mushrooms and toadstools represent only a fraction of the fungal biomass in the soil. Some of these fungi, notably the waxcaps, but also the earth tongues and fairy clubs, are truly enchanting, and it is easy to understand the folklore and taboos with which they are linked in Anglo-Celtic culture. Whilst hiding in the soil, the mycelia of these, and other fungi and bacteria play a central role in the functioning of the ecosystem. However, soil biology is not a 'sexy' subject and it is hard to generate any warm feelings for a mould or bacterium. Nevertheless, the maintenance of biodiversity requires some appreciation of what happens below ground. One way of arousing the interest of members of the public in these processes is to use the larger fungi as 'flagship species', but first it is important to understand what they do and how to manage sites with these organisms in mind.

There is a broad correlation between fungal diversity and plant diversity (with the ratio 6:1 often being quoted; Hawksworth 2001), so that most fungal forays tend to focus on woodlands rather than grasslands. In comparison to animals and plants, fungi are seldom considered in site management plans, and a desire for tidiness (e.g. the burning of fallen timber or the use of leaf-blowers) can often unwittingly have negative impacts on fungal diversity and abundance. Indeed, it was only in 2001 that the first fungal Site of Special Scientific Interest (SSSI) was notified, at Disgwylfa, in the Brecon Beacons, followed by a second at Roecliffe Manor, Leicestershire. Even the lawn at Llanerchaeron, in Ceredigion, with over 27 waxcap species in an area of less than 0.2ha, whilst not under threat, does not currently enjoy statutory protection.

Grasslands are the dominant habitat type in the UK (some 65% of UK land area) and are home to distinct assemblages of macrofungi. This article aims to provide an insight into the diversity of fungi found in grassland habitats, the roles they play in the functioning of these habitats, and the way in which different management practices can affect their diversity and abundance.

Nutrient-cycling in grasslands

In order to understand what the fungi are about and how to conserve them, it is necessary to appreciate how energy sources (sugars or other forms of organic carbon) and nutrients (notably nitrogen and phosphorus, which are needed to make proteins, DNA, etc.) flow through terrestrial ecosystems. Most people are aware that photosynthesis by plants fixes atmospheric carbon dioxide (CO_2) by using sunlight, providing the energy that fuels most life on Earth. However, the other half of the cycle, whereby organic matter is broken down by the process of respiration, is altogether more complex and murky, though it is just as important since it releases nutrients for plant growth and, on a global scale, CO_2 for photosynthesis. Many of the misconceptions about global climate change emanate from a failure to understand the delicate balance that exists among the pools of carbon present in the biomass of living organisms, dead organic matter and atmospheric CO_2. With grasslands comprising such a large proportion of the UK land area, carbon sequestration in these soils is important for UK treaty obligations and, in a large part, it is the fungi which determine rates of decomposition and CO_2 release. Above-ground food webs (most mammals, birds, insects, etc.) make a relatively minor contribution, with most of the CO_2 fixed by plants entering the soil and the realm of the decomposers directly (Swift *et al.* 1979).

It is in soil that the fungi play their key role in the functioning of living ecosystems, with their hyphal filaments burrowing into decaying leaves, secreting an array of enzymes to dissolve plant debris, in particular enzymes that can degrade the cellulose and lignin in plant cell walls (these are the most abundant biopolymers on Earth). The most indigestible part of plant litter is lignin (it imparts to hardwoods their brown appearance), but an 'elite' group of decomposers, the white-rot fungi, play a central role in its breakdown owing to their ability to secrete powerful oxidising enzymes. These white-rot fungi belong mainly to the phylum Basidiomycota, which includes most of the macrofungi with fruiting bodies (toadstools, brackets, etc.) visible to the naked eye.

The other key role played by larger fungi, again mainly basidiomycetes, is to close the nutrient-cycling loop through mycorrhizal associations (Merryweather 2001). By forming intricate sheathing structures on the roots of many shrubby and woody plants, the fungal mycelia act as an extended root system which is efficient at scavenging nutrients from soil organic matter, fuelled by sugars from the host plant.

Types of grassland fungi

The colourful waxcap fungi belonging to the genus *Hygrocybe* and inhabiting long-established, nutrient-poor grasslands are the mycological 'flagships' of northern European grasslands, and these habitats are sometimes called 'waxcap grasslands' to denote the mossy lawns or sheep-grazed pastures where these fungi are found. There are, however, many other macrofungi that inhabit grasslands. Indeed, data for the Netherlands suggest that, out of a total of 3,400 species of macrofungi, about 700 (~20%) have a preference for non-wooded habitats, with about 360 (~10%) preferring grasslands (Arnolds & de Vries 1989).

The absence of woody plants is the reason why ectomycorrhizal fungi are rare in grasslands, while differences in the quality of plant litter (no large branches and twigs, and more fine leaf and stem material) explain the absence of wood-decay fungi. Woodland and grassland soils are not fundamentally dissimilar and, from a below-ground fungal perspective, grasslands could be thought of as 'treeless woodlands'. This oxymoron begins to make a little sense when one learns that, outside Europe, waxcaps are thought of as woodland fungi. For instance, the Pink Meadow Waxcap *Hygrocybe calyptriformis* has been reported from rainforest in the Caribbean, and the great diversity of waxcaps found in Australasia (where they are called wax-gills) and

Box 1 Functional groupings of grassland fungi

Litter saprotrophs In well-established swards, there is a distinct layer of dead plant material ('thatch'), often interspersed with the moss *Rhytidiadelphus squarrosus*. Fungi colonising litter tend to have smaller fruit bodies (often only 1-2mm in diameter), reflecting the smaller underlying biomass.

