# Ecology and Diversity of Waxcap (*Hygrocybe* spp.) Fungi

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#### Summary

Members of the genus *Hygrocybe* are ubiquitous and colourful components of many undisturbed and nutrient-poor grasslands in the UK. Through a number of detailed surveys of the distribution of *Hygrocybe* spp. and of genera showing similar patterns of occurrence (e.g. Clavaria spp., Entoloma spp., Geoglossum spp.) a picture is gradually emerging of the more important 'waxcap grassland' sites, and of those species in greatest need of protection. Waxcap fungi are far from ideal experimental organisms which explains why so little has been published about their biology and ecology. They cannot be cultured on laboratory media and the correct conditions for inducing spores of most species to germinate have yet to be established. Nevertheless approaches such as isotope ratio mass spectrometry and the use of molecular biology techniques are beginning to provide an insight into the role played by these organisms in grassland ecosystems, and why they are so adversely affected by many agricultural practices. Current field experiments at various sites including Sourhope near Kelso will also permit investigations into waxcap ecology to be correlated with parallel studies of other members of the soil biota.

Key words: Ecology, diversity, Hygrocybe spp., Biodiversity Action Plan.

#### Introduction

Grasslands are the product of centuries of human agricultural activity and represent the dominant habitat type in the UK, covering some 65% of the total land area. Semi-natural grasslands in the form of herb-rich meadows also represent a significant but largely neglected reservoir of indigenous biodiversity for the British Isles. However, over the past 50 years it is reliably estimated that in excess of 95% of haymeadow habitats have been destroyed (Lovegrove, Shrubb & Williams, 1995), partly through urban or suburban building programmes but mainly through agricultural intensification in the form of ploughing and increased fertiliser input. The resultant loss of biodiversity among plant and animal species (e.g. orchids, birds, butterflies) has received some public attention but only in recent years has there been a recognition that the distinctive mycota of these unimproved grasslands is also under threat. In the Netherlands with a higher population density and no remote upland areas the situation is much worse and it is estimated that only some 200 ha of 'waxcap grasslands' remain (Arnolds, 1988).

The most distinctive and visible components of the grassland mycota are the waxcap fungi, belonging to the genus *Hygrocybe*. Members of this genus typically possess brightly coloured pilei which are often given a 'shiny' appearance by the presence of a glutinous surface layer. Some 60 species of

Hygrocybe occur in Europe most commonly in Western and Northern regions (Boertmann, 1995). In Europe there is a strong association between Hygrocybe spp. and grassland habitats, though some species, notably H. viola and H. quieta, can also occur in woodlands. The association of the European waxcaps with grassland habitats contrasts with the situation in North America where Hygrocybe spp. are typically considered as woodland species. The reason for this difference in habitat is unclear, though it may be related to similarities in summer soil temperature between the grasslands in the Atlantic regions of Europe and the woodlands of North America. Watling (1984) has suggested that Hygrocybe spp. originally evolved in grassy woodland glades and that historic deforestation and agriculture has in effect expanded the habitat of these fungi.

The aim of this article is to review the literature relating to ecology and distribution of *Hygrocybe* spp. and other grassland macromycetes. Strategies for elucidating the ecological role of these fungi and thereby their habitat requirements are also discussed.

## Waxcaps as ecological indicators

Waxcaps are found on a range of grasslands, ranging from old lawns and graveyards, to upland and calcareous haymeadows. Mainly through the still ongoing waxcap grassland surveys in Wales (Rotheroe et al., 1996; Rotheroe, 2001), Scotland (Newton et al., 2000), Northern Ireland (McHugh et al., in press) and England (Thompson, 2000), a significant body of data is being accumulated as to the types of grassland which contain the greatest diversity of waxcaps and associated taxa. It should also be noted that several important surveys of grassland mycota have been conducted by a previous generation of mycologists (Wilkins & Patrick, 1939; Parker-Rhodes, 1951, 1953, 1955a; Warcup, 1959a, b) and these are potentially invaluable sources of data since they relate to a period which largely pre-dated the widespread use of inorganic fertilisers.

