HARPER ADAMS UNIVERSITY

THE EPIDEMIOLOGY AND INTEGRATED CONTROL OF FAIRY RINGS ON GOLF COURSES

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ABSTRACT

Fairy ring is a common turf disease found on golf courses, but is poorly understood in terms of its epidemiology and control. An online questionnaire was emailed to every golf course in the UK and Ireland (equating to 3,849 recipients) in order to gather information on incidence, distribution and severity of fairy ring. Greenkeepers reported that type-2 fairy ring, where growth of the turf is stimulated, occurred the most frequently and that the impact was predominantly aesthetic. Disease symptoms were at their worst in July and August and were considered more of a problem when occurring on putting greens than any other part of the golf course. Links golf courses had a higher incidence of severe fairy ring than other golf course types and the south-east of Great Britain appeared to be more badly affected than the north-west.

A mycelial growth assay *in vitro* found that propiconazole was significantly more effective at inhibiting growth of some common fairy ring species than fungicides flutolanil, azoxystrobin and pyraclostrobin, and simple salt potassium bicarbonate. Experiments on *Marasmius oreades* and *Agaricus campestris* fairy rings in the field did not provide evidence that any of these chemicals controlled symptoms *in situ*.

The active zones of fairy rings at two golf courses were monitored using a soil moisture meter and a test to detect soil hydrophobicity, a condition whereby water fails to absorb into the soil. A significant moisture deficit and presence of hydrophobicity was detected as early in the year as March. Hydrophobicity was found to be absent from all tested fairy rings by October.

Overall, the project has produced a number of novel and interesting findings that have advanced understanding of fairy ring epidemiology and offer some practical solutions for greenkeepers trying to manage fairy ring symptoms on golf courses.

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1.0 Literature Review

1.1 Introduction

A fairy ring is a circle or partial circle of fungal fruiting bodies, usually mushrooms or puffballs; which are the reproductive structures marking the advancing front of a fungal mycelium existing underneath in the soil. The circular formation is characteristic of the growth habits of many of the fungi, which expand outwards radially from a point of origin. The activity of the fungus in the edaphic environment can affect the growth of neighbouring vegetation, which can have undesirable effects in managed environments such as amenity turf. In this context, the term ‘fairy ring’ refers to a disease of turfgrasses. In sports turf, such as golf courses, cricket pitches and bowling greens, there are numerous species of basidiomycete fungi that cause symptoms in the turf which negatively affect the quality of the game playing surface; causing interference with ball-roll during gameplay and presenting unsightly blemishes on otherwise uniform, close-mown turf (Figure 1). Fairy ring on golf courses can be particularly problematic, especially on putting greens, where smoothness and trueness of the turf surface have a large effect on the vertical and lateral movement of the golf ball, respectively.

Figure 1: Numerous particularly well-defined fairy rings, visible on Google Earth satellite images, growing on a golf course in Oxfordshire

Shantz and Piemeisel (1917) were the first to outline a classification system for fairy ring according to the symptoms expressed in turf; a system that is still in use today. They describe ‘type-1’ fairy ring as a ring or arc of necrotic turf, ‘type-2’ as a ring or arc of taller and/or darker turf where growth has been stimulated, and ‘type-3’ as a ring or arc of fruiting bodies (basidiocarps), which do not exhibit any detrimental effect on the turf (Figure 2).

Figure 2: Symptoms of type-1, -2, and -3 fairy rings, as defined by Shantz and Piemeisel (1917), plus superficial fairy ring patches, also known as thatch fungi. This was the infographic used in the Fairy Ring Questionnaire (Chapter 3) to help responders identify the fairy ring type(s) occurring on their golf course

This classification system can cause confusion in the greenkeeping community, partly as type-1, -2, and -3 symptoms can (and often do) occur simultaneously in one ring and, secondly, as symptoms may change according to environmental conditions. Type-2 symptoms, for example, can develop into type-1 symptoms when soil moisture falls below a certain threshold, with some causal species being more prone to this than others.