Soil/humic saprotrophs Most of the larger grassland fungi are active within the upper soil horizons where the organic content (food) is higher and the environment more stable. These include fairy-ring-forming fungi, such as *Agaricus*, *Lycoperdon* and *Lepista*.

Dung saprotrophs These fungi grow on or near dung and are therefore generally restricted to grazed grasslands. Grazing animals extract only about 10% of the energy from the herbage which they ingest, and when it 'emerges' from them it is extensively comminuted (chewed up) and enriched in available nutrients, representing a very high-quality resource for fungi. Most fungi which colonise dung have dark (melanised) spores which can withstand passage through the digestive tract and are able rapidly to colonise the fresh faeces. There is a classic succession of fungi on dung, from pin moulds to cup fungi to mushrooms, which not only can vary according to the quality of the ingested herbage but also differs according to grazing animals. For instance, the Nail Fungus *Poronia punctata* is associated with horse dung. Dung fungal communities also change if animals receive supplementary feeding with hay or silage. Most fungi considered to be dung fungi grow directly on dung, but some species may also be found growing on soil, suggesting that, once established on dung, the colonies can invade surrounding soil.

Mycorrhizal fungi Mycorrhizas are important in all natural ecosystems, but in grasslands it has generally been assumed that the microscopic arbuscular mycorrhizal fungi are the main players (Merryweather 2001), since ectomycorrhizal basidiomycete fungi are generally associated with woody or shrubby plants. Such hosts are largely absent from grasslands, though on calcareous grassland shrubs such as the Common Rock-rose *Helianthemum nummularium* can host a range of ectomycorrhizal fungi, notably *Cortinarius* species. Mountain Avens *Dryas octopetala* is associated with a diverse range of basidiomycetes. A recent and more surprising discovery is that some sedges *Carex*, generally viewed as non-mycorrhizal plants, can form ectomycorrhizal associations with *Cortinarius cinnamomeus* (Harrington & Mitchell 2002).

Many members of the genus *Mycena* are litter saprotrophs. Gareth Griffith

A young fruit body of the dung fungus *Coprinus niveus*. Gareth Griffith

A *Boletus luridus* growing on Mountain Avens on the Burren in western Ireland. Gareth Griffith

America includes many species common to the UK. Waxcaps are indeed found in UK woodlands (some species, such as *H. quieta*, more so than others), but it is more the great abundance of grassland waxcaps in northern Europe that is so impressive. Speculation as to why this is the case ranges from reduced competition (from other fungi) in grasslands compared with woodland to Bruce Ing's suggestion that the higher summer temperature of temperate grassland soils is a significant factor.

The influential ideas of the Dutch ecologist Frans Vera (2000) have given credence to the notion that northern European grasslands are not a recent creation. It is argued that prehistoric landscapes comprised significant areas of parklands, more like the present-day New Forest, than continuous forest cover, and that grazing by large herbivores such as Aurochsen and Tarpans played an important role. In this context, the theory that areas of woodland may be temporarily treeless is compatible with the idea of a more dynamic landscape, without the strict distinctions between woodland and grassland imposed by man. Thus, if one considers the uplands of Britain as huge forest glades, it is perhaps easier to imagine how waxcaps and other grassland fungi existed prior to the advent of agriculture.

Distinct correlations between plant communities or species and the presence of certain fungi do

Box 2 Fairy rings

Many grassland macrofungi form fairy rings. They inspire both fear and wonderment, as encapsulated by Shakespeare's Prospero: '…You demi-puppets that/ By moonshine do the green sour ringlets make/ Whereof the ewe not bites; and you, whose pastime/ Is to make midnight mushrooms…'. Readers may also be aware of the American 'Humungus Fungus' (see http://botit.botany.wisc.edu/toms_fungi/ apr2002.html), but aerial photographs of grasslands reveal the presence of some large fungi in the UK, too. Some of these fungi (which are genetically a single individual) can form rings of 10m or more in diameter in grasslands (with radial growth rates of 5-50cm/yr) and, like their American counterparts, they are very old and very large organisms. Turfgrass managers, who, along with many gardeners, sadly detest fairy rings, identify three types of ring, depending on whether vegetation is killed at the ring margin (type 1), grows more vigorously (type 2) or is unaffected (type 3). The classic Fairy Ring Champignon *Marasmius oreades* forms type 1 rings, as a result of increased hydrophobicity of the soil causing symptoms of drought, whilst the Field Mushroom *Agaricus campestris* forms type 2 rings, probably as a result of increased release of soil nutrients. Some 50 species of basidiomycete are known to form type 1 or type 2 rings, but a much larger number of macrofungi, including many of the CHEG fungi, will grow in this centripetal manner. The visibility of type 1 and 2 fairy rings is highly variable and requires appropriate climatic conditions. However, the occurrence of dozens of often overlapping fairy rings at certain times does serve to illustrate that the macrofungi are much more widely distributed in grasslands than fruit-body surveys tend to suggest.