The greatest diversity of waxcaps are found on mesotrophic grasslands (MG5 (Rodwell, 1992)), though upland (U4) and calcicolous (CG1, CG2) grasslands can also be very productive. Some species such as *H. calciphila* and *H. laeta* are restricted to more alkaline or acid sites respectively, with others such as *H. chlorophana* showing no preference and even occurring in wetter mire communities (M23, M24, M25) (Rotheroe, 1999; Thompson, 2000; Rotheroe, 2001). One as yet unexplained feature of some of the best waxcap grasslands is that several have been subject to some agricultural improvement or overgrazing and are consequently considered botanically mundane (e.g. the MG6 Lolium-Cynosurus grassland at Waunlas, NBGW) (Rotheroe, 2001). Thompson (2000) found that fields which had been partly improved (MG6a, MG6b) had on average (but not always) retained their fungal diversity well.

A feature common to all sites with diverse waxcap populations is that they are grazed or mown regularly and that there has been no recent fertiliser application. Ploughing is the most drastic form of disturbance for a grassland and many (possibly most) of the best 'waxcap grasslands' have probably experienced ploughing at some point in the past. However, conclusive proof (by examination of soil profiles) of the total absence of ploughing is difficult to obtain and it appears that many good waxcap grasslands were ploughed during and immediately after World War II. The time elapsed since last ploughing/fertiliser application is difficult to measure, though it is likely that recovery after fertiliser application occurs more quickly than after ploughing. Ejrnaes & Bruun (1995) found that species such as *H. virginea* and *H. conica* reappear after c. 10 yr but that more sensitive species (e.g. H. splendidissima) may not reappear for >30 yr.

The effect of inorganic fertilisers on fruit body production is known to be immediate but it is not known how well the underlying mycelial system can withstand eutrophic conditions. It may be the case that sporadic fertiliser application (e.g. once every 5-10 years) is less damaging, permitting the mycelium to recover between inputs. Fertiliser application to grasslands did take place during the 19th century but overall application rates remained low until the late 1940s. There has been a sixty-fold increase in use of nitrate fertilisers since 1930 (Frink, Waggoner & Ausubel, 1999) with UK grasslands now receiving on average 120 kg ha<sup>-1</sup>yr<sup>-1</sup> of nitrogen (compared to 263 kg ha<sup>-1</sup>yr<sup>-1</sup> for the Netherlands; data from FAO for 1996; http://www.fertilizer.org). Over the course of the past century there has also been an increase (up to two-fold from c. 5-7 kg ha<sup>-1</sup>yr<sup>-1</sup> to 8-14 kg ha-1yr-1 (Frink et al., 1999)) in the aerial deposition of nitrogen (important for upland grasslands), due to increased air pollution and fertiliser use. High sensitivity to elevated nutrient levels may explain why waxcaps seem to provide a better indication of the continuity of more extensive management regimes than do vascular plants, since they very probably establish more slowly than vascular plants and are longer-lived (Keizer, 1993).

The absence of mowing or grazing also has a deleterious effect on fruit body production, ultimately leading to successional changes in the plant communities with which waxcaps are associated (Keizer, 1993). It is difficult to conceive how mycelial systems might be adversely affected by taller above-ground vegetation and it seems more likely in the short term that factors such as the effect of microclimate on the development of fruit body primordia may be responsible. Furthermore, cropped vegetation does make it very much easier for the mycologist to find and thus record fruit bodies (Thompson, 2000), especially for smaller species such as *H. glutinipes*. Grazing (and dung input) as opposed to mowing (with or without removal of hay or cuttings) will alter nutrient flows within the soil, though no significant differences in mycoflora have been observed. In some circumstances, mowing is logistically easier (Nauta & Jalink, 2001), though grazing (particularly by sheep or rabbits (Rotheroe, 2001)) generally produces closer cropping of the vegetation and a greater diversity of niches (from fresh dung to patches of scrub) for fungal colonisation.

Another consistent feature of habitats in which waxcaps occur (in the UK at least) is the presence of moss cover (usually Rhytidiadelphus squarrosus in Wales). Arnolds (1981, 1982), in an extensive study of the ecology of grassland fungi in the Drenthe region of the Netherlands, classified several Hygrocybe spp. (including H. psittacina, H. glutinipes and H. miniata) as 'saprophytic fungi, associated with bryophytes', noting a positive correlation with the occurrence of several species with pleurocarpous mosses, such as R. squarrosus and Pseudo-scleropodium purum.