Death of turf associated with type-1 symptoms is largely attributed to drought stress caused by the soil hydrophobicity induced when the waxy, water-repellent fungal mycelium becomes so abundant in the soil that water fails to penetrate (Figure 3). An accumulation of phytotoxic levels of ammonium, hydrogen sulphide and potassium resulting from impaired microbial activity under hydrophobic conditions may also contribute to necrosis of the turf (Fidanza *et al*., 2007). The luxuriant growth of turf displayed in type-2 symptoms is a result of the grass plant taking up the nitrogenous compounds being released as the fungus decomposes organic material (Smith, 1965).

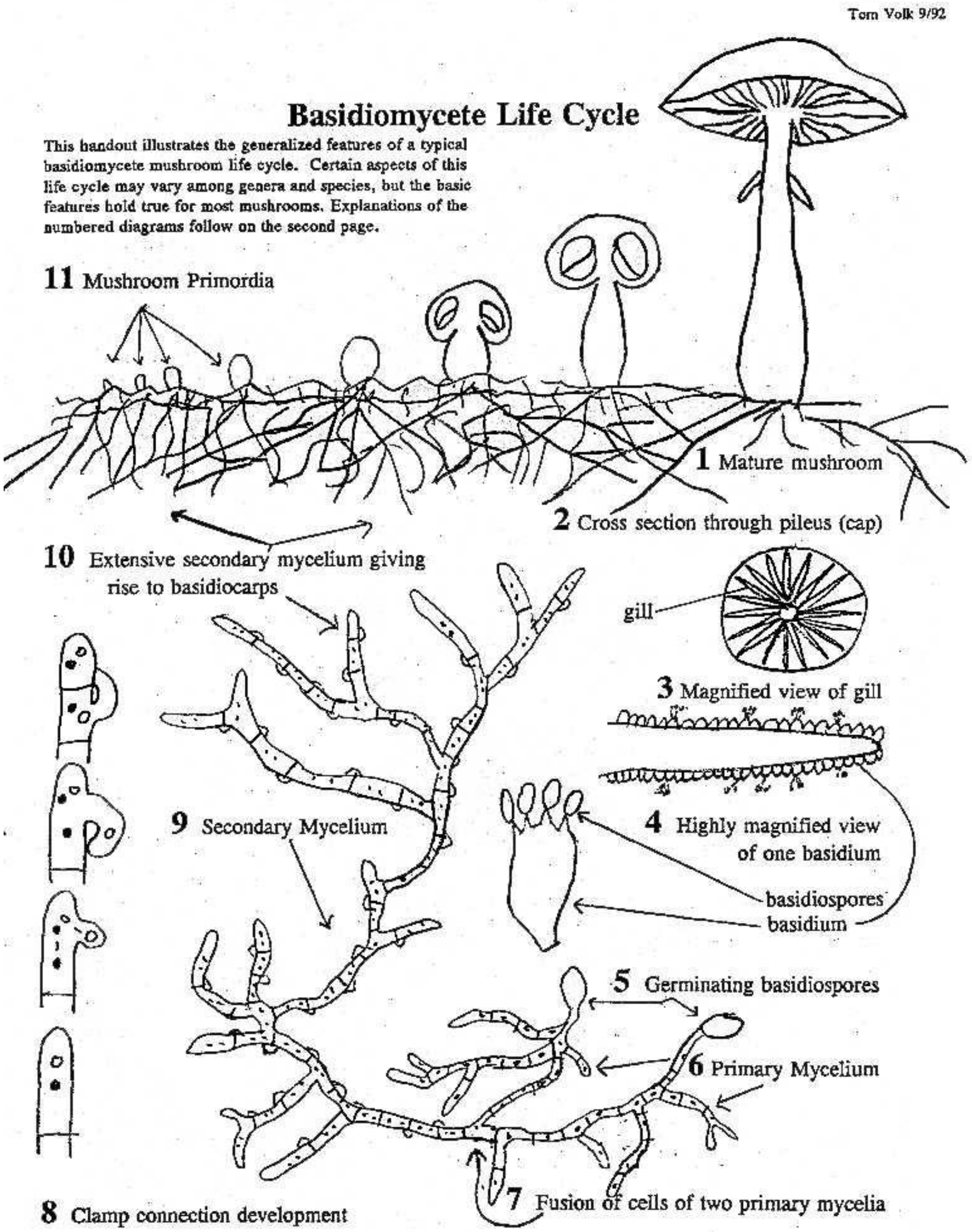
Figure 3: Turf layer removed mechanically to reveal the circular mycelium of an unidentified fairy ring fungus growing in the soil beneath
(photograph courtesy of Campey)