A fairy ring of *Marasmius oreades*.
Paul Sterry/Nature Photographers

occur, but only rarely. For instance, the presence of Mountain Avens *Dryas octopetala* is correlated with the occurrence of many ectomycorrhizal species. Factors, such as soil pH, which greatly influence plant-community composition have a lesser effect on the diversity of waxcap fungi (there are exceptions, such as *Hygrocybe calciphila* and the acid-loving *H. laeta*), though they seem to prefer drier areas, such as banks, and avoid wetter areas. Thus, waxcaps are found in a wide variety of grassland communities ranging from calcicolous (National Vegetation Classification: CG1, CG2), through mesotrophic (MG5, MG6) to acidic grasslands (U4, U5), as well as heaths, mires, sand dunes and maritime cliffs (Rotheroe 2001; Thompson 2000). Of particular note is the lack of correlation between waxcap diversity and plant diversity. Rotheroe (2001) and others have noted that high fungal diversity can often occur on semi-improved and botanically mundane MG6 grasslands.

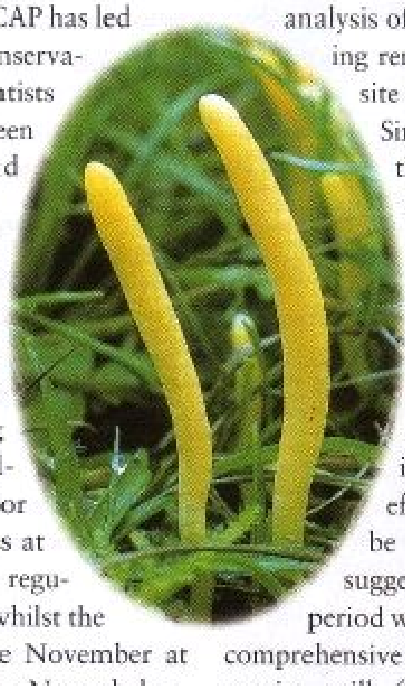
The earth tongue *Trichoglossum hirsutum*. Gareth Griffith

Even though there are more species of *Hygrocybe* (>40 species found in the UK) than in any other genus of grassland fungi, the other 300 or so species of the latter do deserve some attention. From a conservation perspective, it is more useful to group these by ecological function than by taxonomic affiliation. As described in Box 1, most grassland fungi fit into one of four broad functional groupings, namely dung fungi (inkcaps *Coprinus*), litter-decomposers (e.g. *Mycena* species), terricolous species (*Agaricus*), and mycorrhizal species (*Cortinarius*). The remainder, including the waxcaps, are less easy to define but, despite being taxonomically quite unrelated, they show uncanny ecological similarities. The term which we have chosen to use for this group is the 'CHEG' fungi, a term coined by Rotheroe (2001) and based on the initials of the four main taxonomic groups involved: Clavariaceae (fairy clubs), *Hygrocybe* (waxcaps), Entolomataceae (pink gills) and Geoglossaceae (earth tongues). The abundance and diversity of members of all four groupings show distinct similarities, and in several recent studies sites are given a CHEG score rather than a score for waxcaps alone. It was the uncertain ecological status of this last group, combined with its susceptibility to many forms of grassland management, that has inspired us to conduct research into the role that they play in grassland soil processes.

Monitoring of grassland fungi

Despite the vast areas of grassland in the UK, it was only in the 1990s that Rotheroe *et al.* (1996) and others, including Peter Marren (1998) in this journal, highlighted the threatened status of grassland fungi in the UK, notably the loss of diversity of waxcap fungi. The publication of a wonderful key to the genus *Hygrocybe* by David Boertmann (1995) has made waxcap classification more accessible and stable, whilst the recognition that only 200ha of waxcap grassland remained in the Netherlands (Arnolds 1988) has persuaded UK mycologists that we have something special here. This increased interest in the waxcap fungi has reflected more general concerns about the biodiversity of grassland habitats, the loss of hay meadows and the effects of more recent agricultural innovations, such as silage-making. These initial concerns persist, as reform of the CAP has led to a convergence of interests of conservationists and agricultural scientists aiming to find a balance between maintenance of biodiversity and agricultural productivity.

There has been a chronic under-recording of all groups of macrofungi, mainly because of a dearth of suitably experienced recorders. Added to this is the ephemeral nature of most fungal fruiting structures and the distinct seasonality of fruiting of most species. For instance, on the university campus at Aberystwyth, *Hygrocybe conica* is regularly located only in mid-August, whilst the same species appears only in late November at other sites within a few kilometres. Nevertheless, there is a tendency for waxcaps to fruit later in the season, often as late as mid-December, while other grassland taxa tend to fruit earlier in the season, for instance *Agaricus* (August) and *Entoloma* species (October).



The fairy club *Clavulinopsis helvola*. Bob Gibbons

Grassland fungi are even more sensitive than their woodland counterparts to fluctuations in soil moisture levels. For instance, at one of our field sites, at Sourhope, near Kelso, we recorded 995 and 775 *Hygrocybe* fruit bodies in 2001 and 2002 respectively, but only seven in the dry autumn of 2003. Changes in site management from year to year can lead to changes in sward length. Not only does this affect the visibility of fruit bodies to surveyors, but, in rank vegetation, the altered microclimatic conditions (e.g. higher humidity and less light) inhibit fruiting.