In an on-going study at a Scottish field site (Sourhope, nr. Kelso; U4b habitat) managed through the NERC Soil Biodiversity Initiative, we have

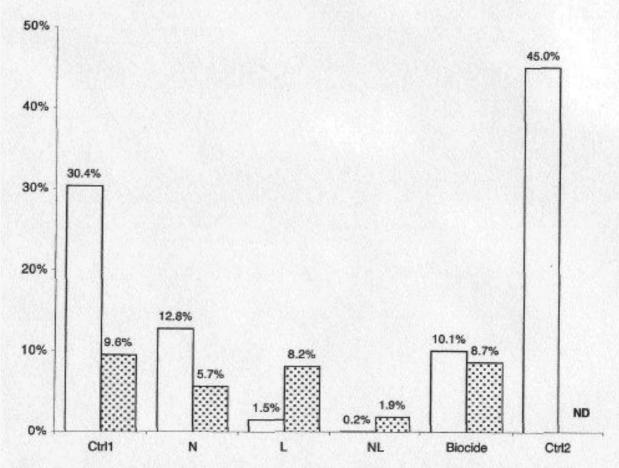


Fig. 1. Percentage of fruit bodies found in each plot at Sourhope during autumn 2001 (clear) and % cover of the moss Rhytidiadelphus squarrosus in July 2000 (shaded). Treatments began in May 1999 (N = 12 g.m<sup>-2</sup> granular Ammonium nitrate; L = 6 t ha<sup>-1</sup> yr<sup>-1</sup> CaCO<sub>3</sub>; Biocide = Dursban 4 added at 1.5 l ha<sup>-1</sup> monthly during the summer). Monthly mowing (cuttings removed) during summer period. Further treatment details are provided at http://mwnta.nmw.ac.uk/soilbio/ sourhope\_treatments.htm. (G.L. Easton, unpublished data; moss data from (Buckland, 2001)).

recorded fruit body abundance of *Hygrocybe* spp. in response to a range of replicated plot treatments (Fig. 1). Further details of the experimental design can be found at http://mwnta.nmw.ac.uk/soilbio/Sourhope\_Design .htm. As Arnolds (1982, 1989) found, the application of nitrogen (NH4NO3 at a rate of 120 kg ha<sup>-1</sup>) led to an approximate four-fold decrease in fruit body production. Application of a pesticide (Dursban) also caused a reduction in fruit body production, possibly due to the inhibition of grazing of mycelia by soil animals. The results of a parallel Soil Biodiversity project on saprotrophic fungi (based at King's College, London) found a contrasting pattern of fruit body production for *Psilocybe semilanceata*, which fruited in great abundance on the pesticide-treated plots (Deacon, 2001).

There were some differences between the two control plots (Ctrl1 and Ctrl2), probably because the former has been subject to much soil sampling and disturbance by previous research, while the latter has been left essentially untouched. Data from vegetation analysis in 2000 (Buckland, 2001) shows that the effect of these treatments on the distribution of the moss *R. squarrosus* mirrors that for *Hygrocybe* fruit bodies.

More surprising was the near-absence of any waxcap fruit bodies on the plots treated with lime as CaCo<sub>3</sub> (6 t ha<sup>-1</sup> yr<sup>-1</sup> annually since 1999). Since many waxcaps are found on calcareous grasslands, it might be expected that species other than the acidophilic *H. laeta* (second commonest at this site; pH of control plot soil is c. 4.7) would be unaffected. Furthermore, previous studies of grassland fungi have found a greater diversity of *Hygrocybe* spp. species on sites with neutral (6.5-7.5) rather than acidic pH (Wilkins & Patrick, 1940; Warcup, 1959b).

Liming (like fertiliser input) is known to cause general changes in the soil microflora, with an increase in bacterial biomass (and activity) occurring at the expense of the fungi (Bardgett, 1996). However, in a similar experiment in Wales, a single application of lime produced a significant increase in fruiting by waxcaps after 3-5 yr (J.N. Hedger, unpublished data). Furthermore, at Llanerchaeron there is a slight increase in soil pH nearer the house (thought to be due to run-off from application of limewash) and some unusual species, notably Microglossum olivaceum, occur specifically in this area (M. Rotheroe, pers. comm.). Therefore, both the dosage (the 18 t ha<sup>-1</sup> over 3 yr at Sourhope is a high rate of application) and the time elapsed since treatment may affect fruiting of these species. The effect of lime application was found by Hora (1959) to inhibit fruiting of some woodland fungi but to spectacularly promote fruiting of others.

