Contract FC-73-01-403: Mycological survey of selected semi-natural grasslands in Wales.
Final Report August 2006.

GW Griffith, C. Aron, DA. Evans, S. Evans, A. Graham, L. Holden, D. Mitchel.

1. Executive Summary

There has been considerable interest in the distinctive macrofungi inhabiting semi-natural grasslands in North-Western Europe, in particular the colourful members of the genus *Hygrocybe* but also including taxa such as *Entoloma*, Clavariaceae, Geoglossaceae, *Dermoloma*, *Porpoloma* and *Camarophyllopsis* (collectively grouped as CHEGD fungi). The BMS database contains over 8500 *Hygrocybe* records from >900 sites in Wales but there has to date been no systematic survey. The present investigation surveyed in detail 51 sites spread throughout Wales. In addition to surveys of whole sites, conducted twice at each site between 2003 and 2005, permanent quadrats were also set and surveyed twice (87 in total with 2-6 quadrats being set at larger sites). This approach allowed the same small area to be re-surveyed (early and late in different seasons) and for detailed measurements of sward height and fruitbody abundance to be quantified. No correlation was found between CHEGD species diversity or fruitbody abundance with either sward management regime or sward height. Although the commonest NVC habitat type was MG5a, higher diversity of CHEGD species was found on upland grasslands (NVC class U; 20.1 spp./site) than in calcareous (CG; 19.1 spp./site) or mesotrophic grasslands (MG; 15.0 spp./site).

A total of 112 CHEGD species were found during the survey, including 17 spp. new to Wales (two of which are new UK records). Additional sites for three species for which Species Biodiversity Action Plans exist were also discovered. The timing of surveys was found to influence the taxa encountered, with fewer *Entoloma* spp. found later in the autumn. The quadrat survey approach was not designed as a surrogate for a full site survey but there was a positive correlation between CHEGD species diversity at sites and diversity in quadrats at these sites. Measures of fruitbody abundance for quadrat surveys established that commoner species (with wider distributions) fruited more abundantly, though there were large differences in fruiting abundance between taxa irrespective of frequency of occurrence. The number of *Hygrocybe* spp. was positively correlated with numbers of Clavariaceae for individual quadrat surveys. The strongest positive correlation between diversity of *Hygrocybe* and *Entoloma* was found when data for whole sites (including both 'early' and 'late' visits) were analysed suggesting that not only do these taxa fruit at different times but also that they may prefer different sub-habitats. Apart from evidence of different habitat preferences between these genera, there was no evidence that different types of grassland fungal communities exist.

Although several weighted scoring systems (giving higher scores to good indicator species) have been developed to assist in the identification of sites with diverse grassland fungi, the identification of good indicator species has hitherto been done subjectively. We used an index of association for each *Hygrocybe* spp. to identify those taxa found most consistently in association with large numbers of other grassland fungi. The results of these analyses suggested that some species (e.g. *H. quieta*, *H. irrigata*) should be demoted and others (*H. flavipes*, *H.intermedia*) in the ranking system of McHugh *et al*. However, we note that the sites surveyed here were generally of high quality with a mean of 14.6 *Hygrocybe* spp./site. Based on two visits, 31% of the sites were ranked as of national importance (≥17 spp.) and 8% (4/51) of international importance (≥22 spp.) according to the classification of Rald. Based on single visits, 35% of the sites ranked as of international importance (≥15 spp.) base don the Rald system. These figures are indicative of the exceptional diversity of grassland fungi found in Wales. A ranking using data from the BMSFRD, revealed that the majority of the UK's top 20 sites for *Hygrocybe* spp. are found in Wales, notably Epynt, Trawscoed and Garn Ddyrys, each with ≥30 spp. We are not aware of any sites outside the UK where such high levels of diversity exist. As such the waxcap grasslands of Wales are of international importance.

Contract FC-73-01-403: Arolwg Mycolegol o Lastiroedd Lled-naturiol yng Nghymru.
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GW Griffith, C. Aron, DA. Evans, S. Evans, A. Graham, L. Holden, D. Mitchel.

1. Crynodeb Gweithredol

Bu diddordeb mawr yn ffyngau nodweddiadol glastiroedd lled-naturiol Gogledd-Orllewin Ewrop, yn enwedig yr aelodau lliwgar o'r genws *Hygrocybe* (y capiau cwyr) ond hefyd taxa fel *Entoloma*, *Clavariacaeae*, *Geoglossaceae*, *Dermoloma*, *Porpoloma* a *Camarophyllopsis* (gelwir y rhain yn grŵp ffyngau CHEGD). Er bod >8500 cofnod *Hygrocybe* o >900 safle yng Nghymru ar gronfa ddata y BMSFRD, ni fu arolwg ffurfiol erioed. Yn ystod yr ymchwiliad presennol arolygwyd 51 safle ledled Cymru yn fanwl. Yn ogystal ag arolygon safleoedd cyfan, a wnaethpwyd ddwywaith rhwng 2003 a 2005, gosodwyd quadratau parhaol o fewn pob safle (cyfanswm o 87; 2-6 quadrat ar y safleoedd mwy). Trwy hyn roedd modd ail-arolygu yr un ardal fechan er mwyn mesur hyd y glaswellt a niferoedd y madarch. Ni chanfuwyd unrhyw gydberthynas rhwng amrywiaeth na helaethrwydd y ffyngau CHEGD gydag un ai hyd y glaswellt na'r dull o bori. Er mai MG5a oedd y grŵp NVC mwyaf cyffredin yn y quadratau, canfuwyd mwy o rywogaethau CHEGD ar lastiroedd ucheldir (dosbarth NVC U; 20.1 rh./safle) na mewn glastiroedd calchog (dosbarth CG; 19.1 rh./safle) neu lastiroedd mesotroffig (dosbarth CG; 15.0 rh./safle).

Darganfuwyd 112 rhywogaeth o'r ffyngau CHEGD yn ystod yr arolwg, yn cynnwys 17 rhywogaeth newydd i Gymru (a dwy o'r rhain yn newydd i Brydain). Darganfuwyd hefyd sawl safle ychwanegol i'r tair rhywogaeth a ddynodwyd mewn Cynlluniau SAP. Cafodd amseru'r arolwg effaith sylweddol ar y rhywogaethau o fadarch a ddarganfuwyd ar y safleoedd, gyda llai o *Entoloma* spp. yn hwyrach yn yr hydref. Er nad oedd yr arolwg quadrat wedi ei gynllunio fel dull symlach, i'w ddefnyddio yn lle arolygu safleoedd cyfan, roedd perthynas agos rhwng nifer y rhywogaethau ar y quadratau a'r safleoedd cyfan. Trwy fesur nifer y madarch ar y quadratau roedd yn amlwg fod y rhywogaethau mwy cyffredin (h.y. lletach eu dosbarthiad) hefyd yn ffrwytho yn fwy toreithiog, er bod gwahaniaethau sylweddol yn y patrymau ffrwytho rhwng y rhywogaethau gwahanol. Roedd cydberthynas positif cryf rhwng nifer y rhywogaethau o Clavariacaeae a *Hygrocybe* yn y quadratau unigol. Canfuwyd y berthynas gryfaf rhwng niferoedd rhywogaethau *Hygrocybe* ac *Entoloma* gyda data o'r safleoedd cyfan (h.y. yn cynnwys data o'r arolygon 'cynnar' a 'hwyr'). Mae hyn yn awgrymu fod y grwpiau hyn yn ffafrio is-cynefinoedd gwahanol yn ogystal ag amseroedd ffrwytho gwahanol. Heblaw am y gwahaniaethau uchod, nid oedd unrhyw dystiolaeth i awgrymu fod gwahanol fathau o gymunedau madarch yn y glastiroedd.

Er bod nifer o systemau sgorio pwysedig (yn dynodi sgoriau uwch i rywogaethau sydd yn 'ddangosyddion' da) wedi eu datblygu er mwyn adnabod safleoedd o ansawdd uchel, 'does hyd yn hyn ddim sail rifol i'r sgoriau a ddynodwyd. Yn yr astudiaeth bresennol cyfrifwyd 'mynegai cyswllt' ar gyfer pob rhywogaeth *Hygrocybe* er mwyn adnabod y rhywogaethau a oedd yn gysylltiedig yn rheolaidd â niferoedd uchel o rywogaethau eraill. Awgrym y mynegai cyswllt oedd y dylid diraddio rhai rhywogaethau (e.e. *H. quieta*, *H. irrigata*) a dyrchafu eraill (e.e. *H. flavipes*, *H.intermedia*) o fewn system rengu McHugh *et al.* Er hynny, rydym yn nodi fod y safleoedd a arolygwyd gennym yn rhai o ansawdd uchel ar y cyfan gyda chyfartaledd o 14.6 rhywogaeth o *Hygrocybe* ar bob safle. Ar sail y ddau arolwg, roedd 31% o'r safleoedd o bwysigrwydd cenedlaethol (≥17 rh.) ac 8% (4/51) o bwysigrwydd rhyngwladol (≥22 rh.) yn ôl system Rald. Mae'r ffigyrau hyn yn arwyddocaol o'r bioamrywiaeth arbennig o ffyngau a geir yng nglastiroedd Cymru. Trwy ddefnyddio cronfa ddata BMSFRD i adnabod y safleoedd gorau yn y Deyrnas Unedig ar gyfer *Hygrocybe*, fe amlygwyd y ffaith fod y mwyafrif o'r 20 safle gorau yng Nghymru. Yn benodol, darganfuwyd ≥30 rhywogaeth o *Hygrocybe* ar safleoedd Epynt, Trawscoed a Garn Ddyrys. Ni wyddom am unrhyw safle y tu allan i'r DU gyda lefel cymharol o fioamrywiaeth o gapiau cwyr. Felly mae glastiroedd capiau cwyr Cymru o bwysigrwydd rhyngwladol.

Report Structure

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3. AIMS AND OBJECTIVES

The aim of this project is to conduct a mycological survey of 50 selected semi-natural grasslands in Wales, each site to be surveyed twice during 2003-2005 at different times during the fruiting season. The macrofungi to be recorded are the members of the genus *Hygrocybe* and grassland representatives of the families Entolomataceae, Clavariaceae, Geoglossaceae (also *Camarophyllopsis*, *Dermoloma* and *Porpoloma* spp.). It is expected that data from this survey will provide an insight into the relationship between macrofungal diversity and both grassland plant communities and different management regimes.

4. INTRODUCTION

It is estimated that the past 75 years have witnessed a decline of more than 97% of unimproved lowland grasslands in England and Wales, mainly due to agricultural intensification (Fuller, 1987). The threats to higher plant and bird populations as a consequence are well-recognised but the effect of agricultural intensification on grassland macrofungi has only received attention in the last 10-20 years [>97% of Welsh *Hygrocybe* records on the BMS field records database date from 1985 onwards]. The diversity of macrofungi in woodland habitats is greater than that of grasslands; it is estimated that about 400 taxa (some 10% of the total number of species) are specific to, or mainly found in grasslands (Arnolds & de Vries, 1989), with members of the genus *Hygrocybe*, ('waxcaps') and grassland representatives of the families Entolomataceae ('pink gills'), Clavariaceae ('fairy clubs') and Geoglossaceae (earth tongues) (also *Camarophyllopsis*, *Dermoloma* and *Porpoloma* spp.) comprising about half of this number.