Besides the aforementioned type-1, -2, and -3 fairy rings, thatch fungi, also known as ‘superficial fairy ring’, are categorised as the fourth type of fairy ring, as they are also basidiomycetes (Figure 2). Unlike the other types, superficial fairy ring occurs as rings or patches of discolouration and/or depression in the turf and is caused by lectophilic (thatch dwelling) fungi feeding in the litter and thatch layers rather than edaphically in the soil (Smith and Jackson, 1981). Superficial fairy ring symptoms may vary considerably according to the causal species, many of which are yet to be identified (Watschke *et al*., 1995), and, like the other types, may also express basidiocarps, stimulated turf growth or turf necrosis due to soil hydrophobicity (Smith and Jackson, 1981).

1.2 Fairy ring-forming fungi

The common characteristic that all of the fairy ring types share is that they are caused by fungi of the division Basidiomycota (formerly known as the Basidiomycetes), which is generally known as encompassing the mushroom-forming fungi, which often form the conspicuous fruiting bodies (basidiocarps) with which many of us are familiar.

The division Basidiomycota comprises filamentous fungi, generally characterised by their ability to produce sexually using basidiospores produced on specialist club-shaped end cells called basidia (Kirk *et al*., 2008). Volk’s (1992) representation of a basidiomycete’s life cycle is shown at Figure 4.



This handout illustrates the generalized features of a typical basidiomycete mushroom life cycle. Certain aspects of this life cycle may vary among genera and species, but the basic features hold true for most mushrooms. Explanations of the numbered diagrams follow on the second page.

Figure 4: Diagrammatic representation of the life cycle of a basidiomycete (Volk, 1992)

The primary difficulty in managing fairy ring as a turf disease lies in the fact that there are numerous causal species, which have the potential to vary in their response to control methods. This makes species identification an important aspect when considering treatment options. Whilst identification is straightforward in the presence of basidiocarps, many fairy rings will not produce basidiocarps, especially in frequently mowed areas such as golf putting greens, so symptom type may be the only diagnostic information available to the turf manager.

Although rather basic information, symptom type and location on the golf course do hold some value as diagnostic tools, as certain species may be more likely to be associated with certain symptoms in certain areas (Ainsworth and Bisby, 1950, York, 1998). Puffballs, for example, such as *Lycoperdon* spp. are frequently found on putting greens, where they are often responsible for type-2 symptoms (Smith, 1965, York, 1998). The most-frequently studied fairy ring forming species is *Marasmius oreades* (the fairy ring champignon), which produces small, brown, edible mushrooms and can commonly be found causing aggressive type-1 symptoms, particularly on golf fairways (Smith, 1965, York, 1998).

Most of the current literature refers to there being 60+ different species of basidiomycete that form fairy rings; an estimate which appears to originate from Ainsworth & Bisby (1950). The estimated number of species, therefore, has remained unchanged for over half a century. Both Gregory (1982) and Harding (2008), however, claim that there are over 100 fairy ring-forming fungus species, but make no reference as to from where they obtained this figure. It could be argued, however, that, under consistent growing conditions, any basidiomycete has potential to grow in fairy ring formation.

A collation of the available literature from around the world has shown that, to date, at least 78 species of fungi have been associated with the formation of fairy rings or superficial patches (see Table 1). There are likely to be more. This figure is devised from scientific papers spanning more than a century, during which time considerable changes have occurred in fungal classification and nomenclature. Species listed in

Table 1, therefore, were checked for current legitimacy of scientific names using www.mycobank.org and adjusted accordingly.