#### Conservation issues

The association of waxcaps with unimproved grasslands was first recognised by Schweers (1949) who introduced the term *Hygrophorus* meadow. With the aim of being able to identify more objectively the better waxcap grasslands, a number of authors have proposed the ranking of sites based on the number of *Hygrocybe* spp. present. The classification of Rald (1985), based on numbers of *Hygrocybe* spp. found during a single or multiple visit, has proven very useful for identifying grasslands with high conservation value.

The 'CHEG profile' devised by Rotheroe et al. (1996) modified this approach by including other grassland taxa (as originally suggested by Nitare, 1988) characteristic of oligotrophic grasslands (C – clavarioid species (Clavariopsis spp. etc.; fairy clubs), H – Hygrocybe spp. (including Dermoloma and Porpoloma spp.) E – Entolomatcaeae (pink gills) and G – Geoglossaceae (earth tongues)) such that a site like Sourhope would be defined as C4, H12, E3, G3 if there were four species of fairy club, twelve waxcaps, etc. By Rald's definition (Table 1), the same site (twelve Hygrocybe spp. have been found at Sourhope) would be classified as of regional importance.

More recently Rotheroe (1999) has suggested a more refined system to give added weighting to the presence of certain indicator species. The 'Top twenty-four' system is based on two sets of twelve species (category A (top 12) includes six *Hygrocybe* spp. and six other taxa, whilst category B includes a further eleven *Hygrocybe* spp. and any earth tongue, except for *Geoglossum fallax*), such that Sourhope with one species from the A-list (*H. splendidissima*) and two from the B-list (*H. irrigata* and *H. colemanniana*) is defined as an A1B2 waxcap grassland. There are some UK sites where in excess of 30 *Hygrocybe* spp. have been recorded (e.g. The Patches in the Forest of Dean (Marren, 1998), though the area surveyed is often not noted and may vary considerably. For example, the

Table 1.	Rald's (1985) guidelines for assessing the quality of waxcap
	grasslands. Numbers in brackets refer to a single visit.

Conservation value	Total no. of Hygrocybe species	
Nationally important	17-32 (11-20)	
Regionally important	9-16 (6-10)	
Locally important	4-8 (3-5)	
Of no importance	1-3 (1-2)	

lawn at Llanerchaeron in Ceredigion has yielded 27 Hygrocybe spp. in an area of <0.2 ha (Rotheroe, 1995).

As noted by Parker-Rhodes (1955b), Watling (1995) and others, there is a correlation between species counts and the intensity of the surveying, with single forays seldom encountering even 25% of the total number of species present at a given site. As with all fungi, sporadic occurrence and the small number of field mycologists means that even at the small proportion of sites for which records exist, *Hygrocybe* spp. are significantly under-recorded. The vagaries of fruit body production patterns can also be responsible for significant variation, as illustrated by a recently published twenty-one-year study for a woodland site (Straatsma, Ayer & Egli, 2001) where a >10-fold difference in the number of species recorded between years. For this reason Orton (1986) suggested that data from 10 years are required to provide a comprehensive picture of the mycota of a given site.

Feest (2000) has pointed out the need for more rigorous survey techniques similar to those used by entomologists and ornithologists. At the Sourhope site we have tested the use of a novel surveying tool, namely differential global positioning system (dGPS). Like the now commonplace handheld GPS systems (accurate to c. 10 m), dGPS receives signals from orbiting satellites but correlation with a 'differential' signal from a fixed radio transmitter removes some of the errors associated with the satellite signal. This permits increased positional precision, such that submetre accuracy (in our experience c. 50 cm radius) can routinely be attained. Furthermore, a handheld computer permits logging of large numbers of datapoints and their subsequent transfer into GIS (geographical information system) software packages for analysis in comparison with other datasets. As can be seen in Fig. 2, not only have we been able to map fruit bodies to an accuracy of <50 cm (this will permit comparison with future surveys and also correlation with spatial data obtained by soil scientists and plant ecologists) but the diligence of the surveyor (i.e. any areas not well surveyed) can be recorded objectively (Fig. 2) by recording positional information at one second intervals. Such information is likely to be significantly more useful when examining larger, less well-marked field sites.