Concerns were first raised in mainland Europe, notably the Netherlands (Arnolds, 1982, 1988) and Scandinavia (Rald, 1985; Nitare, 1988; Jordal, 1997). Arnolds warned that only ca. 200 ha of 'waxcap grassland' habitat remained in the Netherlands. Subsequent surveys in the UK (e.g. Rotheroe *et al*., 1996) indicated not only that the UK contains a relatively large number of sites with a high species-richness of grassland fungal diversity (perhaps unsurprising given that grassland habitats comprise some 70% of the UK land area; however, most of these grasslands are 'improved [ploughed, re-seeded, fertilised] and thus of little mycological interest) but also the extent of under-recording of these taxa. This latter issue has led to a great increase in interest in grassland fungi, a positive development that has been greatly assisted by the publication of Boertmann's (1995) monograph of the genus *Hygrocybe* and more recently an updated reprint (with photographs) of Noordeloos' (1992) *Entoloma* monograph (Noordeloos, 2004). The publication of other keys either in hard copy (e.g. Henrici, 1996; Silverside, 1997; Spooner, 2000) or as web resources (e.g. by Noordeloos [http://www.entoloma.nl/html/engels.html] and Ridge [http://fungus.org.uk/nwfg/irene.htm]) have also made identification of these taxa accessible to a wider audience. Lastly, the recent publication of the UK Checklist of Basidiomycota (Legon & Henrici, 2005) permits a consistent approach to the naming of most of these taxa.

The conservation of grassland fungi has received further attention through the inclusion of three taxa (*Hygrocybe calyptriformis*, *H. spadicea* and *Microglossum olivaceum*) in the UK Biodiversity Action Plan (BAP; Anon, 1999). Species dossiers have also been produced for these species (Newton *et al*., 2000a,b; Silverside, 2000; http://www.ukbap.org.uk/SpeciesGroup.aspx?ID=15). The BAP process is currently undergoing review, with the possibility that a single BAP will encompass a range of threatened grassland fungi. Concurrently, there is ongoing research into the ecology of waxcaps and other grassland fungi at UW Aberystwyth (Griffith *et al*., 2002, 2004). This work funded originally by NERC and more recently through a Memorandum of Agreement between the UK statutory bodies plus Plantlife and the British Mycological Society aims to establish how these fungi derive their nutrition, how they are affected by management practises and their interaction with plants in grassland communities. It is hoped that results of these studies will serve to inform the BAP process.

The earliest Welsh record of any grassland fungus date from 1776 (*Clavaria fragilis* at Norton's Hill, Tintern; Evans & Holden, 2003). The earliest records of *Hygrocybe* in Wales are those of Davies (1813) whose book *Welsh Botanology* mentions the presence of several *Hygrocybe* spp. in Anglesey (e.g. *Agaricus coccinnus* [sic] Bolt. & W., *A. pratensis*, *A. psittacinus* ["and a number of beautiful varieties" - these may include several other similar species], *A. niveus*, *A. virgineus* as well as some other possible grassland taxa (e.g. *Clavaria corralloides* ["and a white variety on Malltraeth"] and *C. vermiculata*). Davies also mentions some other grassland fungi (e.g. *Agaricus columbarius* "in old pasture" and *Agaricus dillennii* ["I have for several successive years found the plant, to which I give this specific name, in abundance in an old pasture between Bodgylchad and Cefn Coch. See *With*. p.206, and *R. Syn*. p3. n.13."]). However, it seems that *A. dillennii* most likely corresponds to *Lepista* *nuda* [conceivably an *Entoloma*] (Peter Roberts, pers. comm., Feb. 2006). According to Griffith, (1913), Davies' herbarium was transferred to the British Museum and later to Kew. Sadly, no fungal material appears to be present amongst the remaining collection (Peter Roberts, pers. comm., Feb. 2006). Since Davies' pioneering work, there are sparse records of grassland fungi from the 19th century (29 records mostly from Coed Coch, Denbighshire, home of the Wynne family; Evans & Holden, 2003) and early 20th century (249 records from 1900 to 1960; Evans & Holden, 2003) but even in the era of modern macrofungal recording (post-1960) there are relatively few records of grassland fungi compared to woodland (1206 grassland records from 1960 up to 1994). More generally, most of the general biological surveys conducted at sites in Wales have seldom even mentioned macrofungi. A pertinent example is Jackson & Foster's (1994) survey of the National Trust owned Mynachdy'r Graig site (site 32 in the present survey) reporting little biological value for grassland areas of the site. However, to be fair to the authors of this and similar surveys, field work is generally restricted to a few days in summer and until recently accessible keys to macrofungi were not available.

The past decade has seen a large increase in the recording of grassland fungi (especially *Hygrocybe* spp.), mostly through non-systematic surveys by volunteers and also through the BMS Upland Forays (http://www.britmycolsoc.org.uk/meetings.asp). In large part recording of grassland fungi in Wales was inspired by the activities of the late Maurice Rotheroe in the 1990s. It was fortuitous that David Boertmann's (1995) key to *Hygrocybe* was published at around this time. A number of site surveys (funded by a range of organisations, including CCW, National Trust and MoD; e.g. in Wales Rotheroe, [1999- Carmarthenshire; 2002 –Hafod / Caeau Cae'r Meirch; 2003 -Epynt], Griffith *et al.*, 2004 [Powis Castle]; Evans, 2005 [Chirk Castle]) have also been published, as well as some more systematic surveys in Somerset (Thompson, 2000) and Scotland (Newton *et al.*, 2002, 2003). A survey of Northern Irish grasslands was also completed in 2004 and its results are currently being prepared for publication (David Mitchel, pers. comm.). Collations of existing data from England (>20000 records) and Wales (>8000 records at 911 sites; 77% of which are *Hygrocybe* spp.) have also been published recently, funded respectively by EN (Evans, 2003) and CCW (Evans & Holden, 2003).

The recording activities outlined above have led to the identification of a number of sites in the UK at which very high diversities of waxcap fungi are found. As a result four sites in the UK are now notified as SSSIs on the basis of their grassland mycota, the first being lawn at Roecliffe Manor, Leciestershire (SK532126) two in Wales (Disgwylfa, Powys SN991438 [part of Epynt ranges]; Llanishen Reservoir, Cardiff [ST186815] notified in early 2006) and one in Northern Ireland (Binevenagh ASSI is notified with grassland fungi as a feature of the site).

As well as identifying sites of high species richness for grassland fungi, effective conservation and protection of these fungal communities requires some knowledge of the ecological requirements of the taxa in question. More fundamental ecological research is underway at UWA focusing on lab-based investigations and surveys at replicated field sites. However, it is also important to ascertain the extent to which particular assemblages of grassland fungi are associated with particular plant communities and how various forms of grassland management affect fruiting of these fungi. Rotheroe (2001) noted that diverse grassland fungi were often associated with sites that were 'semi-improved' and botanically mundane. Furthermore, it is often noted by field mycologists that fruiting of grassland fungi is inhibited by rank swards, though to some extent fruitbodies (FBs) are more difficult to observe in longer swards.

The CCW Lowland Grassland Survey (Howe *et al.*, 2005) detailed vegetation mapping (Phase 2 survey) has been conducted at 1070 grassland sites in Wales, a number of which are already known to be home to diverse grassland fungal populations. The aim of this study is to conduct mycological surveys at a range of sites around Wales, with the aim of exploring possible relationships between macrofungal diversity and plant communities/management regimes at each site (see project specification Appendix A).

5. METHODOLOGY

a. Survey Protocols

The specification for this work is described in Appendix A. The protocol for survey (standard operating procedures; SOP) is detailed in Appendix B. Sample completed survey sheet (Allt Goch, Gwynedd; surveyed by Debbie Evans) is also included as Appendix C.

The original SOP was modified in October 2003 to reduce the original quadrat area from $2500m^2$ to $900m^2$, though sites already surveyed at the larger size (17 quadrats at 9 sites) were re-surveyed at the same size (all by Liz Holden in NE Wales). Two quadrats were smaller than $900m^2$ due to the small size of the whole site or small area of grassland present (Bodrwyn and Dolobran).

Surveyors were asked to survey quadrats 'early' and 'late' in the different years. Constraints of surveyor availability limited, in some cases, the extent to which this was done and in part site-related (altitude, rainfall, soil drainage etc.) and seasonal differences (e.g. dry early autumn in 2003) in peak fruiting times made it difficult to define these times precisely. Therefore, survey times have been classified as early (on or before 24th October), middle (25th Oct to 7th Nov) or late (on or after 8th Nov).

There were some quadrats (Maes Caradog 2003/04; Bodwryn 2004; Cors Geirch 2005; Bryn-Gwenyn 2005; Dolobran 2005; Foel Fawr 2005) at which sward height was estimated rather than measured using the sward stick method. There is some variation in the details provided which would facilitate re-location of the quadrats (e.g. photographs of marked quadrat supplied for some sites). All are supplied with grid reference to 10m accuracy and nearly all are marked in one corner with a yellow plastic peg, in addition to photographs showing the quadrat marked out with tapes.

b. Calculations of CHEGD and *Hygrocybe* scores

For grassland fungi, much weight is placed on total numbers of *Hygrocybe* taxa at a given site and to a lesser extent the total number of target grassland taxa (usually given as a CHEG/CHEGD count). There is scope for confusion, not only in the way taxa are counted (e.g. counting of subspecies ["taxa"] or species only, inclusion of related non-grassland taxa from woodland areas adjacent to grassland sites) and also which species are included among the target species (e.g. inclusion of *Dermoloma* spp. in the 'H' count, suggested by Rotheroe [2001], counting of *Camarophyllopsis* or not). There is no explicit guidance as to how this is done so comparisons between sites are difficult unless species lists are provided. Furthermore, until the recent publication of the Basidiomycota checklist (Legon & Hernici, 2005), there was some doubt as to taxa should be counted as species. Although neither Boertmann (1995) nor Legon & Henrici (2005) have listed *H. berkeleyi* (instead this taxon is listed as *H. pratensis* var. *pallida*), some authors of this report consider that *H. berkeleyi* should be reinstated as a species (based on its distinctive cap colour, ecological distribution and fruiting time). However, Boertmann (pers. comm., 2006) still considers this to be a variety rather than a species. Therefore, in this report, this taxon is counted as a subspecies of *H. pratensis*.

The general approach adopted here is to be strict with regard to taxon names and status (i.e. counting species rather than taxa; using the Legon & Henrici (2005) checklist for guidance). However, we have also been inclusive with regard to the taxa counted as 'grassland fungi' so that taxa which are occasionally found in grasslands but more frequently in woodland or other habitats (e.g. dune, heath, moorland) are counted. A complete list of all species and subspecies belonging to the genera listed above (including woodland taxa and listing habitat) is provided in Appendix D. Numbers of taxa for each group in Wales and the UK are also summarised in Table 6. The maximal CHEGD score possible using the rules listed above is 24:51:91:12:10 = 188 based on current UK records according to Legon & Henrici (2005). For Wales the maximal score would be 17:42:44:12:6 = 121.

The scoring method used here is as follows:-

All Basidiomycete taxon names follow those of Legon & Henrici (2005) and all taxa not exclusively found in woodland are included.

C = Species of the genera *Clavaria*, *Clavulina*, *Clavariopsis*, *Ramariopsis* including those taxa found in both woodland and grassland but not taxa recorded in Legon & Henrici (2005) as woodland only.