From the above, the reader will gather that fungal surveying is a process akin to the mapping of oak woodlands based on the distribution of acorns! Indeed, detailed DNA-based studies by ourselves and others (e.g. Horton & Bruns 2001) have shown that there is often a poor correlation between numbers of fruit bodies produced above ground and the abundance of the underlying mycelium. Moreover, genetic analysis of mycelia from soil often reveals the presence of species which have never been seen to fruit at a given site. Therefore, some fungi which are regarded as rare may in fact be species which reproduce only very infrequently. Nevertheless, in the absence of multi-million-pound budgets for the genetic analysis of grassland soils, fruit-body surveying remains the best practical option for site assessment.

Since most of the people who possess the skills to identify fungi tend to be more interested in taxonomy than in ecology, it is not surprising that methodologies for fungal recording are crude. The majority of fungal surveys are quantitative only insofar as numbers of species encountered are obtained. As the intensity of surveying has a large effect, comparison between sites can be difficult. Peter Orton (1986) suggested that data over a ten-year period were necessary in order to build up a comprehensive inventory for a site, since some species will fruit only rarely. The work of Straatsma *et al.* (2001), using 22 years of data, shows the extent of annual variation in fruiting. In practice, however, site surveys will provide only a

Hygrocybe chlorophana. Bob Gibbons

Hygrocybe coccinea. Bob Gibbons/Woodfall Wild Images

limited snapshot and, as noted by Alan Feest (2000), it is important therefore to consider survey methodology.

To date, there have been a number of detailed waxcap surveys in the British Isles, including Somerset (Thompson 2000), Ireland (McHugh *et al.* 2001), Scotland (Newton *et al.* 2003) and Carmarthenshire (Rotheroe 2001). In each case, the aim was to identify and rank sites of conservation interest. Since Rald's (1985) use of numbers of waxcap species to rank site quality (with >17 species on a single visit indicating a site of national importance), various other systems, elegantly described by McHugh *et al.* (2001), have been devised. For instance, the 'CHEG' profile devised by Rotheroe *et al.* (1996) includes a broader range of macrofungi (see above; the genera *Dermoloma* and *Porpoloma* are also included, and merged with counts of *Hygrocybe* species for convenience), whilst McHugh *et al.* (2001), recognising that some species are better indicators than others, placed the CHEG fungi into three categories (A, B or C). The presence of one or more members of category A (which consists of 12 species, including the Crimson Waxcap *H. punicea*, the Purple Fairy Club *Clavaria zollingerii* and the Olivaceous Earth Tongue *M. olivaceum*) is the best indicator of a high-quality site, whilst the 28 species in category B (despite including rare species such as the Date-coloured Waxcap *H. spadicea*) are inferior indica-

Box 3 A novice's guide to fungal diversity at grassland sites

Small (<2cm cap diameter) brown/grey/white basidiomes (basidiomycete fruit bodies)	☐ (1 pt)
Basidiomes on dung	☐ (1 pt)
Field mushroom (*Agaricus*) basidiomes	☐ (1 pt)
Puffballs or other larger basidiomes (Cap >4cm diameter)	☐ (1 pt per species)
Pink gills (*Entoloma*) – narrow pink/off-white gills; caps white to brown	☐ (2 pts per species)
Earth tongues (black/dark green; tough) or fairy clubs (yellow/white; fragile)	**☐ (5 pts per species)**
Purple Fairy Club *Clavaria zollingerii* (distinctive forked structures)	**☐ (10 pts)**

Waxcaps *Hygrocybe* (caps often slimy when wetted; gills thick, well spaced and white or similar colour to cap)

Cap colour	white (*H. virginea*)	☐ (2 pts)
	orange/yellow, turning black (*H. conica*)	**☐ (2 pts)**
	yellow (*H. chlorophana, glutinipes*)	**☐ (2 pts)**
	green (*H. psittacina*)	**☐ (2 pts)**
	light brown (*H. pratensis*)	☐ (3 pts)
	orange (*H. reidii*; honey odour *H. laeta*)	☐ (3 pts)
	red (*H. coccinea, punicea, splendidissima*, etc.)	**☐ (7 pts)**
	pink (*H. calyptriformis*)	**☐ (10 pts)**

Based on a single visit:
Scores of <10 would be expected for more intensively managed grasslands
Scores of 10-30 indicate some mycological potential
Scores of >30 would indicate a good 'waxcap grassland'
For species read distinct morphological type. The groups shown in bold are those which are fairly unmistakable. Note that sites should not be 'written off' because of a low score; scores will be higher from mid-October onwards.

***Hygrocybe psittacina*.** David Osborn/Nature Photographers

***Hygrocybe conica*.** Paul Sterry/Nature Photographers

tors. There are several waxcaps, such as *H. virginea* or *H. conica*, which appear to be early colonisers of previously disturbed grassland and are thus often found alone. However, the relatively common *H. pratensis* was found by Thompson (2000) to be a good indicator of high-quality sites, since it generally co-occurred with nine or more other waxcap species. On the other hand, Newton *et al*. (2003) found that the best sites for waxcaps tended not to be the best ones for other CHEG species, with the best waxcap site in Scotland (Rassal, in Western Highland Region, with 27 waxcap taxa) ranked only 230th for fairy clubs out of the 621 sites surveyed.

The Curragh of Kildare, which covers thousands of hectares, and the lawn at Llanerchaeron, covering only a few hundred square metres, are home to 33 and 27 species of *Hygrocybe* respectively, and both score highly in any of the ranking systems outlined above. However, no account is taken of the huge differences in site area. In seeking to provide objective criteria for site assessment, Thompson (2000) has noted that it is necessary to consider the size of the site (or, more precisely, the area of grassland) and to standardise both the surveying methodology (random walk, mowing transect or quadrat) and the intensity of surveying (number of visits per season and number of years).