Table 1: List of species that grow in fairy ring formation, as derived from existing literature

Fairy ring species	Type	Ecology	Reference
Agaricus arvensis Schaeff.	Type-2 Type-3	Saprophyte	(Couch, 1986, Halisky and Peterson, 1970, Shantz and Piemeisel, 1917)
Agaricus campestris L.	Type-2 Type-3	Saprophyte	(Couch, 1986, Halisky and Peterson, 1970)
Amanita muscaria (L.) Lam.	Type-3	Mycorrhizal	(Couch, 1986)
Amanita phalloides (Fr.) Link	Type-3	Mycorrhizal	(Couch, 1986)
Arachnion album Schwein.	Type-2	Unconfirmed	(Miller, 2010)
Bovista dermoxantha (Vittad.) De Toni	Type-2 Type-3	Unconfirmed	(Miller, 2010)
Calocybe carnea (Bull.) Donk	Type-3	Mycorrhizal	(Smith, 1957)
Calocybe gambosa (Fr.) Donk	Type-1	Unconfirmed	(Ainsworth and Bisby, 1950, Bayliss-Elliott, 1926)
Calvatia cyathiformis (Bosc.) Morgan	Type-2	Unconfirmed	(Couch, 1986, Halisky and Peterson, 1970)
Calvatia fragilis (Vittad.) Morgan	Unconfirmed	Unconfirmed	(Couch, 1986)
Cantharellus cibarius Fr.	Type-3	Mycorrhizal	(Couch, 1986)
Cantharellus cinereus Pers.	Type-3	Mycorrhizal	(Couch, 1986)
Chlorophyllum molybdites (G. Mey.) Massee	Type-3	Saprophyte	(Ainsworth and Bisby, 1950)
Clavulinopsis corniculata (Schaeff.) Corner	Unconfirmed	Unconfirmed	(Smith, 1957)
Clitocybe dealbata (Sowerby) P. Kumm.	Type-3	Saprophyte	(Halisky and Peterson, 1970)
Clitocybe geotropa (Bull. ex DC.) Quél	Type-2	Saprophyte	(Ramsbottom, 1953)
Clitocybe maxima (P. Gaertn., G. Mey. & Scherb.) P. Kumm.	Unconfirmed	Saprophyte	(Couch, 1986)
Clitocybe nebularis (Batsch) P. Kumm.	Unconfirmed	Saprophyte	(Couch, 1986)
Clitocybe phaeophthalma (Pers.) Kuyper	Unconfirmed	Saprophyte	(Halisky and Peterson, 1970, Halisky and Buckley, 1993)

Table 1: List of species that grow in fairy ring formation, as derived from existing literature (continued)