H = Species of *Hygrocybe*, *sensu* Legon & Henrici (2005) Checklist and Boertmann (1995),

E= Species of *Entoloma* noted by Legon & Henrici (2005) as occurring in grasslands (and also *E. hebes* and *E. conferendum* found in both woodland and grassland).

G = Non-woodland taxa are counted. Geoglossaceae present a problem since there is no definitive checklist (see Ridge, 1996 at http://fungus.org.uk/nwfg/irene.htm; also Silverside, 1997 and Spooner,2000). *G. arenarium* is not mentioned by Ridge and was not found in the present study. Other species mentioned by Ridge (1996) but not found in the present study include *Geoglossum vleugelianum* Nannf., *Geoglossum elongatum* Starb., *Geoglossum peckianum* Cooke. The taxonomy of *G. atropurpureum* is contentious (=*Thueminidium atropurpureum* (Batsch ex Fr) O. Kuntze = *Corynetes atopurpureus* (Batsch ex Fr) Durand.) but there seems little dispute as to the validity of the species itself. *Trichoglossum tetrasporum* Sinden & Fitzpat. (not found in this study) may be a variant of *T. hirsutum*. In the absence of collated data as to known distributions of these taxa it is difficult to ascertain whether *T. variabile* is a new Welsh record.

D = All species of *Dermoloma* and *Camarophyllopsis* plus *Porpoloma metapodium* recorded as occurring in grasslands by Legon & Henrici (2005). From discussions with a range of mycologists, there is no consensus as to whether *Camarophyllopsis* spp. should be included. The rationale applied here is that it is a genus closely related to *Hygrocybe* and frequently found in grassland, though often near shrubs/trees. Furthermore, Nitare (1988), in one of the earliest systematic surveys of grassland fungi, also included this genus.

The system described above aims to provide a consistent counting system (but as with any system contains an element of the authors' subjectivity). Inclusion of members of the CHEGD taxa which are not exclusively found in woodland is consistent with the broader observation that most of the taxa found in grasslands in Europe are found in woodland in other parts of the world.

It is usually the case that sites are ranked according to numbers of *Hygrocybe* spp., as initially devised by Rald (1985). Nitare (1988) extended this system to include the other CHEGD target taxa. In the present study, we make some use of CHEGD totals for sites / quadrat. To our knowledge this report is the first use of CHEGD species total. However, we recognise that such totals are unduly influenced by numbers of *Entoloma* species. Not only is this the most numerous grassland taxon, its members are also the most difficult to identify. Thus high CHEGD scores may in part reflect the skill of the surveyor in *Entoloma* identification. In several site reports unidentified *Entoloma* spp. are noted but not included in CHEGD scores.

6. RESULTS / DISCUSSION

a. Site choice

Choice of the 50 sites to be surveyed, was initially guided by prior knowledge derived from both the CCW lowland grassland survey and also information collated by Evans & Holden (2003), as well as input from CCW District Officers and sites identified by surveyors. It was unfortunate that this project began in 2003, since the early autumn period was very dry and not conducive to fungal fruiting. As a result, a number of the sites which were visited in October 2003 were unproductive despite surveyors noting that these sites might well be more promising if visited under better conditions. Thus of 56 sites visited in 2003, quadrats were only set at 20 sites. Information about the non-quadratted sites (as well as other sites visited informally by surveyors during the period 2003-5) is included in Appendix H. It was also intended to choose sites in such a way that there was good geographical coverage over the whole of Wales (Figure 1). The 51 sites surveyed (listed in Table 1) are located in 47 different 10 km grid squares with 4 pairs of sites being located in the same 10 km square (Bryn Alyn/Minera; Bryn Cul/Maes Caradog; Hafod/Caeau Cae'r Meirch; Gilwern Hill/Garn Ddyrys). As can be seen in Figure 1, few sites in Carmarthenshire (surveyed intensively by Rotheroe [1999] and also by Nigel Stringer [Evans & Holden, 2003]), the 'M4 corridor' (West Glamorgan/ Mid and south Glamorgan / Gwent) and the area north/east of Aberystwyth were studied in this survey, probably reflecting a paucity of grassland sites identified through the CCW Phase II surveys of these areas. Between 2 (VCs 35, 50, 51) and 9 (VC 48) sites were located in each of the 13 Welsh Vice-Counties (see Table 1). With regard to the present 23 Administrative Areas (post-1974 Councils) of Wales, sites were located in 14 areas, the 9 areas not represented being all located in the Vice-Counties of Glamorgan (VC41) or Monmouth (VC35).

b. Summary of 'quadratted' sites, quadrat number and size.

Quadrats were placed at 51 sites (Table 1) with 20 surveyed in 2003, 49 in 2004 and 30 in 2005. At 15 sites, more than one quadrat was surveyed on each visit (six sites with 2 quadrats; eight sites with 3 quadrats; two sites with 5 quadrats and one site with 6 quadrats). Thus a total of 88 quadrats were surveyed during 2003-5. However, of these only 79 quadrats were surveyed twice (i.e. the total number of quadrat surveys was 168). Philip Jones only re-surveyed one of the 3 sites allocated to him. GWG did not re-survey Caeau Cae'r Meirch but conducted a repeat survey of one quadrat in 2005 (Mynachdy'r Graig Q1). David Mitchel was forced to re-locate one quadrat at Epynt (originally in a live firing area!) and also added 3 quadrats which were only surveyed in 2005 (two extra at Old Castle Down, one extra at Troed-Rhiw-Drain; fruiting conditions were not good enough in 2004 to identify the best areas for grassland fungi). Andrew Graham added a third quadrat at Trawscoed in 2005 on land (Hafod yr Wyn), which he purchased in early 2005 (and which lies within the Migneint-Arenig-Dduallt SSSI).

Sixty nine of the quadrats were 900m^2. However, 17 quadrats at nine sites (the first to be set in Sept 2003; all Liz Holden in NE Wales) covered an area of 2500 m^2. It was subsequently judged that this was unfeasibly large for many sites (difficult to find large enough areas of homogeneous vegetation), so surveyors were subsequently advised to do 900m^2 quadrats (though once set, quadrats were re-surveyed at the same size). Two quadrats were set smaller due to the size of the site (Bodwryn at 24x32 m [768m^2]) or the available area of contiguous grassland (Dolobran at 24 x 28m [644 m^2]). The data for quadrats was analysed to investigate whether there was any correlation between quadrat area and CHEGD species diversity. The mean number of species found in 900m^2 quadrats was 12.05±5.78 compared to 8.79±6.93 in the 2500 m^2 quadrats. Linear correlation analysis of CHEGD species diversity and abundance suggested a slight negative correlation with quadrat area ($P=0.03$), though this is most likely because the 17 larger quadrats were initially surveyed in early October 2003 during a period of dry weather which was unfavourable for fruiting of *Hygrocybe* spp. (see below).

It is generally the case that fungal records are associated with nearby landmarks listed on OS maps. For the present survey, it was deemed important to define 'sites' more precisely. For most sites boundaries corresponded to ownership (17/51), SSSI (19/51) or Phase 2 NVC vegetation survey (2/51) boundaries. However, for the remaining 13 sites, boundaries were determined arbitrarily (centred upon areas of unimproved grassland; see Table 1 and also MapInfo files). As described in the SOP (see Appendix A) multiple quadrats were set at larger sites in rough proportion to site area, so that quadrats could cover different sub-habitats within these larger sites. The areas of the sites surveyed during this study ranged from 0.11 ha to >ca.15,000 ha and in many cases only parts of the sites were suitable for grassland fungi. Of the 21 sites larger than 40 ha that were surveyed, multiple quadrats were placed at 13 of these (Tables 1 and 2) with five quadrats at Halkyn Common (772 ha) and Epynt (14,568 ha) and six at Maelienydd (342 ha). Multiple quadrats were placed at five smaller sites (e.g. 2 and 3 quadrats respectively at Llanishen and Gilfach Farm respectively - both ca 6 ha).

Informal feedback from surveyors indicates that sites consisting of up to ca. 40-50 ha of grassland could be surveyed reasonably well during the course of a single day (6-8 hrs). In many cases, however, sites consisted of large areas of non-grassland habitats (bracken, moorland, heathland, woodland, wetland) such that the area of grassland at a site was much less than that stated in Table 1. For NVC sites it would be possible to calculate the area of grassland and to ensure that these areas are visited where possible but for other sites, it is less easy to identify suitable areas. However, at some large sites (e.g. Castlemartin [1,880 ha] and Epynt [ca. 15,000 ha], a comprehensive survey would take several days, even when non-grassland areas are discounted. At other large sites (e.g. Bryn Alyn and Halkyn) it is likely that the availability of NVC maps and advice from CCW staff will have resulted in relatively good coverage of promising grassland areas. Similarly, other larger sites such as Trawscoed (418 ha total area) could be well surveyed in a single day, since the area of mesotrophic (MG) grassland is only ca. 32 ha. One surveyor (Liz Holden) indicated on a map the approximate path which she had taken to cover the site (see Figure 2). This type of information, combined with an indication of the time spent on the survey, is very useful for estimating how well a site has been covered. In most cases recording intensity is only recorded as number of visits. However, some indication of surveying intensity is useful when making inter-site comparisons.

c. Timing of Surveys

Sites were surveyed between 20th Sept and 4th Dec (30th Sept and 4th Dec 2003; 22nd Sept and 4th Dec 2004, 20th Sept and 18th Nov 2005) as shown in Table 2. However, it is not uncommon to find some *Hygrocybe* spp. as early as July and as late as mid-January and some species are known to regularly fruit outside the main fruiting season, usually considered to be mid-September to late November (e.g. *H. intermedia* [early] and *H. punicea* [late]; in some frost-free locations such as Lundy, significant numbers of FBs can last well into January [John Hedger, pers. comm, Feb 2006]). *H. intermedia* was observed in large numbers (>80) at Cae Trefor though not at all during the survey visits (25/8/03).

As stated above, the precise definition of 'early' and 'late' surveys is difficult, so taking the 1st Nov as the peak fruiting date, survey dates have been classified as 'mid' (within a week of peak fruiting date), early or late. Had the 'mid' period not been defined then sites surveyed only a few days apart in different years (e.g. Cae Trefor surveyed on 29/10/03 and 4/11/04) could be artificially defined as having been surveyed early and late. Therefore, of the 79 twice-surveyed quadrats, only 33 were surveyed early and late (with six, two and two quadrats respectively being surveyed only 'early', 'mid' or late only; the remaining 36 quadrats were thus surveyed early/mid or mid/late). Figure 3a shows the distribution of survey dates during the three years illustrating the fact that most surveys were conducted between the 6 weeks from 4th October to 21st November.

Analysis of temporal patterns of fruiting showed that there was no significant correlation between the diversity (no. spp found per quadrat)/ abundance (total no. FBs per quadrat) and survey date (expressed as no. days after 1st September) (Figures 3b,c). However, when target groups were analysed separately, both statistically significant correlations were observed (analysed by Pearson's Correlation analysis in Minitab v. 12) (Figure 3d). Taking all three survey years together, more *Hygrocybe* spp. and Geoglossaceae species were present at later survey dates whilst the opposite was the case for *Entoloma* spp. However, as noted earlier, the 2003 season was generally poor for grassland fungi due to the lack of rain in the early autumn period. More detailed analysis of fruiting patterns in the individual survey years revealed that for *Hygrocybe* spp. there was a significant correlation between species diversity/abundance only in 2003. In contrast the greater diversity /abundance of *Entoloma* spp. earlier in the season was significant in all three years. This is consistent with the findings of Newton *et al.* (2003) and informal observations by a number of surveyors. It may be the case that more detailed analysis (e.g. Canonical Correspondence Analysis) may reveal more specific differences in fruiting patterns (e.g. late fruiting of *H. punicea*).