In an ongoing project funded by CCW, we are testing the use of 900m² quadrats, surveyed by the 'mowing method' (walking back and forth at approximately 2m intervals), either early or late in the season in two successive years. Larger sites involve more quadrats, and surveyors are also making counts of numbers of fruit bodies for each species and total number of species for each site. It is hoped that this system will provide a cost-effec-

***Hygrocybe punicea*.** Gareth Griffith

A primordium of *Hygrocybe reidii*. Gareth Griffith

***Clavaria zollingerii*.** Gareth Griffith

Box 4 Key recommendations for grassland fungal management

For agricultural grasslands:
- Avoid ploughing and application of fertilisers or pesticides
- Application of lime is potentially harmful, so should generally be avoided
- Application of farmyard manure can lead to increase of soil nutrient levels, so should be avoided
- Reduction in grazing levels can lead to increase in sward length and soil nutrient levels
- Keep the sward short by grazing during the autumn period

For lawns/churchyards:
- Do not use lawncare products or any fertiliser
- Removal of clippings is important to maintain low soil nutrient levels
- Good waxcap sites have a healthy moss layer, so avoid scarifying or application of mosskiller
- Trampling, especially in late summer/autumn, can damage fruit-body primordia and reduce fruiting

In all cases, site visits in the autumn to observe fruiting will indicate which areas are good for macrofungi. Encourage use of the novice key (Box 3) for landowners/site-managers. Collection of a few fruit bodies (dried over a radiator and kept in an envelope) will allow more precise identification at a later date.

tive method of site assessment, whilst allowing resurveying of defined areas over the longer term. For more detailed surveys, we use differential GPS to record fruit-body positions to within 1m (Griffith *et al.* 2002), but this is a much more time-consuming process.

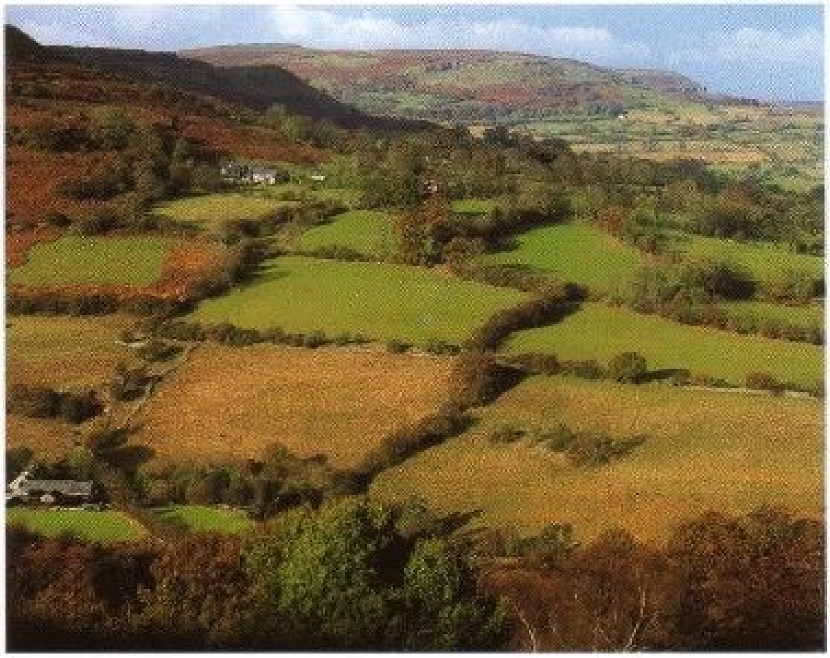
One further idea, untested at present but which overcomes the problem of a dearth of suitably experienced surveyors, is a phase 1-type survey in which waxcaps are classified rather crudely by colour (red, pink, green, blackening, etc.). The inspiration for this was Plantlife's recent Pink Waxcap Survey (see www.plantlife.org.uk/waxcap/home.htm), and also the idea of recognisable taxonomic units (RTU) for rapid diversity assessment of invertebrates (Beattie & Oliver 1994). Box 3 contains a suggested checklist which readers may like to try out this autumn. At the very least, this approach may identify sites worthy of closer attention, although we emphasise that it should not be used to discount sites with a low score, since dry weather or the presence of rank vegetation due to temporary absence of grazing could cause poor fruiting in a given year, even though the underlying mycelium is in good health.

Effects of management

Many of the practices of modern agriculture are harmful to macrofungi. In particular, ploughing destroys subterranean mycelial networks, a trauma from which these slow-growing fungi can take decades to recover. Equally harmful, though more subtle, are the effects of fertilisers, which alter the way in which nutrients flow through the soil and can devalue the fungal 'currency' in finely balanced mycorrhizal interactions.