Fairy ring species	Type	Ecology	Reference
Clitocybe praemagna (Murrill.) Murrill.	Unconfirmed	Mycorrhizal	(Ramaley, 1916)
Clitocybe rivulosa (Pers.) P. Kumm.	Type-2	Saprophyte	(Smith, 1957)
Coprinopsis atramentaria (Bull.) Redhead, Vilgalys & Moncalvo	Unconfirmed	Saprophyte	(Smith, 1957)
Coprinopsis kubickae (Pilát & Svrcek) Redhead, Vilgalys & Moncalvo	Unconfirmed	Saprophyte	(Redhead and Smith, 1981)
Cortinarius armillatus (Fr.) Fr.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Cortinarius traganus (Fr.) Fr.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Crepidotus sp.	Unconfirmed	Unconfirmed	(Smith, 1957)
Cyathus stercoreus (Schwein.) De Toni	Type-3	Coprophyte	(Mercier *et al*., 1999)
Disciseda subterranea (Peck) Coker & Couch	Type-2	Unconfirmed	(Couch, 1986)
Entoloma conferendum (Britzelm.) Noordel	Unconfirmed	Unconfirmed	(Smith, 1957)
Gliophorus psitticina (Schaeff.) Herink	Type-3	Mycorrhizal	(Smith, 1965)
Gymnopus confluens (Pers.) Antonín, Halling & Noordel	Unconfirmed	Unconfirmed	(Couch, 1986)
Gymnopus peronatus (Bolton) Gray	Unconfirmed	Saprophyte	(Couch, 1986)
Handkea utriformis (Bull.) Kriesel	Type-2	Saprophyte	(Couch, 1986)
Hebeloma crustuliniforme (Bull.) Quél.	Type-3	Mycorrhizal	(Couch, 1986)
Hydnellum compactum (Pers.) P. Karst.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Hydnellum suaveolens (Scop.) P. Karst.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Hydnum repandum L.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Hygrocybe coccinea (Scop.) P. Kumm.	Type-3	Mycorrhizal	(Smith, 1957)
Hygrocybe pratensis (Schaeff.) Murrill.	Type-3	Unconfirmed	(Smith, 1957)
Hygrocybe reidii (Maire) J. E. Lange	Type-3	Unconfirmed	(Smith, 1957)
Hygrocybe virginea (Wulfen) P. D. Orton & Watling	Type-3	Unconfirmed	(Couch, 1986)
Hygrophoropsis aurantiaca (Wulfen) Maire	Type-3	Saprophyte	(Couch, 1986)

Table 1: List of species that grow in fairy ring formation, as derived from existing literature (continued)

Fairy ring species	Type	Ecology	Reference
Lactarius zonarius (Bull) Fr.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Lactarius torminosus (Schaeff.) Pers.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Lactifluus piperatus (L.) Pers.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Lepista nuda (Bull.) Cooke	Type-3	Saprophyte	(Halisky and Buckley, 1993)
Lepista panaeolus (Fr.) P Karst	Unconfirmed	Saprophyte	(Couch, 1986)
Lepista personata (Fr.) Cooke	Type-2	Saprophyte	(Ainsworth and Bisby, 1950)
Lepista sordida (Schumach.) Singer	Type-3	Saprophyte	(Ainsworth and Bisby, 1950)
Leucoagaricus leucothites (Vittad.) Wasser	Unconfirmed	Unconfirmed	(Couch, 1986, Halisky and Peterson, 1970)
Leucopaxillus giganteus (Sowerby) Singer	Type-1	Saprophyte	(Bayliss, 1911, Halisky and Peterson, 1970)
Lycoperdon hiemale Bull.	Unconfirmed	Unconfirmed	(Smith, 1965)
Lycoperdon perlatum Pers.	Type-2	Saprophyte	(Miller *et al*., 2007; Ainsworth and Bisby, 1950)
Lycoperdon pusillum Fr.	Type-2	Unconfirmed	(Terashima *et al*., 2002)
Lycoperdon spadiceum Schaeff.	Type-2	Unconfirmed	(Smith, 1965)
Macrolepiota procera (Scop.) Singer	Type-2 Type-3	Saprophyte	(Couch, 1986)
Marasmius oreades (Bolton) Fr.	Type-1 Type-2 Type-3	Saprophyte	(Couch, 1986, Halisky and Peterson, 1970, Bayliss, 1911, Shantz and Piemeisel, 1917)
Melanoleuca grammopodia (Bull.) Fayod	Unconfirmed	Saprophyte	(Couch, 1986)
Melanoleuca melaleuca (Pers.) Murrill	Unconfirmed	Saprophyte	(Couch, 1986)
Mycena flavoalba (Fr.) Quél	Unconfirmed	Saprophyte	(Smith, 1957)
Panaeolina foenisecii (Pers.) Maire	Type-2	Saprophyte	(Halisky and Peterson, 1970)
Panaeolus papilionaceus (Bull. ex. Fries) Quélet	Type-3	Coprophyte	(Smith, 1957, Halisky and Peterson, 1970)
Paralepista flaccida (Sowerby) Pat.	Type-3	Saprophyte	(Couch, 1986)
Paxillus involutus (Batsch.) Fr.	Type-3	Mycorrhizal	(Couch, 1986)
Polyporus varius (Pers.) Fr.	Unconfirmed	Unconfirmed	(Couch, 1986)