In order to assess the effect of survey date on the taxa found, the correlation between the diversity and abundance of CHEGD species relative to survey date (expressed as the number of days after 1st September; Figures 3b,e) was explored. Overall CHEGD species number per quadrat and CHEGD species abundance (the sum of the number of FBs for each species -this was estimated from the abundance data supplied by surveyors [see Appendix B: SOP; further details below]) were compared. Analysis of all quadrat data showed little effect of survey date on CHEGD diversity and abundance per quadrat. However, more detailed examination of individual taxa, showed a clear negative correlation between the number and abundance of *Entoloma* spp. and later survey dates and also a positive correlation for *Hygrocybe* spp. and Geoglossaceae. Examination of diversity / abundance data for individual years shows that the overall positive correlation of *Hygrocybe* spp. and Geoglossaceae was in fact the result of a very strong correlation in the autumn of 2003, the early part of which was very dry. However, in 2004 and 2005 there was little correlation. In contrast, the negative correlation of *Entoloma* diversity/abundance with survey date was clear in all three survey years.

<u>d. Taxon abundance and diversity in different sites</u>

A total of 35 *Hygrocybe* spp. (+4 subspecies), 44 *Entoloma* spp. (+1 subspecies), 17 Clavariaceae spp., 9 Geoglossaceae spp. (+1 subspecies), 4 *Camarophyllopsis* spp., 2 *Dermoloma* spp. and one *Porpoloma* spp. were found during the course of this survey (grand total CHEGD score of 112 [17:35:44:9:7]; see Table 3). These include several new Welsh and new UK records as detailed below (section 8; Table 6). As shown in Table 4, the commonest species were *H. virginea* (50/51 sites), *H. pratensis* (48/51 sites) and *H. psittacina* (47/51) with 13 of the CHEGD species (including 9 *Hygrocybe* spp.) being found at 35 or more sites. Of the 112 CHEGD species found in these surveys, 22 species were found at only a single site.

The number of CHEGD species found at the 51 different sites ranged from 3 (Foel Fawr) to 56 (Halkyn) with a mean of 27.3 species/site (Tables 2,3). Table 2 shows the ranking of the 51 sites according to CHEGD total score. The number of *Hygrocybe* spp. found ranged from 3 to 25 spp (mean 14.6 spp.; see Table 2). Numbers of *Entoloma* spp were more variable with none being found at five sites but 25 spp. at one site (Halkyn Common) (mean was 5.5spp. per site). With regard to the worst site (Foel Fawr), it would appear that the timing of the two surveys was unfortunate since previous surveys have recorded 26 *Hygrocybe* spp and the site is ranked in the top 20 for the UK (Table 12). Overall, all but six of the 51 sites would be considered as of at least regional importance (9-16 spp.) according to Rald's (1985) classification (as adapted by Versterholt *et al.*, 1999). Using the same classification 15/51 sites are ranked as of national importance and 5/51 as of international importance (based on two visits). It should be noted that Rald (1985) proposed less stringent classification for single visits. When the 99 (3/51 sites not re-surveyed) separate site visits are considered, 58/99 were classed as of national importance (11-14 spp.) and 26/99 visits (18 different sites) were of international importance (see Rald and Nitare Tables in Figure 13).

One or more of the three current BAP species were found at 30/51 reflecting the fact that many of the sites selected were previously known to be home to diverse populations of grassland fungi. Furthermore, it must be appreciated that sites which proved to be poor during initial visits were not quadratted and generally not re-surveyed. The distribution of BAP and other rare taxa is discussed below.

Sites varied considerably in area (from 0.25 ha to ca. 15,000 ha). There was no significant correlation (r^2 = 0.23) between site area and CHEGD species diversity. However, as noted above the percentage of the area of each site that was a suitable habitat for the CHEGD species was very variable (100% for many of the smaller sites) and for the larger sites (21/51 sites were larger than 50 ha), coverage of the whole site in a single visit would not have been complete. However, the seven sites with the highest CHEGD scores were all large (>80 ha) and of the largest sites >80 ha, only Castlemartin, Rhossili and Cors Geirch had fewer than 30 CHEGD species. Large sites would be more likely to encompass a larger range of grassland habitat types and thus potentially different grassland fungal communities.

Evans and Holden (2003) have collated ca. 8500 records for grassland fungi from Wales. More recent records, including all those from the present survey, have been added to the Welsh database by David Mitchel (pers. comm, 2006).

e. Taxon abundance and diversity in different quadrats

Of the 112 CHEGD species found at the 51 sites surveyed, only three species (+one variety) were not found in any quadrat (*E. ochromicaceum*, *E. sodale*, *T. variabile, M. olivaceum* var. *fuscorubens*). That the 88 quadrats (of which only 79 were re-surveyed -see above) covering ca. 10 ha in total should contain such a high proportion of the diversity found at sites which together covered an area ca. 2000 times larger (>400 larger even when Epynt and Castlemartin are excluded) with a median area of 33 ha (mean 406 ha, skewed by a few very large sites) is perhaps surprising. However, it should be noted that quadrats were set in the areas of highest CHEGD species diversity and that many sites consisted of large areas of unsuitable (non-grassland) habitat. Indeed the most prolific quadrat (Gilwern Hill; see Table 9) with 41 CHEGD species (10:23:4:4:0) after two surveys and 35 CHEGD species in a single survey (8:20:3:4:0) contained all the species found at this quite large site (81 ha). The quadrats ranked 2nd to 5th were also found to contain a high percentage (64-92%) of the CHEGD species found at the whole site [36/56 at Halkyn Q2; 35/40 at Somerton; 35/56 at Halkyn Q4; 34/37 at Ogof Foel]. At the other extreme there was a poorer correlation, with the three poorest quadrats having 17-67% of the site totals (3/18 at Dugoed Q2; 2/8 at Gweunydd Llechwedd Newydd; 2/3 at Foel Fawr). In the case of these three sites it is clear that low diversity in these quadrats was the result of placing quadrats in an unsuitable area having first visited the site during a poor fruiting period. The commonest species in the quadrats were the same as the species common at whole sites with *H. psittacina*, *H. pratensis* and *H. virginea* found in 113, 108, 87 quadrats respectively (out of a possible 168; Table 5).

Quadrats can be assessed on the basis of individual visits or by summing the CHEGD species found on both visits (as above). It is further possible to sum the CHEGD species diversity at all the quadrats on a given site (on the 15 sites where >1 quadrat was set). In all three cases (see Figure 6), there was a significant correlation (r2 = 0.439 or high; P<0.01) between quadrat diversity and site diversity. This was the case even when the five smallest sites (where quadrat size was >10% of site area) were excluded. However, it is important to note that whilst there is a general correlation, the quadrat approach was never intended to be a surrogate for a wider site survey. The dangers of extrapolating from a single quadrat survey are illustrated in Figure 6c where a number of points lie at some distance from the two lines of best fit. Only when multiple year and multiple quadrat data are combined (Figure 6a) did the correlation coefficient reach a level (r^2=0.875) where such an approach might be considered.

Use of the quadrats facilitated collection of quantitative data, in particular the use (for the most part) of a standard survey area. Additionally, surveyors were asked to estimate abundance of FBs of the CHEGD taxa. This information was recorded on a base 3 scale of 0-5 (0; 1-3; 4-9; 10-27;28-81;>81 FBs). For data analysis, these data were converted to actual numbers (i.e.0, 2, 6, 18, 54, 100 FBs).

Most CHEGD species were found at low abundance (<20 FBs) within quadrats but several were found in larger numbers. In some cases, exceptionally large number of FBs were also noted (e.g. >1000 FBs of *H. chlorophana* in Trawscoed Q2 in 2005) but this information was not used for quantitative analysis of FB abundance. When average abundance (either for individual quadrat surveys or summed data from both surveys of a given quadrat) was plotted against the frequency with which a particular species occurred in different quadrats, a positive correlation was found (Figures 4a, 4b). Thus more species with a more widespread distribution generally fruited more prolifically. Of greater conservation significance in the converse situation, whereby rarer species are likely to occur in lower numbers (*E. caesiocinctum* is a stark exception here- found on a single occasion but in large numbers), since they are less likely to be discovered by surveyors. With regard to individual species *H. laeta* when found occurred in very large numbers (mean 40 FBs/quadrat), as was the case for *H. coccinea*, *H. reidii*, *H. pratensis* and *H.ceracea* (i.e. above the lines of best fit in Figure 4a, 4b). However, *H. quieta, D. cuneifolium*, *E. conferendum* and *Cp. corniculata*, though frequently encountered (on >40/168 quadrats) were found on average at low abundance.

Surveyors also noted that during quadrat surveys they found FBs that they would very likely have missed had they not been required to conduct a detailed ('lawnmowing') survey of the quadrat area.

f. Relative occurrence of different CHEGD taxa

The mean number of CHEGD species found per site was 27.4 (range 3 to 56; see Table 2). The most diverse taxon was *Hygrocybe* (mean 14.6 spp [range 3 to 25]), followed by *Entoloma* (mean 5.5 spp [range 0 to 25]) with Clavariaceae, Geoglossaceae and *'Dermoloma'* being present at lower levels (mean 4.6, 1.8 and 0.9 spp. respectively). Survey data were analysed in various ways (using species lists based on whole sites, individual quadrat surveys or summed quadrat surveys) to examine whether the species diversity of the various CHEGD groups were correlated. As shown in Figure 5, there was a significant correlation between the occurrence of all CHEGD groups when site survey data were examined, the coefficient of correlation being highest ($r^2 = 0.598$***) between *Hygrocybe* and *Entoloma* species diversity and lowest for Clavariaceae vs. *Entoloma* ($r^2 = 0.316$*). However, when data from quadrats were examined a much weaker correlation was observed for *Hygrocybe* vs. *Entoloma*. Given the different temporal fruiting patterns it is perhaps unsurprising that no significant correlation was observed for individual quadrat surveys (ie. depending on whether the survey was conducted early or late in the season different taxa would be found). Even when summed quadrat data were analysed, only a weak correlation was observed ($r^2 = 0.265$*), suggesting that *Entoloma* and *Hygrocybe* spp, would tend to occur at different locations (habitats) within individual sites. For Clavariaeceae vs *Hygrocybe,* there was a strong correlation ($r^2 > 0.497$; $P<0.001$) whichever way the data were analysed. With regard to Geoglossaceae, the strongest correlation was with Clavariaceae.

Table 4 shows the rankings of sites for the various taxa, showing that the best 3 sites for *Entoloma* were only 9th, 17th and 21st for *Hygrocybe*. Conversely, the best three *Hygrocybe* sites were 4th, 8th and 18th for *Entoloma*. These correlations are broadly comparable to those found by Newton *et al.*, who also found a strong correlation between *Hygrocybe* and Clavariaceae diversity but no strong correlation between *Hygrocybe* and *Entoloma* spp. This lack of agreement may be explained by the fact that the Scottish survey conducted single surveys, such that the timing of the survey would have a strong influence on which taxa were present. The re-surveying of sites/quadrats in the present study to some extent removed the bias of survey date.