Due to the continued loss of unimproved grassland habitats, waxcap grasslands generally and certain waxcap species in particular have become more restricted in their European distribution. Of particular concern are two species, *H.* spadicea (date-coloured waxcap) and *H. calyptriformis* (pink meadow waxcap), with the former appearing in the highest category of Ing's provisional European Red Data List (Ing, 1993) and the latter appearing in the second category. In fact,

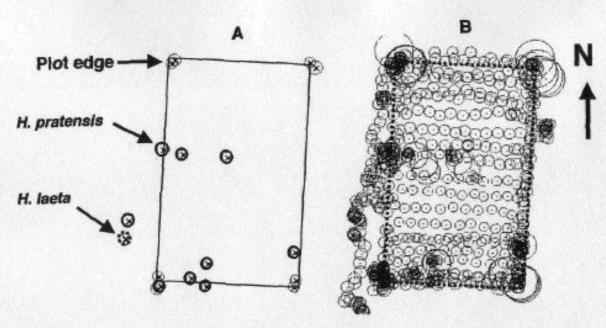


Fig. 2. The use of differential Global Positioning System (shoulder-mounted) for mapping of fruit body positions. A control plot (12 × 20 m) at the Sourhope site (plus adjacent regions) is shown with the rectangle indicating the dimensions of the treatment plot. Diagram A shows the position of the waxcap fruit bodies (bold or dotted circles) and the plot corner posts (faint circles). Diagram B shows the route taken during the surveying of the plot with positional measurements being taken every second. The accuracy of each reading is shown by the circles surrounding each point (mostly <50 cm radius).

the majority of Hygrocybe spp. (89% according to Arnolds & de Vries, 1993) appear on a Red Data List somewhere in Europe.

It is very pleasing to see that in recent years, the statutory bodies responsible for upkeep of the British countryside have devised effective action plans for waxcap fungi. The involvement of Plantlife as a campaigning organisation is also very welcome. Biodiversity Action Plans (BAPs) have been developed for two species, *H. spadicea* (date-coloured waxcap) and *H. calyptriformis* (pink meadow waxcap) (Fig. 3), as well as for the earth tongue *Microglossum olivaceum* which also inhabits oligotrophic grasslands (Fleming, 2001).

The absence of rare plant taxa can make it difficult to accord protected status to a site, though at least two UK sites (Disgwylfa, in the Eppynt Mountains, Powys and Roecliffe Manor, Leicestershire) have been denoted SSSIs based on their grassland mycota, with a third site (the lawn at Llanerchaeron, Ceredigion (Rotheroe, 1995)) currently in the process of notification. Both Welsh sites contain *H. calyptriformis* but *H. spadicea* has not been found at any of these three sites, though in Aberystwyth both species occur within 1 km on similarly managed sites.

As mentioned earlier, there is a problem of under-recording, a problem compounded by the possibility that abundance of a particular species can only be inferred from the occurrence of fruit bodies and that some rare species (e.g. at the limits of their ecological range) may only fruit very occasionally. It is useful to

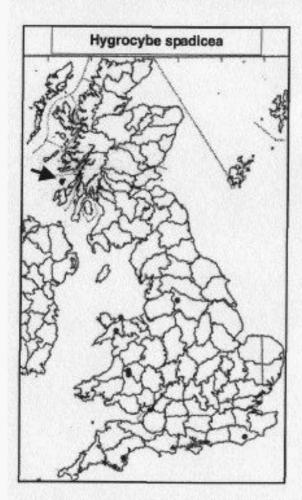




Fig. 3. Distribution maps for two Hygrocybe spp. for which Biodiversity Action Plans currently exist. The arrow denotes the site of the only H. spadicea record for Scotland (Isle of Colonsay). Data from http://194.131.255.3/bmspages/BMSFRD/bmsfrd.htm

consider the example of ectomycorrhizal species in which above-ground fruit body abundance is compared to the mycelial symbiont (probably a better measure of biomass than fruit bodies), as assessed by analysis of PCR-amplified fungal sequences (Horton & Bruns, 2001). Such studies have consistently shown that the correlation between above- and below-ground abundance is very poor and that species whose fruit bodies are only very sparsely recorded can be widespread below ground.