Table 1: List of species that grow in fairy ring formation, as derived from existing literature (continued)

Fairy ring species	Type	Ecology	Reference
Psilocybe semilanceata (Fr.) P. Kumm.	Type-3	Saprophyte	(Smith, 1957)
Rhodocollybia butyracea (Bull.) Lennox	Unconfirmed	Saprophyte	(Couch, 1986)
*Sarcoscypha coccinea** (Scop.) Sacc.	Type-3	Saprophyte	(Smith, 1957)
Scleroderma verrucosum (Bull.) Pers.	Type-3	Mycorrhizal	(Smith, 1965)
Suillus bovinus (L.) Roussel	Type-3	Mycorrhizal	(Couch, 1986)
Suillus variegatus (Sw.) Kuntze	Type-3	Mycorrhizal	(Couch, 1986)
Trechispora cohaerens (Schwein.) Jülich & Stalpers	Unconfirmed	Unconfirmed	(Smiley *et al*., 2005)
Trechispora farinacea (Pers.) Liberta	Unconfirmed	Unconfirmed	(Smiley *et al*., 2005)
Tricholoma columbetta (Fr.) P. Kumm.	Unconfirmed	Mycorrhizal	(Couch, 1986)
Tricholoma terreum (Schaeff.) P. Kumm.	Type-3	Mycorrhizal	(Couch, 1986)
Tuber melanosporum Vittad.	Type-1	Mycorrhizal	(Ainsworth and Bisby, 1950)
Vascellum curtisii (Berk.) Kreisel	Type-2	Unconfirmed	(Miller, 2010)
Vascellum pratense (Pers.) Kreisel	Type-2	Unconfirmed	(Miller and Tredway, 2009b)

* Ascomycete

This research has shown that fairy ring causing basidiomycetes can be further categorised into the subdivision Agaricomycotina and then further, into the class Agaricomycetes. In 2008, the class Agaricomycetes was thought to contain approximately 21,000 species (Kirk, 2008).

Gregory (1982) makes a clear distinction between grassland fairy rings, which he terms ‘free’, as they can spread through soil organic matter unrestricted, and woodland fairy rings, which he refers to as ‘tethered’ due to their reliance on tree root systems as ectomycorrhizae. Whilst grassland species would be expected to be found most commonly on golf courses, it is possible that any part of the golf course with trees in close proximity will be subject to the encroachment of so-called woodland fairy rings onto areas of turf. Hence, Table 1 is a comprehensive list of all grassland and woodland fairy ring-forming fungi, both of which have been found to occur on golf courses (Couch, 1986).

A recent study by Miller (2010) used DNA analysis techniques to identify fairy ring-forming species from golf courses across eight American states. The investigation was restricted to putting greens only, of which he sampled 45 (obtaining 122 samples), resulting in identification of five causal species, all of the puffball family *Lycoperdaceae*. Whilst reiterating Smith (1965) and York's (1998) claims that puffballs are mainly responsible for symptoms on golf putting greens, restricting sampling to only one part of the golf course may have excluded species causing notable symptoms elsewhere, including the type-1 formers, which are less common on greens (Smith 1965). As different species may occupy different parts of the golf course, a more holistic approach to sampling would perhaps give a more representative estimate of the number of species present throughout.