When presence/absence data for quadrats was plotted against FB abundance for each taxon (see Figure 4) there was a clear correlation irrespective of whether data from individual or summed quadrat surveys were used. Therefore, commoner species (i.e. those found in more quadrats) formed larger numbers of FBs. Conversely, it was clear that rarer species formed smaller numbers of FBs. For instance the 57 taxa found in 6 or fewer quadrats in total, formed an average of 6.6 FBs per quadrat whereas the 20 taxa found in 30 or more quadrats formed a mean of 20.9 FBs.

g. Site management

The 51 sites were managed in various ways, a small number (4) by mowing or haymaking (part of one site) and the majority by sheep grazing, with the remainder being grazed by other herbivores (cattle, horses, rabbits and guanaco [Esgyrn Farm]; see Tables 1 and 9). Sward heights were measured at all quadrats (ranging from 16.5 cm to 0.78 cm; mean 4.97 cm for all quadrats). At some sites there were noticeable differences in grazing regime in the two survey years. For example at Great Orme Q2, Gloddaeth Q1, Trawscoed Q1and Hafod Q1/Q2, the sward height in the quadrats was 4-8 cm longer during the 2nd visit. However, for 70/79 quadrats the inter-visit difference in sward height was less than 3 cm. Survey data were analysed alongside data for sward height or sward management regime but as shown in Figure 7 no significant correlations were found. Quadrats with a longer sward (>9cm; 12 quadrats) did have a lower CHEGD species diversity (7.67 spp.) than sites with shorter swards. ANOVA (analysis of variance; conducted using Minitab version 13) did not reveal any significant difference.

It is important to note that quadrat locations within sites were selected because they were home to diverse populations of grassland fungi, rather than being chosen at random or as representative of sites. Since grassland fungi are more easily noticed when fruiting in a short sward, there may have been bias in the location of quadrats. It is widely observed that fewer grassland fungi are observed in rank grassland but the extent to which this is due to problems in finding FBs or because fruiting is inhibited by the microclimatic conditions at soil level in longer swards is not known. Furthermore, it is quite possible that defoliation by mowing or grazing may be important in induction of fruiting and/or mycelial growth. At Hafod fewer fungi were observed in the 2nd survey when sward height was significantly longer but at the Great Orme (Q2) a less clear pattern was observed (more CHEGD taxa but fewer FBs).

Lastly, it is important to appreciate that FB presence/abundance may not correlate with abundance of mycelium in the soil. For ectomycorrhizal fungi in woodlands, a number of studies have shown that abundance of mycorrhizal root tips does not always correlate well with FB abundance (Horton & Bruns, 2001) and there is little detailed information about whether fruiting is an indication of health or stress in the underlying mycelium. Apart from the effect of climatic conditions (e.g. the dry October of 2003), grassland fungi may respond in different ways to different management regimes such that the timing of mowing or high/low grazing pressure may have an effect of FB presence/abundance later in the year. The sward height measurements used here provided a snapshot of sward management and it is likely that a more detailed study of mowing regimes/stocking levels is required to detect any correlations. Changes in management may have an effect over the longer term (several years) as the soil biota adapt to changes above ground. It was noted at RAE Aberporth that areas classified as 'scrub regeneration' in the 1998 Phase II survey, were now closely mown (assumed in Table 11 now to be MG1/MG5a like adjacent lawn areas).

h. Relationship between Grassland fungi and vegetation

NVC data from the CCW Lowland Grassland Survey were available for 29 of the sites (Appendix E). Information about vegetation cover was available for some other sites (e.g. Jackson & Foster [1994] for Mynachdy'r Graig; Stevens [2004] for Dolobran), though vegetation analyses did not always involve quadrats and precise definition of NVC types. Of the 87 quadrats, 42 coincided with areas for which NVC was obtained, including 38 of the twice-surveyed quadrats (see Table 8) and GIS (MapInfo) was used to identify the vegetation types found in each quadrat. In most cases, quadrats covered 2/3 different plant communities. A total of 28 NVC types and other communities were represented (CG1e[1], CG2a [1], CG2c [7], CG2d [3], CG10a [3], H4, H8b [1], H8c [2], H10c [1], Maritime Grassland [2], MG1 [3], MG5a [13], MG5b [2], MG5c [2], MG6 [5], Sanddune [1], Semi-Improved Calacareous Grassland [SICG; 1], Semi-Improved Grassland [SIG; 4], U1 [1], U3 [1], U4a [1], U4b [11], U4c [2], U4e [1], U5 [1], U5a [1], U5d [2], U20 - Bracken [2]). There were also areas of vegetational mosaic [4] at too small a scale to be mapped. For quadrats with mixed vegetation types, the three dominant types are listed in order of prevalence (% of quadrat area) in Table 8. However, due to concerns about precise location of quadrats, geo-referencing of NVC data and the likelihood that vegetation boundaries might have moved in the time elapsed since the NVC survey a precise breakdown was not attempted.

Surveyors did not by and large record locations of FBs within quadrats but it is likely that FBs were not found in areas of bracken or heath (H) even if these habitat types were present in the quadrat. The fact that most quadrats contained several vegetation types may of itself be significant. Since quadrat placement aimed to encompass areas of high fungal diversity, it may be that transitional habitats (ecotones) are favoured by grassland fungi. It has previously been noted that grassland macrofungi tend to fruit along ecotones (Thompson, 2000) and base-rich flushes (Watling, 1962; Newton *et al.*, 2003). However, since the some of the NVC data used were collected as far back as 1995, it is possible that some communities have changed or their boundaries moved. Combined with small errors in geo-referencing of NVC data and location of fungal quadrats, it would be unwise to draw firm conclusions from the present dataset. However, a future study using dGPS to map abundance of fungi alongside NVC data could be very useful in elucidating the relationship between NVC communities and NVC boundaries.

Broadly, the commonest vegetation types in these quadrats were MG (25), U (20), CG (15). It is noteworthy that species-poor grassland types (SIG, SICG, MG6) were found within several quadrats, including one of the best quadrats (Halkyn Q4; 34 CHEGD spp.).

Due to the mixture of vegetation types present in most quadrats and other caveats described above, detailed analysis was not possible. However, when the sites were classified according to broad vegetation sites (7xCG; 17xMG; 7xU; 6xother), it was apparent that the mean diversity of *Hygrocybe* spp. was highest in U (11.9 spp.) compared to CG (8.7 spp.) or MG (8.0 spp.). However, diversity of all CHEGD species was similar in U (20.2 spp.) and CG (19.1 spp.) but lower in MG (15.1). Thompson (2000) also assessed macrofungal diversity at sites with different vegetation types, finding that U4/U5d communities supported the highest diversity of *Hygrocybe* spp. and CHEG species, followed by MG5 communities.

Correlation with vegetation data is consistent with the suggestion that CHEGD diversity is greatest at less disturbed grassland sites. However, a number of the sites visited during this survey, including several of the best, are known to have been disturbed in the more distant past (100-200 yrs), including Llanishen Reservoir (embankment completed in 1886) and Garn Ddyrys (ironworks constructed in 1817 but abandoned in 1860 [http://212.219.230.30/$sitepreview/world-heritage-blaenavon.org.uk/whs-info/nomination/hills/hilltram.htm]). Other sites including Ffynnon Tal y Pyst, Gilwern Hill, Halkyn and Great Orme (former quarry) could be considered post-industrial (also some sites not included in this survey, such as the lawn at Llanerchaeron [house completed in 1795] and the coal / iron mining tips at Rhyd-y-Car [abandoned in the 1890's]).

Thus, although most / all of the sites visited during this survey have fortuitously escaped the effects of modern agriculture (farmyard manure or synthetic fertiliser addition and ploughing), several have experienced significant past disturbance but appear to have recovered. The timescale for full recovery would appear to be at least a century. One further possibility given the frequency of 19th century 'post-industrial' grasslands amongst the top sites for grassland fungi is that these fungi are more prolific on recovering sites than at pristine undisturbed sites. All grassland sites are plagio-climactic with succession to woodland prevented by grazing. However, related yet distinct successional processes will also occur in soil following physical disturbance without chemical disturbance (e.g. fertiliser addition, a relatively recent [<50 yrs] phenomenon) but very little is known about these. The occurrence of particular plant species may offer some clue. For instance, absence of *Helianthemum nummularium*, a potential host for several ectomycorrhizal fungi (see below) may be beneficial to waxcaps and other CHEGD taxa. Some CHEGD taxa may prove to be good indicators of sites of past disturbance; for instance *Entoloma incanum* and *Microglossum olivaceum*, which are sometimes found in abandoned limestone quarries.

i. Species found at sites

It is clear from the results of the present survey that the mycota of oligotrophic grasslands in Wales is under-recorded. For all the five target CHEGD groups, the presence of new species in Wales was either confirmed or established, with 17/112 (15%) of the CHEGD taxa found being new to Wales. The distribution of new Welsh/UK records among the various CHEGD taxa further indicates that under-recording is less severe for *Hygrocybe* than for *Entoloma* (ie. >10 *Entoloma* species new to Wales were discovered in this study). This is consistent with the fact that *Hygrocybe* spp. have a higher profile amongst mycologists (and the public) due to their distinctively-pigmented basidiocarps and the existence of a very accessible taxonomic monograph (Boertmann, 1995).

The recently published (very timely with respect to this report) *Checklist of British and Irish Basidiomycota* (Legon & Henrici, 2005) provides an analysis of which species are found within England, Wales, Scotland and Ireland. Data regarding the previous distribution of CHEGD taxa were obtained from this Checklist and are summarised in Appendix D and Table 6. A total of 112 CHEGD species were found during the course of this survey (Table 6). No new *Hygrocybe* spp. were recorded but the presence of *H. ingrata* (previously dubious- no herbarium record, according to Legon & Henrici, [2005]) was confirmed (present at 3 sites). Of the 8 species previously found in Wales but not found during this survey, the majority were rare (*H. auranita*: 3 records; *H. calciphila*: 12 records [incl. Garn Ddyrys]; *H. coccineocrenata*: 3 records; *H. phaeococcinea*: 2 records [incl. Ffynnon Tal y Pyst]), or not normally found in grassland habitats (*H. citrinopallida*, *H. conica* var. *conicoides* [previously found in dune grassland at Castlemartin], *H. radiata*, *H. turunda* [previously found at Trawscoed]). *H. persistens* var *konradii* was also not found, despite being previously recorded at Gilfach Farm, Great Orme and Trawscoed.

Forty four *Entoloma* species were found, equalling the known total for Wales. The three species previously of dubious status in Wales were confirmed and 13 species new to Wales were recorded. These included one woodland species (*E. scabiosum* found at Cefn Rofft; not included in the totals), one species found at a non-quadratted site (*E. fuscomarginatum* at Cors Erddreiniog, Ynys Môn; CEA; 11/9/03) and two new UK records (*E. glaucobasis and E. tibiicystidiatum*). However, it should be noted that critical determination of these novel taxa has yet to be conducted. Twelve grassland *Entoloma* species previously recorded in Wales were not found but all were rare (≤5 records each). The large number of novel taxa suggests that *Entoloma* spp. are significantly under-recorded in Wales, in large part due to the absence of any accessible keys and the high skill level required to negotiate the existing key (Nordeloos, 2004).