There are also problems of distinctiveness, longevity and visibility, all of which can affect how frequently rare species are recorded. *H. calyptriformis* is an example of a European Red Data List species which is considerably less rare in the UK than in other European countries (e.g. only a single site in Denmark (Brandt-Pedersen, 1980)) but which is unmistakable in appearance (definitely the easiest waxcap to identify unambiguously). Recent interest in waxcap fungi has led to the discovery of many more UK sites for this fungus, such that even recent distribution maps (Fig. 3) underestimate how widespread *H. calyptriformis* is in some areas. It appears to be mainly Westerly with its absence from large parts of Scandinavia suggesting an intolerance of low temperatures. However, in Wales *H. calyptriformis* can often be found fruiting very late in the year (e.g. 10.12.00 and 5.12.01 in Aberystywth). Apart from its distinctive appearance and slight preference for acidic/neutral soils (Rotheroe, 1999), *H. calyptriformis* seems

to occur quite often in more 'domesticated' locations (e.g. gardens at Kew, university campus in Aberystwyth) which may be slightly more enriched or have possibly been subject to occasional fertiliser application.

By contrast, *H. spadicea* remains very rare in the UK being found at only ten sites (see Fig. 3; four in Wales, one in Scotland), though on a European level it can be said to be less rare than *H. calyptriformis*. This species is found in well-drained sites often South-facing and frequently on limestone. It is smaller and far less conspicuous than *H. calyptriformis*, suggesting that it is more underrecorded. It should be noted that Rotheroe did not include either of these species in category A of his 'top twenty-four' list, mainly because other species have been found to be better indicators of waxcap-rich grasslands.

### Ecology of Hygrocybe spp.

Over half a century ago Chesters (1949) called the basidiomycete fungi 'the missing link in soil mycology'. In part due to their greater diversity, there has historically been more interest in the woodland fungi (Rayner & Boddy, 1988; Horton & Bruns, 2001). Basidiomycetes (identified in culture by the presence of clamped hyphae) are occasionally isolated from soil on agar media during studies of grassland fungi but there is very little correlation between those isolated on agar media and those taxa whose fruit bodies are observed in the field (Warcup, 1959b).

All current knowledge of the ecology of waxcap fungi is based on field observations of their fruit bodies. This contrast with the situation for many other grassland macromycetes which form fairy rings (such as the puffballs or the fairy ring fungus, Marasmius oreades) which alter the growth of nearby grasses to form a ring of necrotic or more luxuriant vegetation. Furthermore the mycelial systems of these fungi are readily apparent either on the soil surface or within the soil profile, usually because of the hydrophobicity conferred by the presence of the fungal hyphae. Warcup (1959b) found that mycelia of M. oreades and Agaricus arvensis could readily be isolated from colonised soil beneath fruit bodies and were able to map the vertical extent of their mycelia by this method. However, attempts by Warcup to study H. conica and a Clavaria sp. 'proved intractable by the methods used in this study and little information has been gained about their mycelial growth'. This quote summarises a fundamental problem associated with studying the mycelia of waxcap fungi. No member of the genus has been successfully cultured axenically, suggesting some as yet undefined nutritional fastidiousness. Furthermore, their mycelia are not visible macroscopically in the soil profile, though we have, using species-specific PCR primers, been able to demonstrate the presence of mycelia in DNA extracted from soil below fruit bodies (A.W. Jones, unpublished data). Even the basidiospores of these fungi germinate only very slowly on laboratory media with the spores of H. virginea being the only species to show high levels of germination under the conditions we have tested to date. It is perhaps significant that H. virginea is the most frequently encountered waxcap species and also the species most tolerant of disturbance (Wilkins & Patrick, 1940; Thompson, 2000)

Fruit body tissues comprise only a small fraction of the biomass of fungal colonies, so the aforementioned difficulties represent significant obstacles to achieving a greater understanding of waxcap ecology. With other uncultured fungi, including many mycorrhizal taxa, detailed investigation has been possible through the use of laboratory or glasshouse-based microcosms. However, waxcaps and the larger ectomycorrhizal macromycetes (e.g. Russula spp.) operate at spatial scales which are often incompatible with microcosm systems. For instance, one ring of H. splendidissima at the National Botanic Garden of Wales, Middleton is fully 10 m in diameter and the smaller species, such as H. virginea, frequently form rings 1-2 m in diameter. Therefore even field experiments such as that at Sourhope with a  $12 \times 20$  m treatment block are verging on the small side for fungi such as these.