Whilst Miller's (2010) finding of only five species falls well short of the 78-species total estimate, it shows that it is likely that only a small number of species are most-commonly responsible for forming fairy rings on golf courses. Fidanza (2011) claims that the traditional 60+ species estimate can be narrowed down to approximately 10-12 species that are seen most commonly on golf courses (http://www.golfcourseindustry.com/video/disease-digest-podcast-mike-fidanza-fairy-ring/). Most fairy ring research to date, including estimations of the number of causal species, originates from America and, to a lesser extent, Japan. However, considerable attention has been paid to fairy ring in Great Britain by J. D. Smith during his time at the Sports Turf Research Institute, with the majority of his work focussing on the biology and control of the common type-1 former, *Marasmius* *oreades* (Bolton) Fr. There is currently no existing estimation as to how many species are responsible for causing fairy ring on turf in the UK today. An understanding of the species implemented is fundamental in devising control strategies, which may need to be tailored according to varying responses of the species being treated.

1.3 Factors affecting fairy ring development

As with any other living organism, the establishment and development of fairy ring-forming fungi is subject to an array of climatic and environmental variables that may affect growth and persistence (Ingold and Hudson, 1993; Smith, 1965; Couch, 1986; Halisky and Peterson, 1970; Wilkins and Patrick, 1940).

Growth rates, which may also vary by species according to variations in their biology and hyphal architecture (Halley *et al*., 1994), have been calculated for numerous fairy ring-formers and can be used to estimate the age of individual rings, some of which are thought to be hundreds of years old (Shantz and Piemeisel, 1917, Smith, 1957, Bayliss, 1911). Shantz and Piemeisel (1917) found their *Agaricus praerimosus* (now *Agaricus tabularis*) fairy rings in Colorado grew by an average of 12 cm per year, whereas *Calvatia cyathiformis* grew by an average of 24 cm per year.

Due to the uniform nature of constructed surfaces such as golf putting greens, fairy ring fungi are thought to spread faster through sports turf than they do in natural grasslands (Money, 2011), but this will be largely dependent on soil type. *Lepista sordida* is the fastest recorded golf course-dwelling fungus, the rings of which have been found to increase in diameter by over one metre per year (Terashima and Fujiie, 2005).

Despite a long history of research, literature on the epidemiology of fairy rings in turf is relatively sparse, as most studies focus on methods for their elimination. Here, however, some of the key factors attributing to development of fairy ring symptoms will be reviewed.

1.3.1 Climate

Wilkins and Patrick (1940) highlighted the influence of climatic factors on seasonal variation of grassland fungi, finding that temperature and soil moisture content in particular were directly correlated with basidiocarp production. Even where fairy rings do not produce basidiocarps, other symptoms can readily be seen changing throughout the year in response to climate, with symptoms becoming most severe during or after periods of hot, dry weather (Mann, 2011b, Mann, 2007, Mann, 2004) and often subsiding and becoming less visible in the wetter winter months.

1.3.2 Soil moisture

Soil moisture is the primary factor influencing the onset of type-1 fairy ring symptoms, where the grass plant dies from drought stress (Rillig, 2005). Symptoms that persist as type-2 in periods of adequate rainfall can develop into type-1 following situations of drought. From day-to-day observations, agronomist Richard Windows of STRI has found that symptoms are triggered when soil moisture falls below 10%, even for a short amount of time (personal communication 09/10/2012). Richard recalls symptoms worsening on the Old Course at St Andrews in 2010 following just one weekend where soil moisture dropped to 8%. In particular, these ‘flare-ups’ seem highly responsive to certain combinations of wet/dry weather cycles. Observations by Fidanza (2010) on golf course fairways in Pennsylvania, USA correlated a severe outbreak of type-1 fairy ring with a preceding series of weather events that involved approximately three weeks of low rainfall (i.e. a dry cycle), then a week of higher than normal rainfall (i.e. a wet cycle), followed by another dry cycle of 3-4 weeks, in conjunction with with prolonged periods of high humidity and high air temperatures. Fidanza does not name the fairy ring species involved in this set of observations. Evidence relating to response to wet/dry cycles is anecdotal and no controlled experiments appear to have been conducted, despite the potential to investigate this in glasshouses or manipulate conditions in the field. Whilst rainfall is an uncontrollable phenomenon, water input on golf courses can be controlled through supplementary irrigation. A better understanding of the climatic cycles that trigger fairy ring outbreaks would help in predicting and preparing for onset of the disease.