Amongst the other CHEGD taxa, there were four new Welsh records (*Clavulinopsis subtilis* [previously found in woodland at Trawscoed on 1/11/2004], *Trichoglossum variabile*, *Camarophyllopsis atropuncta* and *Dermoloma magicum*) with only *Clavaria rosea*, *Cm. micacea* and *D. pseudocuneifolium* (all rare: 7, 6 and 1 previous Welsh record respectively) not being found. Furthermore *Clavaria krieglsteineri* (new Welsh record) was also found in woodland at Trawscoed in 2005).

Non-target taxa were also recorded by surveyors with varying degrees of consistency (the SOP only specifically mentioned that *Cortinarius* spp. should be recorded). These are listed at the end of each site report (Appendix F; summary in Appendix E). Macrofungi belonging to 36 non-target genera were recorded, the most common being *Mycena* spp. (36/51 sites), with up to 29 species (at Gilfach Farm) being recorded (excluding species associated with trees or woody debris). The most unusual non-target species found was *Sowerbyella radiculata* at Rhosili.

Of particular interest is the occurrence of ectomycorrhizal species (mainly *Cortinarius*, sometimes alongside *Tricholoma*) at 11 sites. The species found included *Cortinarius croceus*, *C. anomalus*, *C epsomiensis*, *C. pratensis* and *Tricholoma hemisulphureum.* Nearly all (9/11) the sites at which these taxa were found were mainly maritime or on limestone. *Cortinarius* spp. were usually found in association with *Helianthemum nummularium*, though in at least some sites (Bryn Alyn, Castlemartin, Gilwern Hill) surveyors noted the presence of *C. croceus* or *C. pratensis* apparently in the absence of any woody / shrubby host. Some *Cortinarius* spp. in grassland have been found to form mycorrhizal associations with *Carex* and possibly other hosts (Harrington and Mitchell, 2002). There is some suspicion that large populations of *Cortinarius* spp. may be negatively correlated with *Hygrocybe* diversity/abundance but there is no clear evidence from the present study that this is the case.

j. Comparative assessment of sites: Use of indicator species and weightings

Increased awareness of grassland fungi has led to an increase in the number of naturalists and conservation professionals aware of (and able to identify to greater or lesser extent) these species. Information from such non-specialists can be important for identification of new sites and 'novice' keys such as that suggested by Griffith *et al.* (2004) may assist in highlighting sites meriting more detailed surveying. However, obtaining a complete (or near-complete) species list for the macrofungi (or even CHEGD fungi) at any grassland site requires many visits by a surveyor competent in the identification of all the target taxa. It is known from species accumulation curves for grassland fungi (e.g. Newton *et al.*, 2003) and fungi in other habitats (Tofts & Orton, 1998) that taxa new to a given site are routinely discovered even after >20 visits covering several fruiting seasons. As shown earlier (Figure4a, 4b), rarer species tend to fruit more infrequently, making it more likely that they will not be recorded if only a few surveys are conducted.

In order to be able to focus recording/conservation effort, there is a requirement for a system to be able to prioritise sites of greatest conservation value for their grassland fungi. Rald (1985) introduced the concept of assessing grassland sites (in Denmark) based on species richness of *Hygrocybe* (excluding subspecies), such that sites could be ranked as of national, regional or local importance. He also attempted to correct for survey intensity by providing a similar ranking system for single visits. Nitare (1988) extended this system to cover counts of other CHEGD taxa during single visits (for Sweden). Following the publication of Boertmann's (1995) monograph and increased interest in grassland fungi amongst mycologists in other parts of Europe, Vesterholt *et al.* (1999) extended Rald's classification to include sites of international importance. In the UK, the same basic system has been applied, though Rotheroe (2001) introduced the concept of counting subspecies, and also of including *Dermoloma*/*Porpoloma*, in the *Hygrocybe* count. Since the 'headline' figure quoted for most UK studies (e.g. Evans, 2003 etc.) follows the Rotheroe convention, there is a danger that like may not be compared to like (given that many good sites may have rather similar numbers of *Hygrocybe* spp.; e.g. Table 11). When comparing the 51 sites surveyed in the present study, the 'Rotheroe' mean score (16.0 taxa) is higher than would be counted by Rald's method (14.6 spp.). However, the counts for the best *Hygrocybe* sites (Trawscoed, Epynt and Blaen Nedd) are 24, 24 and 25 spp. respectively by Rald's method but both 24, 28, 28 taxa by Rotheroe's method). However, when the Rotheroe and Rald counting systems are compared for all 51 sites, there is a very high correlation ($r^2 = 0.998$; Figure 9)

Simple species (or taxon) counts in large part reflect recording effort (as illustrated in Table 11). For sites visited only once the raw species counts also reflect the timing of visits. It is recognised that the presence of the commonest species (e.g. *H. virginea* and *H. psittacina*) is less informative as to the overall diversity of *Hygrocybe* spp. because these species are often found on more disturbed sites, being apparently more tolerant of higher soil nutrients and/or quicker to recolonise (and fruit) on previously disturbed sites. As shown in Figure 4, the commonest species, when present, occur in greater abundance than rarer taxa and are thus more likely to be recorded, especially in a superficial survey. In recognition of this, several attempts, summarised very clearly by McHugh *et al.*, (2001) have been made to attribute increased weighting to species more commonly associated with species-rich sites (Table 12 is adapted from appendix 2 in McHugh *et al.* [2001]). It appears that these quality scoring systems (aka weighting systems) are based on qualitative assessment (to our knowledge they are not quantitatively derived). They are also likely to reflect national/regional diversity, since some species (apparently very rare or absent; e.g. *H. canescens*, *H. spadicea* and *C. zollingeri*) are included in only a few of the weighting systems. This is reflected in the differences between the Red Data Lists for grassland fungi in different European countries. Furthermore, it seems to be implicit in these weighting systems (explicitly stated by Vesterholt, 1999) that more readily identifiable species, less susceptible to climatic variables (ie. *Hygrocybe* rather than *Entoloma*) are preferable as indicators, thus attracting a higher weighting.

The existence of large record databases for the UK and constituent nations (collated in the BMS field record database: http://194.203.77.76/fieldmycology/BMSFRD/bmsfrd.asp) offers the possibility of deriving a weighting system which incorporates quantitative data for taxon occurrence. As can be seen from Table 11 the most useful indicator species (i.e. those given added weight in most of the eight different systems; these are highlighted by shading and the highest 'summed' scores from all the weighting systems are also shown) tend to be (in Wales) of intermediate rarity (e.g. *H. aurantiosplendens*, *E. bloxamii, H. ovina, H. splendidissima*, *H. intermedia*). However, rarity (or moderate rarity) in itself is not necessarily a robust criterion for being a good indicator, since some species (e.g. *H. spadicea*, *E, incanum*) tend to inhabit atypical sites which are not necessarily species-rich for grassland fungi. Nevertheless, the relative abundance of *H. punicea* (12th most abundant species in Wales [318 records] and 10th in Scotland [187 records]) and *H. quieta* (10th in Wales) suggest that the utility of these species as indicators may be over-estimated by qualitative assessments. However, in the case of *H. punicea*, the authors of this report are unanimous is considering this species a very useful indicator of potentially good waxcap grasslands.

A more robust method of identifying good indicator species is to identify taxa which are found at sites where a high number of other CHEGD taxa are found (index of association). Using this approach Thompson (2000) calculated the minimum number of *Hygrocybe* taxa with which each species was associated. For the Somerset dataset (56 fields at 18 sites), Thompson found that *H. pratensis* was found in all fields with 9 or more *Hygrocybe* spp. and never with fewer than 5 other species. *H. intermedia* and *H. reidii* were only found in fields with at least 9 other *Hygrocybe* spp. A related approach was used by Newton *et al*. (2003) for the Scottish waxcap survey where the species associated with the highest mean diversity of other *Hygrocybe* spp. were *H. aurantiosplendens*, *H. ingrata* and *H. ovina*. However, in Scotland *H. pratensis* and *H. reidii* were associated with lower mean numbers of other *Hygrocybe* spp.

The 'CCW Rarity Score' shown in Table 11 gives a weighting (classes are arbitrarily chosen) of 1 for common species (>200 records), 2 to uncommon species (25-200 records) and 3 for rare species (0-25 records) based on the 11844 records (including data from the current survey) for 149 CHEGD grassland taxa. There are obvious biases inherent in using raw field records, including the following: i. there can be multiple records for the same species at the same site but on different dates; ii. recorders may choose to submit records only for some of the species they find (e.g. unusual species, easily identified species, species belonging only to one particular genus etc.). For example, comparison of the Welsh records database with its Scottish equivalent shows that there are ca. twice as many *Hygrocybe* records for Wales (8810 vs. 4460) but fewer records for *Entoloma* (1204 vs. 1774), suggesting that there has probably been more overall recording effort in Wales (e.g. Maurice Rotheroe) but that Scottish recorders have focused more attention on *Entoloma* spp. However, within a single genus such as *Hygrocybe* for which a good key exists such bias is likely to be less significant. Given the fact that *H. calyptriformis* is the easiest waxcap to identify and its recent high profile as a BAP species and subject of a public awareness-raising campaign by Plantlife, it might be expected that there might be some bias in the Welsh records database in its favour (363 records). Figure 12 shows that the numbers of records is significantly higher after 1996 (after publication of the Waxcap-grassland Survey [Rotheroe *et al*., 1996]) but except for 2000, there is no clear pattern of any over-recording of *H. calyptriformis* relative to *H.pratensis* (the commonest *Hygrocybe* spp.; 724 records).

Overall there were 49% as many *H. calyptriformis* records compared to *H.pratensis*, a similar proportion to the 46% (22/48) found in the present systematic survey.

We have calculated an 'index of association' for *Hygrocybe* species (see Table 10; ie. the number of CHEGD species found alongside each given species), as conducted by Newton *et al*. (2003). The numbers of Clavariaceae, *Hygrocybe* spp., *Entoloma* spp. Geoglossaceae and CHEGD fungi associated with each *Hygrocybe* species are shown in Table 10. Statistical analyses were conducted only with numbers of *Hygrocybe* spp. (this group are significantly easier to identify to species than the other taxa, an important consideration for any indicator species). Nine species found at sites with a mean of >19 *Hygrocybe* species are identified as potentially useful indicators, whilst a further thirteen species are identified as poorer indicators (associated with a mean of <16 *Hygrocybe* spp.). Comparison of the species identified as good indicators provides some surprises, notably that *H. punicea* was the species associated with the lowest mean number of other *Hygrocybe* spp. (found with only 5 spp. at one site). Similarly, *H. quieta* was also found to be a poor indicator in these analyses.

As expected many of the poorer indicators are amongst the commonest species, whilst the better indicators are less commonly found. Figure 10 shows that there was a significant negative correlation between the index of association and both the number of sites at which a given species was found during the present survey and also the number of Welsh records for that species. However, two rare species (*H. spadicea* and *H. vitellina*) were found to be poor indicators (ie. low index of association) whereas moderately rare species such as *H. splendidissima*, *H. flavipes* and *H. intermedia* were identified as good indicators.

The quantitative evidence described above provides some objective criteria for choice of indicator species. However, there are other important considerations, such as ease of identification (e.g. *H. miniata,* identified as a good indicator in the present study, can be difficult to identify in the field), visibility and FB longevity. These last two traits are closely linked since the species with large (i.e. visible) FBs tend to be long-lived. The fact that *H. punicea* has been specifically identified as one of the best indicator species by a number of authors (Rotheroe, 1999; Boertmann, 1995; Mc Hugh *et al*., 2001) is very likely due to its high visibility, ease of identification and FB longevity. Several *Hygrocybe* taxa., such as *H. vitellina* (acid bog), *H. cantharellus* / *H turunda* (acid grassland), *H marchii* / *H. conica* var *conicoides* (in sand dunes), are more commonly found in habitats other than mesotrophic grasslands (U/CG/MG in NVC). It is not clear whether it is appropriate to include these species (when they occasionally occur in more typical 'waxcap grassland' sites) as indicators. As such, the high index of association found for *H. miniata* more commonly found in acidic grassland could be misleading.