In the case of nitrogen, usually the main limiting nutrient in natural ecosystems, inputs can come from both natural sources (fixation of atmospheric nitrogen by legumes, addition of dung/urine from grazing animals) and anthropogenic sources (inorganic fertilisers and atmospheric pollution: see below). For macrofungi and other soil organisms, and indirectly higher plants (via mycorrhizal associations), the key factor is the nitrogen status of the soil, and it is the balance between inputs and outputs that is critical. As far back as 1875, J H Gilbert noted that addition of nitrogen (as ammonia or manure) reduced legume cover on the classic Parkgrass plots at Rothamsted and that there was a strong negative correlation between nitrogen addition and both the diversity and the abundance of macrofungi. He found waxcaps mainly restricted to untreated plots or those given superphosphate of lime. Our more recent surveys of established grassland at Sourhope showed the distinct negative effect of nitrogen addition at 240kg N/ha/yr (see Fig. 1) on the abundance and diversity not only of CHEG fungi, but of macrofungi in general. Macrofungal fruiting patterns also have the potential to assist conservation managers in detecting changes in management practices. For example, Dr Nigel Stringer, a mycologist at CCW, was able to identify a case of fertiliser application on an SSSI from changes in macrofungal fruiting patterns where there was no change in higher plant composition. Whilst nitrogen fertiliser is detrimental to CHEG fungi, Lange (1982) found that 24 of 55 macrofungi observed on a 2-7-year-old reseeded lawn were 'nitro-philous' (including most species forming type 1 or type 2 rings) and fruited preferentially in N-treated areas (at 350kg N/ha/yr). The only CHEG fungus recorded in this study was *Entoloma sericeum*, which was inhibited by N applications, as were 11 other 'nitrophobous' species.

Nitrogen levels in soil depend not only on inputs but also on the extent of removal by grazing or mowing. The rearing of grazing animals for meat and milk removes some nitrogen (1-10kg N offtake/ha/yr), but their main effect is to recycle plant nitrogen, since about 90% of ingested forage N is returned as urine and dung. However, in dung and urine patches, locally high 'application rates' (up to 1,000kg N/ha) can occur from which some 30-80% of the nitrogen may be lost through leaching, ammonia volatilisation, and denitrification by bacteria. Hay-making or mowing is potentially a more efficient means of nutrient removal. Bill Adams, at UW Aberystwyth, found that nitrogen removal from two cuts per year from an upland (U4) grassland ranged from 28kg/ha/yr (no nutrient additions) to >80kg/ha/yr (when fertilised at 75kg N/ha/yr). Similarly, at Rothamsted's Parkgrass site, the annual hay cut removes 35kg N/ha/yr from the untreated plots.



Unimproved farmland (foreground) near Abergavenny, Gwent, contains some of the top UK sites for waxcaps. Bob Gibbons

Many readers will now be wondering where all the nitrogen that threatens the waxcaps is coming from. The main natural source is fixation of atmospheric nitrogen by bacteria in the roots of legumes (1-20kg N/ha/yr). Another source is the deposition of nitrogen as ammonia or nitrous/nitric oxides. It is still not widely appreciated that nitrogen-deposition levels have increased several-fold over the past century, with the UK now receiving 5-55kg N/ha/yr. Most nitrogen deposition is anthropogenic, originating mainly from vehicle emissions and intensive animal husbandry. N-deposition levels are a significant cause for concern, particularly in the uplands of England and Wales, where high rainfall leads to deposition levels of 20-30kg N/ha/yr in areas which have historically received very low nutrient inputs. In the Netherlands, nitrogen deposition is even higher (with rates of over 100kg/ha/yr in places), and Eef Arnolds (1988) has identified this factor as the main cause of the declining yields of certain ectomycorrhizal fungi in the post-war period. It could be argued that levels of deposition found in the UK are generally lower, but Lilleskov *et al.* (2001), studying macrofungal fruiting in Alaskan pine forests, found that levels as low as about 7kg N/ha/yr had a significant negative effect. Genera such as *Cortinarius* and *Russula* were found to be highly susceptible in both studies.

Therefore, it is important for conservationists to give some consideration to nitrogen budgets. If none is being removed by grazing or mowing, nitrogen deposition will cause levels in the soil to increase. Although most grasslands are managed agriculturally (i.e. N off-take by grazing, silage, hay-making), mowing is the principal form of management for amenity grasslands. There is a worrying trend here to leave grass-clippings in place, a process termed grass-cycling. Though unsightly, grass-cycling is attractive to cash-strapped local councils, since it simplifies the mowing process and reduces pressure on landfill sites. However, when clippings are left to rot *in situ*, the nutrients are returned to the soil, leading to a build-up of soil nutrient levels. Mycologists have realised that grasslands where clippings are left to rot are fungally depauperate. In churchyards, which include many of the best waxcap sites, hard-pressed volunteers faced with the tricky problem of mowing between graves often resort to using strimmers. However, raking and removal of clippings would not only please relatives of the dead but, if timed correctly to allow grassland

When grass clippings are left *in situ* they can lead to a build-up of soil nutrients. Gareth Griffith

plants to set seed but to keep the sward short towards the end of the summer, would also have a beneficial effect on mycological as well as botanical diversity.

Data on the effects of the other main agricultural fertilisers are less clear-cut than for nitrogen. The availability of phosphorus is highly pH-dependent, and one reason for adding lime to acid grasslands is to increase the availability of phosphates for plant uptake, through increasing turnover of soil organic matter. We have found that very high levels of lime application at Sourhope (6 tonnes/ha/yr) were strongly inhibitory to waxcap fruiting. However, the effect of applications at agriculturally realistic levels (about 6 tonnes/ha in a five-year cycle) is less clear. Indeed, in one experiment on an acidic upland in Wales, fruiting of *Hygrocybe conica*, the only waxcap at the site, was greatly enhanced three years after lime addition (John Hedger pers. comm.).