The upshot of these various problems is that we are forced to build up a picture of waxcap ecology from disparate shreds of often circumstantial evidence, for example the harmful effects of fertiliser addition on fruit body production (Arnolds, 1989). However, it is not known whether this reduction is associated with a reduction in below-ground mycelial growth. Arnolds (1982) considered waxcap fungi to be saprotrophs, some associated with humus and others with bryophytes. The basis of the apparent interaction between *Hygrocybe* spp. and mosses such as *R. squarrosus* is unclear. Several lines of evidence are consistent with some sort of nutritional interaction between these organisms, though the basis of any such interaction remains a mystery.

### Stable isotope analysis

A recent innovation in the field of fungal ecology has been to measure the natural abundance of the stable isotopes of carbon and nitrogen (13C and 15N) in relation to the main isotopic forms (12C and 14N) using isotope ratio mass spectrometry (Unkovich et al., 2001). These heavier isotopes occur naturally at low abundance and their abundance is usually quoted with reference to standards (PeeDee Belemnite limestone for 13C and atmospheric nitrogen for 15N). For fungiproducing macroscopic fruit bodies (>10-20 mg fresh wt, corresponding to >100 µg N), it is possible to measure the natural abundance of these stable isotopes relative to presumed plant or other substrates. Where this is not known it may be possible to infer a particular mode of nutrition based on stable isotope fractionation patterns. Gebauer & Taylor (1999) found different patterns of 15N enrichment in ectomycorrhizal and saprotrophic fungi, which reflected differences in N uptake. Consistent differences in 15N and 13C patterns between mycorrhizal and saprotrophic taxa in several woodland habitats were shown by Kohzu et al. (1999) and Hobbie, Weber & Trappe (2001) with ectomycorrhizal fungi showing greater enrichment for 15N and greater depletion for 13C than saprotrophic fungi.

To date there have been no published investigations of stable isotope natural abundance in grassland fungi. However, our initial investigations have provided some quite unexpected results, with waxcap fungi from Sourhope showing quite extreme patterns of  $^{15}N$  enrichment and  $^{15}C$  depletion compared to data from previous studies (Fig. 4). One initial possibility was that this related to inherent differences between woodland and grassland systems, since habitats with different nutrient inputs and plant communities can show large shifts in overall  $\delta^{15}C$  and  $\delta^{15}N$  values (Stapp, Polis & Pinero, 1999). However, comparison with isotope

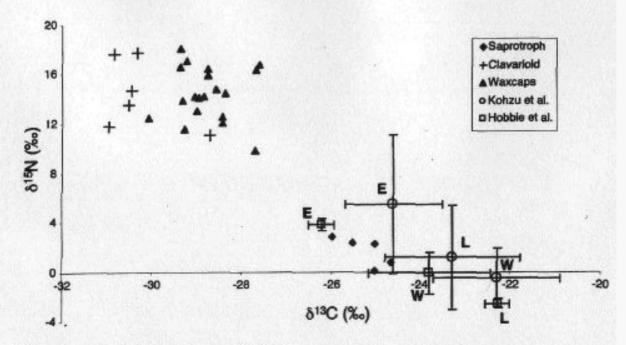


Fig. 4. Natural abundance of δ¹⁵N plotted against δ¹³C natural abundance for fruit bodies of fungi characteristic of waxcap grasslands. All samples were collected from the Sourhope site. The 'Waxcap fungi' included H. pratensis, H. laeta, H. conica, H. splendidissima and H. virginea. 'Saprotrophic fungi' included Cystoderma amianthinum, Panaeolus rickenii, Mycena spp. Entoloma conferendum; 'Clavarioid' fungi included members of the genera Clavulina, Clavulinopsis, Clavaria and Clavulinopsis, as well as the earth tongues Trichoglossum and Geoglossum. Mean data from a previous study of woodland fungal fruit bodies (ectomycorrhizal (E), wood deomposers (W), litter decomposers (L)) in the Far East and the USA by Kohzu et al. (2000) and Hobbie et al. (2001) respectively are shown for comparison. Error bars on mean data represent one standard deviation (G.L. Easton, unpublished data).

ratios of fruit bodies of several known litter saprotrophs and also waxcap fruit bodies collected from dune and upland habitats in Wales confirmed these unusual patterns (waxcaps were c.4% more depleted in <sup>13</sup>C and >10% more enriched in <sup>15</sup>N than saprotrophs). Equally surprising were the similar patterns of <sup>15</sup>N enrichment in the fruit bodies of several other waxcap grassland fungi (earth tongues and fairy clubs), which showed even greater levels of <sup>13</sup>C depletion (Fig. 4).