Compared to the two earlier systematic surveys of grassland fungi (Thompson, 2000; Newton *et al*., 2003), the set of sites surveyed here were very species rich for grassland fungi. The mean number of *Hygrocybe* spp. was 14.6, with 20/51 sites (31%) of the sites being of national importance (>17spp) and 4/51 (8%) of international importance (this is a very conservative estimate since it is highly unlikely that two visits to each site would have discovered all the *Hygrocybe* spp. present). Indeed only 5/51 sites (9.8%) had fewer than 9 species present. Thus the choice of sites and quadrats in the present study was clearly not random but rather skewed towards areas known or suspected to host diverse grassland fungal populations. In contrast, the mean no. *Hygrocybe* spp. found at 474 Scottish sites surveyed by Newton *et al*. (2003) was 6.18± 0.41 (includes ca. 80 zero records) and for the 18 Somerset sites surveyed by Thompson (2000) the mean was 7.41. The statistical analysis conducted here is inevitably directed towards separating good from very good sites. Thompson (2000) identified *H. pratensis*, *H. punicea*, *H. splendidissima* as readily identifiable species consistently associated with communities of ≥5, ≥10, ≥13 , which broadly corresponds with Rald's definitions of locally, regionally and nationally important sites.

k. Grassland fungi as indicators of soil conditions
As well as being indicators of diverse waxcap populations, grassland fungi are also ecological indicators of soil conditions, in particular the absence of ploughing or fertiliser/lime input over long timescales. As such they also represent valuable indicators of ancient grassland (>100 yrs). However, it is likely that, prior to the age of modern agriculture, not all ancient pastures would be equally good for waxcaps, even though most would probably host some species. The soil conditions favour the development of highly diverse (as opposed to moderately diverse) waxcap communities are also unknown. For sites recovering from past fertiliser addition etc. there appears to be a chrono-sequence by which different species reappear (ie, produce FBs), initially *H. conica*, then *H. virginea* etc, such that at the most diverse sites the early-colonising species persist even when other more fastidious species have become established. Why this should be the case is unclear since no clear physiological or other differences between early and later-colonising species have been identified to date. It may be that successional processes occur over longer timescales (decades) which subtly modify soil nutrient, or microbial/plant communities such that these oligotrophic macrofungi are able to grow. A further observation from the sites surveyed during the present study, is that several of the best sites (e.g. Llanishen, Garn Ddyrys) are old but not ancient grasslands since they are known to have been disturbed/created during the 19th Century (see above). Thus diverse populations of *Hygrocybe* spp. (the evidence for other CHEGD taxa such as *Entoloma* spp. is less clear) can re-establish following habitat destruction but it is a process that can take a century or more.

It is important to appreciate that FB abundance is potentially an imprecise surrogate for mycelial abundance in the soil. It may be the case that apparent early-colonisers may grow and fruit more rapidly, whereas the apparent later-colonisers may have become established early in the chronosequence but simply grow more slowly and or only form FBs when a suitably large amount of mycelial biomass has developed below ground. Species with larger FBs (e.g *H. splendidissima*, *H. autantiosplendens, H. punicea*) generally occur later in the chrono-sequence may be significant. Lastly, many *Hygrocybe* spp. are geographically widespread and are not restricted to grassland (in the New World and Australasia they are typically found in woodlands; Young, 2006). It may be that the some of rarer species are those at the edge of their ecological range and thus struggling to survive (and therefore forming FBs only occasionally and in small numbers).

l. The International importance of Welsh waxcap grasslands
18 of the 51 sites (35.3%) surveyed in this survey yielded ≥15 *Hygrocybe* species on a single visit and are thus of international importance according to the Rald classification (for total numbers of *Hygrocybe* species based on only two visits); this attests to the exceptional diversity and abundance of waxcaps and other grassland fungi in Wales. A further 16/51 (31.3%) of the sites were ranked as nationally important using the same criteria. Indeed the 0.09 ha quadrats at Gilwern Hill and Garn Ddyrys (18 and 20 spp on a single visit respectively) themselves qualify as of international importance. This is all the more remarkable given that Wales comprises only 8.6% of UK land area (2,064,000 ha). A partial explanation for these unusually high levels of diversity is the relatively large area of grassland (73% of total land area is classified as 'grasses and rough grazing'; compared to a UK average of 51%) and that in upland areas poor access for mechanised farm vehicles has mitigated against agricultural improvement. Heavy grazing by high sheep populations, generally percieved as having a wholly negative effect on wildlife, may also be a positive contributory factor in this case.

Nine of the 18 internationally important sites and 14/16 of the nationally important sites had not previously been surveyed, so it is likely that further surveys would yield further species. Table 12 shows a list of the best 30 *Hygrocybe* sites in the British Isles. The majority of these top sites, including the best two, are in Wales (17/30, of which 15 were included in this survey). It is apparent that most of these sites have been intensively surveyed (≥10 visits), so sites such as Blaen Nedd with 25 spp. after only two visits (as part of this survey) will very likely rise in the ranking with further surveying. The majority of the top 30 sites are large (>50 ha) and comprise a range of grassland habitats, so it is relevant to draw particular attention to some of the smaller very diverse sites such as

Garn Ddyrys, Llanishen (also Hopetoun House and Rassal in Scotland) where the high grassland fungal diversity on small, botanically mundane and homogeneous grasslands is particularly noteworthy.

Superficially at odds with the above, Welsh sites fare less well in the ranking of CHEGD species totals (the top five are all in England or Scotland). This reflects the under-recording of *Entoloma* spp, in Wales. Eight sites were ranked as of national importance for *Entoloma* spp. (≥9 spp.) by Nitare's (1988) classification system and on several of these sites species new to Wales were discovered.

Waxcap grassland habitats are limited to Northern / Central Europe but it is in the countries bordering the Atlantic Ocean (Scandinavia, Netherlands, UK, Ireland) that the diversity of grassland fungi has attracted most interest. It appears that an oceanic climate (mild winter, moderate to high rainfall) is conducive to fruiting of grassland fungi. Outside Europe, *Hygrocybe* spp. are found as a rather minor component of the woodland mycota and there are to our knowledge very few instances where *Hygrocybe* spp. are found in grasslands in other continents (*H. conica* in churchyards in New Zealand [Patrick Leonard, pers. comm.] and in abandoned agricultural sites in Michigan [Greg Thorn, pers. comm.]).

Even in Central Europe, where traditional pastures have survived in greater numbers than in the UK, diversity of *Hygrocybe* is much lower (the best site in Slovakia has 18 spp. [Adamik & Kautmanova, 2006]), though it is likely that grassland fungi are under-recorded to a greater extent than in Scandinavia, the Netherlands and the British Isles. Outside the UK, the best waxcap grasslands are found in Scandinavia, though even here (with a longer history of intensive recording) Denmark and Norway only have one site each with ≥28 *Hygrocybe* spp. (Jordal, pers. comm.).

7. CONCLUSIONS

a. Statutory considerations and BAP species.

31 of the 51 sites surveyed already have some measure of protection since they are in part or as a whole notified as SSSIs. However, it is worrying to note that the three best sites (Epynt, Trawscoed and Garn Ddyrys; all in the UK top 5) have no or little statutory protection. The three grassland species identified as UK BAP Priority Species as part of the UK Biodiversity Action Plan (BAP) for which Species Action Plans have been prepared were all found during this survey. Only one new site was found for *H. spadicea* (Somerton Farm) but *M. olivaceum* and *H. calyptriformis* occurred at 14 and 22 sites respectively. The total number of sites for these three species is now at 12 (19 records), 33 (60 records) and >160 (362 records) respectively. A review of the UK BAP is currently underway and it has been agreed that *H. calyptriformis* will not be proposed on the new list (but retained as a flagship species), although *E. bloxamii* and *G. atropurpureum* will be added (found at 7 and 3 sites respectively during this survey).

b. Conservation importance of Welsh Grassland Fungi.

As described above, many of the sites surveyed during this study are of international importance for *Hygrocybe* spp. and the best two (Epynt and Trawscoed), as based on present knowledge, are the best sites known anywhere in the world. With the possible exception of the bryophyte populations of 'Atlantic' oak woodlands, it is difficult to find examples of habitats, species or communities in Wales which could be considered of global importance. These unique grassland fungal communities are the fortuitous result of a conducive oceanic climate, a long history of human impact of grasslands through livestock farming and a hilly topography which has provided some protection against the ravages of post-War agricultural intensification. Whilst not as charismatic and attractive to the general public as red kites, it is important that the existence and significance of these grassland macrofungal populations merits a higher profile among conservation professionals and the general public.

c. Effects of sward management

Attempts to find correlations between the grassland management regimes (including sward height measurements) and grassland fungal diversity were inconclusive. Although changes in grazing management can have a radical effect on plant communities, it must be remembered that fungi inhabit the soil and are thus isolated from many above-ground events. Fruiting of macrofungi is generally inhibited by the presence of rank vegetation during the autumn period, though in the present study quadrats were not set on areas of rank vegetation since the aim was to focus on areas of high grassland fungal diversity. Similarly, there was no clear correlation between NVC communities on a subset of quadrats and macrofungal diversity. Previous studies have not found any association between any particular plant species and any of the CHEGD fungi (excepting *Agrostis* spp. and *Festuca* spp.), though *Rhytidiadelphus squarrosus* and *Luzula campstris* are commonly found in association with these fungi (Ray Woods, pers. comm.; Griffith, unpublished data). We also note that waxcaps are occasionally found in woodland habitats in the UK in the absence of any of the plant species listed above. It is noteworthy that in the present study as in previous surveys of grassland fungi (e.g. Thompson, 2000), some botanically poor (e.g. MG6) grasslands were home to diverse CHEGD fungal populations. Thus, there is not a complete correlation between plant and fungal diversity.

d. Tools for the identification of important grassland sites

The Griffith *et al.* (2004) 'novices' guide mentioned above might be useful for a superficial survey by a non-specialist (e.g. Tir Gofal officer). However, there is also a need a system whereby such superficial surveys can trigger a follow-up survey by an experienced mycologists in order to assess the conservation value of the site. There is a requirement for a scoring system that efficiently ranks sites in terms of conservation importance and which recognises that only a subset of the species present will be fruiting during a single visit even if the visit is well-timed. As discussed above, the early simple species counting systems of Rald and others have been modified to include some weighting for species of higher conservation value (e.g. McHugh *et al.*, 2001) but usually based on subjective criteria. The quantitative analysis conducted here (Table 11), which is consistent with results from the systematic surveys of Thompson (2000) and Newton *et al*. (2003), provides some grounds for modifying these earlier quality scoring systems. The species included in the McHugh system are well-chosen (they exclude many of the less distinctive *Entoloma* spp. which require very specialist knowledge and keys for identification but this system also includes as groups the less distinctive Geoglossaceae and Clavariaceae). Specifically, it is suggested that (i) *H. flavipes* and *H. intermedia* be promoted to class A, (ii) *H. punicea* demoted to class B, (iii) *H. quieta* and *H. irrigata* demoted to class C and (iv) *H. miniata* promoted to class C. Several species in the McHugh quality scores (*H. calciphila*, *H. citrinopallida*, *H. constrictospora*, *H. phaeococcinia*, *H. radiata*, *H. xanthocroa*) have not been found or are extremely rare in Wales.