A turf containing *Hygrocybe calyptriformis* as part of a translocation experiment. Gareth Griffith

Habitat restoration

The prevailing wisdom suggests that low soil fertility is correlated with high fungal diversity, particularly in the case of CHEG fungi, mirroring the trend in plant diversity. Microbiological studies have found that at low fertility the importance of fungi relative to bacteria is enhanced (Bardgett *et al.* 1996). The term 'fungally dominated soil' has been used, and it has been suggested that ratios of bacterial and fungal biomarkers in soil might provide an index of 'restorability'.

Many of the best waxcap grasslands are churchyards, lawns or upland grasslands which have been spared the ravages of ploughing and fertiliser application wreaked by post-war agriculture. Sadly, there seem to be few detailed records of the diversity and abundance of grassland fungi in the pre-war period, but it is often quoted for several of the best waxcap grasslands that they have 'not been ploughed within living memory', though Keizer (1993) is more specific and mentions the period of at least 20-30 years. One apparent exception has come to our attention recently. This is St Dunstan's Farm, in Sussex, which, despite having been ploughed and fertilised in the 1980s, is now home to 23 waxcap species (Russell 2004). It would be fascinating to learn what management has been in place here in more recent decades.

Fungi and other soil organisms are affected primarily by soil-nutrient status and not directly by plant-community structure. However, as mentioned earlier, few macrofungi are observed if the vegetation is rank. This is due in large part to unsuitable microclimatic conditions, and, since the underlying mycelium is probably unaffected, changes in sward management to ensure a sward height of 10cm or less in late summer and autumn are likely to lead to increased macrofungal fruiting. Although the majority of good waxcap sites are sheep-grazed, the type of grazing animal (rabbit/sheep/cow) does not, to our knowledge, have a great influence on waxcap diversity. However, other grassland animals such as moles and ants, through the soil disturbance that they cause, may be more damaging (Ray Woods pers. comm.). Another factor emerging from our Sourhope studies is the possible effect of trampling. Fig. 1 shows that 50% more fruit bodies were found on the Control 2 plots compared with Control 1 plots. These untreated plots differed only in that the latter was subject to frequent visits by scientists involved in the NERC Soil Biodiversity Project (of which our work was a part). Thus, trampling by people or grazing animals may be a significant factor in inhibiting fruiting, presumably through damage of fruit-body primordia rather than through soil compaction.

Given the disparities noted above between mycelial abundance in the soil and macrofungal fruiting, it would be fascinating to know whether fertiliser applications merely inhibit or actually kill the mycelia of CHEG fungi. When considering restoration of grasslands, we simply do not know whether recolonisation involves incoming airborne spores or regrowth from residual patches of mycelium. The first waxcaps to re-occur on previously disturbed sites are often *Hygrocybe virginea* and *H. conica*. This may be because they can survive disturbance better, grow more quickly or have a lower critical biomass level for fruit-body production. As in higher plants, both vegetative growth rates and the time lapse between seed/spore germination and fruiting can vary considerably among different species.

The Bronydd Mawr restoration experiment provides an opportunity to record how waxcap populations recover from intensive management. The site was last ploughed in about 1970 and was managed intensively until 1991. It contains plots receiving fertiliser and lime applications at pre-1991 levels (150kg N/ha/yr, 25kg P/ha/yr, 50kg K/ha/yr and lime at 5 tonnes/ha every five years), as well as plots receiving P/K/lime, lime only or no additions (sward height in all plots is kept at 3-4cm by sheep grazing). Botanical diversity has increased only slightly, but untreated plots showed a significant increase in fungal diversity. As Keizer (1993) has also noted, mycologically rich grasslands are sometimes poor in vascular plants and may therefore not receive the conservation attention which they merit.

Hygrocybe virginea. Gareth Griffith

Habitat restoration often involves nutrient removal, and strategies for achieving or maintaining low nitrogen levels are described above. However, the more extreme approaches, such as soil-stripping (to remove high levels of phos-

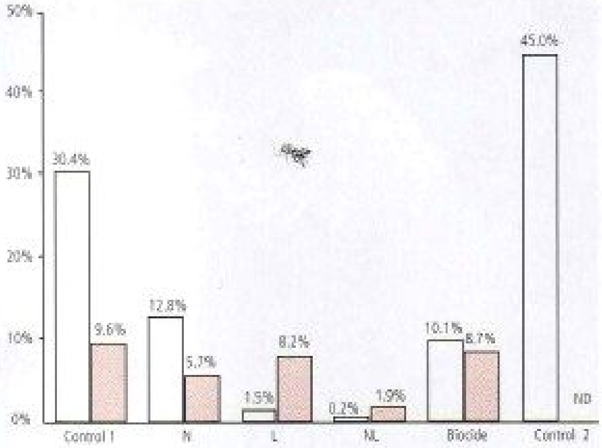


Figure 1 The effect of various treatments on waxcap fruiting and abundance of moss at Sourhope field site. Open columns indicate percentage of all waxcaps found on a given plot. Tinted columns indicate percentage moss cover on the plots. N= nitrogen; L = lime; NL= nitrogen and lime; Biocide = Dursban added at 1.5l/ha/mth during the summer period. ND = not determined.

phate), will damage mycelial networks in soil and these may be very slow to regenerate. Fungal spores are highly mobile and will quickly colonise sites of suitable nutrient status, although there may be a very long time lag before the colony is of sufficient size to form fruit bodies. We have experimented with translocation of turves containing mycelium in order to elucidate which stage of the life cycle is most critical, but it may be many years before we know if the fungal community survives such treatment.