We recognise the risks of attempting to infer too much at this early stage. However, these data are consistent with suggestions that these fungi are humic saprotrophs, since recalcitrant compounds in lower soil horizons tend to be enriched for <sup>15</sup>N (Gebauer & Taylor, 1999). It is interesting to note that members of the genus *Cortinarius* showed significant levels of <sup>15</sup>N enrichment (up to +15.4%) (Taylor *et al.*, 1997). These fungi are also considered to be adapted to N-poor environments and thus particularly sensitive to anthropogenic N enrichment by atmospheric deposition (Arnolds, 1991).

## Breeding biology

Investigations of the ecology of basidiomycete fungi tend to revolve around

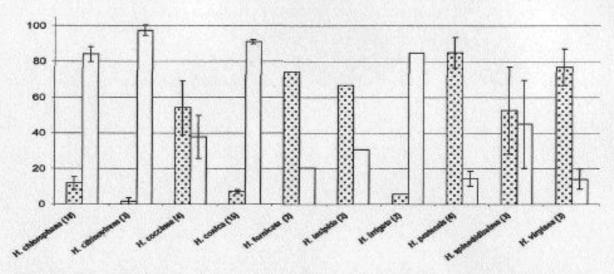


Fig. 5. Proportions of uninucleate (shaded) and binucleate (clear) basidiospores in ten Hygrocybe spp. Numbers in brackets indicate the number of samples examined (A.W. Jones, unpublished data).

either the production of fruit bodies or the dynamics of mycelial growth and nutrient acquisition. Little attention is paid to the process of colony establishment via basidiospores, in part because the mycelial systems of many larger basidiomycetes are considered to be very long-lived (estimated to be up to 5000 yr for Armillaria bulbosa (Smith, Bruhn & Anderson, 1992) and >1-600 yr for various fairy ring fungi in grasslands (Shantz & Piemeisel, 1917)). In these and other fungi, contrasting population structures can arise depending on whether mating between compatible primary mycelia (i.e. outcrossing) is required for colony establishment (Griffith & Hedger, 1994). The alternative non-outcrossing strategy involves production of two binucleate basidiospores per basidium, each capable of establishment and growth without mating. Insight into the breeding biology of basidiomycetes can be obtained by examination of the nuclear condition of basidiospores (corresponding to the number of spores per basidium), in some cases revealing cryptic speciation events (Griffith & Hedger, 1994). In the case of H. conica, the occurrence of fruit bodies with either 4-spored or 2-spored basidia (Kühner, 1979) is suggestive of some form of cryptic speciation, with the former being outcrossing and the latter non-outcrossing. Examination of the nuclear condition of basidiospores from several Hygrocybe spp. (Fig. 5) showed H. pratensis, H. virginea and H. fornicata are outcrossing with predominantly uninucleate basidiospores, whilst H. chlorophana, H. citrinovirens, H. conica and H. irrigata are predominantly non-outcrossing. As can be seen from the size of the standard deviation error bars, H. coccinea and H. splendidissima showed considerable variation between fruit bodies from different sites with the percentage of uninucleate spores ranging from 14 to 80%.

#### Conclusions

This review has focused more on issues of the biology of these fungi rather than the more practical issue of how to conserve these fungi. However, an understanding of the autecology of waxcaps has the potential to be useful for devising effective conservation strategies, in particular to define more precisely how sensitive these fungi are to fertiliser/lime inputs and to what extent they can recover from such enrichment events. Whilst we are undertaking such basic investigations at Aberystwyth (funded in part through the NERC Soil Biodiversity Initiative), for the next few years at least, conservation strategies will be guided by the results of initiatives such as the BMS waxcap grasslands survey (Rotheroe et al., 1996), the Somerset Grasslands survey (Thompson, 2000) and the on-going Scottish Waxcap survey (Newton et al., 2000).

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