The extent to which climatic, habitat and topographical differences might affect the diversity of grassland fungi in different countries is more difficult to explore, though the fact several *Hygrocybe* spp. are found in Scotland (and Scandinavia) but not Wales (*H. lilacina*, *H. xanthocroa*, *H. citrinopallida*; see Appendix D) is probably due to the paucity of arctic-alpine habitats in Wales.

e. Validity of comparative site and quadrat surveys

For all forms of biodiversity assessment there is an evolutionary process, beginning with identification of the taxa present and progressing towards the development of standardised, quantitative survey methodologies. It is clear from the number of taxa new to Wales (and the UK) discovered in this survey that for macrofungi we are a long way behind ornithologists, mammalologists and botanists, for the most part due to the obvious constraints of taxonomy (few experts, lack of good keys, need for microscopic confirmation) and the ephemeral/unpredictable nature of macrofungal fruiting. The present project represents a significant element of this 'evolutionary process', in particular with the recognition of the importance of defining the area which was surveyed (with more quadrats on larger sites) and attempting through repeat visits to 'catch' both early and late fruiting species. With respect to the site surveys, it is difficult to quantify or monitor how well a site has been covered, since this can vary greatly according to surveyor behaviour (affected by weather, number of species present, personal preferences etc.) and site-specific differences (site area, topography, range of habitats present). In the future logging GPS devices could be used to establish which areas have been covered, though drawing a map of the approximate route of the surveying (e.g. Figure 2) together with indication of the time spent at the site can provide a good indication of coverage. For sites >50ha (depending on the percentage of grassland habitat present) it is quite possible for good areas of grassland to be missed and for sites >3-400 ha it is not possible for a full survey to be completed in a single day visit. Such large sites should be divided into 'surveyable' packets.

It was not intended for the quadrat surveys to act as a surrogate for site surveys, though the correlation between site and quadrat diversity was surprisingly high (Figure 6), improving as data for repeat surveys and multiple quadrats were combined. This suggests that surveyors were proficient at identifying areas of high macrofungal diversity when setting quadrats. The requirement to survey a small area in detail was effective in locating less conspicuous FBs (in the opinion of several surveyors) and the approximate abundance data collected is useful not only for the analyses conducted in this report but also potentially for improving the sensitivity of any longer term monitoring to changes in macrofungal populations. Attempts to quantitatively establish optimal quadrat sizes (i.e. species-area curves) for grassland fungi are in progress at UW Aberystwyth but the fact that it was necessary to reduce the initial 0.25 ha quadrat size to 0.09 ha suggests that the latter figure is very unlikely to be too small. The fact that 5/6 quadrats could set and surveyed within a single day (Halkyn and Maelienydd; in addition to the site survey) suggests that neither is 0.09 ha an inconveniently large area to survey in detail within 60-90 minutes.

There have been numerous previous studies of macrofungi using permanent quadrats, mostly, though not exclusively, in woodlands. For instance, Harrington (2003) assessed 0.01 ha quadrats in *Dryas octopetala* grassland habitats in the Burren, incorporating measurements of FB biomass, soil depth, soil nutrients and plant cover, whilst Rolstad *et al.* (2004) assessed quadrats containing different amounts of dead wood for diversity and abundance of wood-decay fungi. In the specific context of grassland fungi, the Somerset survey (Thompson, 2000) used the approach developed by Feest (1999). The Feest method (based on a butterfly survey protocol) involves assessing FB diversity and abundance in forty 12.6m^2 circles (ca. 0.05 ha total) at 10m intervals along a transect line (with 135° turns at field boundaries) across the field being surveyed (ranging in size from 0.6 to 11.5 ha). This is a more objective method for assessing FB diversity/abundance across the whole survey area, though it is not clear how quickly the data could be collected and how the method would work in more heterogeneous habitats (e.g. grassland interspersed with bracken/shrubs/ rocky areas). In the two other systematic waxcap surveys in Scotland (Newton *et al*., 2003) and Northern Ireland (Mitchel *et al*., in preparation) less emphasis was placed on survey methodology, since the aim was to identify sites in single visits across a larger area (in NI all 10 km squares were covered, whilst in Scotland over 500 sites were surveyed). In terms of the discovery of new sites meriting more detailed re-surveying it is clear that these latter approaches are more effective.

8. IMPLICATIONS

a. Statutory considerations and BAP species.

The results of the current and previous grassland fungal surveys suggest that several other CHEGD taxa should be considered for future inclusion. It is unfortunate that the Species Selection Criteria for UK BAP criteria (http://www.ukbap.org.uk/GenPageText.aspx?id=60) were designed more with birds / mammals in mind than fungi. As such criteria such as endemism (very unusual for fungi due to their mobile propagules -unless they are specific to an endemic host), evidence of decline (25% or 50% decline in the past 25 years) and rarity (presence in ≤15 10 km^2 squares) clearly work against fungi (and other less well-recorded groups) such that recent recording efforts in the context of a paucity of historical records give the appearance that species are becoming more common, whereas the drastic decline in unimproved grassland habitats would strongly suggest that all CHEGD have suffered significant decline (much more than 50%) in the post-War period. A consequence of these deficiencies in the UK BAP is that relatively common bird species are included as ‘Species of Conservation Concern' or 'UK BAP Priority Species’ whereas very rare fungi are excluded.

One strategy which would appear highly appropriate for grassland fungi is to produce a Grouped Species Action Plan. Such plans are appropriate for large groups of species with very similar ecological requirements and facing similar threats. A group SAP has already been produced for the stipitate hydnoid fungi and would be very appropriate for the grassland fungi, since the evidence for any ecological difference between most species is very limited (excepting the few calcicolous, calcifugous, heathland species). The UK BAP also includes Habitat Action Plans (HAPs) divided into Broad Habitats and Priority Habitats. The Priority Habitats include several where diverse grassland fungal populations can occur (Coastal sand dunes, Limestone pavements, Lowland calcareous grassland, Lowland dry acid grassland, Lowland heathland, Lowland meadows, Machair, Maritime cliff and slopes, Upland calcareous grassland and Upland hay meadows) but only one (Lowland wood-pasture and parkland) makes ANY mention of fungi. This is a situation that merits urgent attention given the importance of UK BAP in driving conservation policy.

b. Implications for management

Recent changes to DEFRA regulations (Environmental Impact Assessment (Agriculture) Regulations; http://www.defra.gov.uk/corporate/consult/eia2005/index.htm) mean that the cultivation or improvement (change of use) of semi-natural grassland now requires permission from the appropriate national authority (the Welsh Assembly Government in Wales). Granting of such permission requires a site visit to assess the conservation value of the area in question. At present such assessments are based solely on botanical criteria. We are aware of instances where permission to improve sites of high fungal diversity but relatively low botanical interest has been granted. We would suggest that site visits in October when both plant and fungal diversity can be assessed could avoid future occurrences and that assessment of fungal diversity is included as standard in such assessments. The 'novice's guide to grassland fungi' published by Griffith *et al*. (2004) would provide sufficient information for a crude assessment of grassland fungi to be made by a non-specialist.

Similarly, Agri-Environment schemes such as Tir Gofal do not formally take account of macrofungal diversity. This has potentially damaging consequences for grassland fungi, particularly in Wales. Reduction of stocking levels on more upland areas can lead to increased grazing pressure on adjacent *ffridd* grasslands. In order to sustain stock on a restricted area, farmers are more likely to increase fertiliser inputs on these grassland areas. Where no fertiliser has previously been added, the effect of such a change in management will decimate any CHEGD fungi that are present and recovery from such damaging events will potentially take decades. It is therefore important that preliminary assessments of populations of grassland fungi are incorporated as standard into Agri-Environment schemes such as *Tir Gofal* Failure to do so, as at present, will potentially lead to the addition of fertilisers to sites where there are diverse grassland fungi is avoided. As above, it is recommended that site visits are conducted in October, in order to identify areas hosting grassland fungal populations.

9. FUTURE WORK.

a. There is still a lack of wider appreciation of the international significance of 'waxcap grassland' habitats in Wales. It is hoped that this report will contribute towards a wider recognition and that with more amateurs and conservation professionals 'switched on' to these fungi that more good sites will be identified. The crude scoring system suggested by Griffith *et al*. (2004) may have some role in initial identification of such sites by non-professionals but there is still a requirement for a standardised and validated system for rapid site assessment (based on a single ca. 2hr visit by an experienced mycologist). Relative to its area, there is a good density of suitably experienced Wales-based mycologists (including many of the authors of this report) and the recent establishment of the Pembrokeshire Fungus Recording Network (http://www.pembsfungi.org.uk/) combined with the re-establishment of the Gwent Fungus Group suggests that there will be more expertise available in the future.

b. In the course of this project, well-marked permanent quadrats were used (with 8 figure grid references, locations marked on a MapInfo layer, a discrete yellow plastic marker in place and adjacent landmarks identified) such that in the future it will be possible to re-survey these quadrats (and sites) to monitor any long term changes in macrofungal diversity in response to changes in grazing management, air pollution (especially nitrogen deposition) or climate change. It is hoped that informal re-survey of some of the quadrats will be conducted (by the surveyors involved in this project or others) such that a longer term dataset can be accumulated (e.g. Mynachdy'r Graig, Trawscoed, Penrallt, Lligwy, Somerton, Garn Ddyrys, Hay Common). The approach developed in the course of this project could be applied at the permanent quadrats which are monitored by the UK Environmental Change Network (ECN; http://www.ecn.ac.uk/). Only one such grassland site exists in Wales (Snowdon; SH637551; CHEGD score of 0:3:2:1:0 from samples collected during a single visit by Matt Murphy on 27/10/04), but there are plans to expand this network and possibly include the monitoring of macrofungi.

c. The link between macrofungal and plant diversity in waxcap grasslands remains unclear. It is clear from this and previous surveys that some botanically mundane grasslands are excellent for macrofungi. However, previous work has shown that highly improved grasslands are almost always of little interest (Arnolds, 1982). It would potentially be very useful (if possible) if the quadrats not already covered by NVC survey information could be surveyed by an experienced field botanist. Accurate assessment of NVC communities and plant diversity more generally would be very useful for elucidating any links between plant and fungal diversity.

d. Attempts to correlate grazing regime (including sward height) with CHEGD species diversity were unsuccessful. It is possible that some correlation between management and fungal diversity might be observed if detailed information about past and current management was available. Such correlations are more likely to emerge from manipulation experiments in replicated field trials, ideally preceded by baseline surveys. A number of such field sites have been surveyed over the past 5 years at UWA (some details in Griffith *et al*., 2004; manuscripts in preparation) and some future follow-on experiments are planned to assess the effect of sward height (and timing/severity of cutting/grazing regimes) on fruiting.

e. It is intended that the key findings from this project will be written up for publication in a peer-reviewed journal.

f. As many sites included in the survey have received as little as two survey visits, additional future visits are desirable, at least for the higher ranking sites, to confirm or otherwise each site's conservation value. Visits in the early autumn would help to address the comparative dearth of *Entoloma* records on Welsh sites.

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