What do waxcaps do in soil?

Most of our knowledge about CHEG fungi comes from field observations, yet effective management plans also require some information about the ecological function of these fungi. To date, this information has been lacking, because waxcaps, along with other CHEG species, are not easy organisms to study. Their spores barely germinate under lab conditions and they are extremely difficult to maintain in culture, a characteristic shared by many ectomycorrhizal fungi such as Cep *Boletus edulis* and Chanterelle *Cantharellus cibarius*. We have found that the spores of most species fail to germinate at all and that even the commoner species, such as *Hygrocybe virginea*, seem to stop

The 'pink ballerina' *Hygrocybe calyptriformis*. Bob Gibbons

species) and that other CHEG fungi shared these distinctive patterns (Griffith *et al.* 2002). The finding that the CHEG fungi, despite being taxonomically very diverse, share distinctive isotope signatures as well as ecological distributions is strong evidence that they also perform similar ecological functions in grasslands. One possibility is that these fungi, adapted for growth in very low-nutrient soils, are able to access nitrogen locked up in soil organic matter. Thus, addition of nitrogen would allow other microbes adapted to uptake of more accessible nutrients to outcompete and exclude the waxcaps.

growing after limited growth in laboratory conditions. Indeed, the closest relatives of the waxcaps are members of the woodland-dwelling and ectomycorrhizal genus *Hygrophorus*, and it has been suggested by Kreisel (1987) that waxcaps may be mycorrhizal, though most sources list them as saprotrophs. The observation that *Cortinarius cinnamomeus* forms ectomycorrhizas on sedges *Carex* in calcareous grasslands (Harrington & Mitchell 2002) illustrates that ectomycorrhizas can also occur with herbaceous hosts. No such association has hitherto been shown for waxcaps, but Seviour *et al.* (1973) did observe a *Clavaria* species associated with the roots of *Azalea indica*. Despite the lack of evidence of any mycorrhizal association, grasslands which are home to diverse CHEG fungi also tend to be very mossy, and we have observed a close correlation between moss abundance and waxcap fruiting at Sourhope. Furthermore, Ray Woods (pers. comm.) has observed that the moss *Rhytidiadelphus squarrosus* (along with Common Bent *Agrostis capillaris* and Field Wood-rush *Luzula campestris*) is the most constant plant associate of *Hygrocybe calyptriformis*.

Another avenue of investigation which we have followed is that of examining the natural abundance of carbon and nitrogen isotopes, a technique widely used in ecology to determine feeding behaviour (for example, vegetarians have different isotope signatures from those of meat-eaters). These analyses revealed that waxcaps have particularly distinctive isotope patterns (very different from those of other grassland and woodland

Statutory considerations

As mentioned earlier, only two SSSIs have been designated on the basis of their fungi. This stems from the fact that there are very few mycologists (compared with, say, ornithologists) and also that most fungi are visible for only a few weeks during the year. Thus, it is difficult to establish which fungi are in decline and which require protective measures, although 268 grassland macrofungi are included in Red Data lists across Europe (Arnolds & de Vries 1993). Consequently, only 13 Species Action Plans have been prepared for fungi (excluding the 36 lichenised fungi). This represents 0.1% of fungal species (even fewer if one considers that 95% of fungi remain undiscovered: Hawksworth 2001), compared with a figure of 6.7% for birds (Fleming 2001).

Of these 13 species, three (the Pink Meadow Waxcap *Hygrocybe calyptriformis*, the Date-coloured Waxcap *H. spadicea* and the Olivaceous Earth Tongue *Microglossum olivaceum*) are considered grassland species. It is interesting to note that only *M. olivaceum* appears in the McHugh *et al.* (2001) Category A. *H. calyptriformis* is by far the least rare of these species and, following the more intensive monitoring that has taken place in recent years, as well as Plantlife's Pink Waxcap Survey, it has been recorded from well over 200 sites. However, *M. olivaceum* (<70 sites) and particularly *H. spadicea* (<20 UK sites), as well as being less distinctive in appearance, are genuinely limited in their distribution. In the case of *H. spadicea*, its rarity would appear to stem from its preference for atypical grassland sites (south-facing, dry slopes with thin soils, usually within a few kilometres of the sea), possibly indicating a preference for higher soil temperatures or tolerance of droughting.

Conclusions

Great progress has been made over the past decade in highlighting the mycological value of oligotrophic grasslands. The beauty and colour of the waxcap fungi are complemented by the strange fruit bodies of the earth tongues and fairy clubs, making the CHEG fungi in general, and the waxcaps in particular, a group of organisms which are easy to 'sell' to the public. This will, one hopes, help to revise the traditional Anglo-Celtic prejudices about fungi and aid an appreciation of the importance of fungi in ecosystems. Agri-environment schemes now provide an incentive for maintaining the biodiversity of grasslands, and we hope that this article has illustrated that sites of little botanical interest may be worthy of a field visit during the autumn. Managing for fungal biodiversity also requires careful consideration of grazing, with a close-cropped sward in the later part of the year being beneficial for fungal fruiting, and also for removal of nutrients. Further details of waxcap research at the University of Wales Aberystwyth can be found at http://www.aber.ac.uk/waxcap/.

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