1.3.3 Temperature

As a major influencer of fungal growth, temperature is the other climatic variable that can, potentially, affect severity of fairy ring symptoms. Each fungus species has an optimum temperature for growth, which is usually 22-27˚C (Ingold and Hudson, 1993). During an eight-year study in Japan, *Lepista sordida* fairy rings were found to increase in size at the fastest rate in June and September, when temperatures averaged 21-24˚C (Terashima and Fujiie, 2005). *In vitro* experiments on the same species confirmed that its optimum temperature for growth was 25˚C (Terashima and Fujiie, 2005). Fairy rings growing under optimum temperatures will not only expand outwards at a higher rate, as shown by Terashima & Fujiie (2005), but may also increase mycelial density in the soil, encouraging soil hydrophobicity and the development of type-1 symptoms, although there is no evidence of this in the existing literature. Anecdotal evidence shows that, if a fairy ring fungus is subjected to a series of favourable climatic conditions, such as a hot, dry summer followed by a warm, wet autumn, disease symptoms in the turf are likely to be more severe (Mann, 2004; Mann, 2007; Mann, 2011b).

1.3.4 Soil structure

The rate at which a fairy ring fungus develops is largely dependent on the soil in which it grows. Soil structure determines the spread of the fungus horizontally and also how deep into the soil profile the mycelium penetrates vertically (York, 1998). Mycelia spread most readily through sandy soils, which provide the least resistance; allowing fungi to reach depths of 30-50cm (Smith *et al*., 1989). In heavy, clay soils fungi will grow more slowly and may be restricted to growing within the top 2.5-3.0 cm of the soil profile (Smith *et al*.; 1989, York, 1998). It could be hypothesised, therefore, that traditional ‘links’ golf courses (which occupy coastal areas), with their sandy rootzones, could be more extensively affected by fairy ring.

1.3.5 Soil pH

Fairy rings of *M. oreades* have been recorded on sports turf with soil pH’s varying from 5.1 to 7.4 (i.e. throughout the range at which sports turf is usually maintained) and *in vitro* experiments have shown the optimum medium pH for growth of fairy ring species to be a very slightly acidic 6.0 (Smith, 1965). Most natural grassland basidiomycetes from Warcup’s (1951) study (including *M. oreades* and other fairy ring-formers) were from soils of pH 6.4 and 7.0. This suggests that fairy ring fungi favour similar pH ranges to the grasses with which they co-exist.

1.3.6 Nutrients

Smith (1957) stipulates that fairy rings are found more frequently in infertile soils. Whilst this may be the case for *M. oreades*, the species Smith makes most reference to, there is contradictory evidence to suggest that species differ in their response to nutrient status and input. The fertilisation, aeration and irrigation of intensively managed areas such as golf putting greens does seem to discourage the development of type-1 symptoms (Smith 1965), such as those associated with *M. oreades*, but this is not always the case. In a three-year study on Kentucky bluegrass, abundance of *Tricholoma sordidum* (now *Lepista sordida*) fairy rings increased following applications of nitrogen (Beard *et al*., 1973). This reinforces the importance of species identification prior to treatment, due to the danger of exacerbating fairy ring symptoms.

1.3.7 Organic Matter

Basidiomycetes feed on organic matter, be it in the leaf litter, the thatch layer or in the soil. Generally, the more abundant the food source, the more an organism will thrive. It is not uncommon, therefore, to find fairy rings where organic matter is high, such as on areas that were previously pasture, or where tree stumps or lumber is buried (Watschke *et al*., 1995).

1.3.8 Vegetation

Fairy ring affects all turfgrass species, alongside a number of agricultural crops, and there is no evidence to suggest that some grasses are more susceptible to symptoms than others (Couch, 1986; Watschke *et al*., 1995). Some fairy ring-forming species, such as *Amanita muscaria* (Couch, 1986), are ectomycorrhizal; meaning they can only exist in association with certain trees (in this case birch and pine). It is, therefore, worth noting proximity and species of neighbouring trees when considering which species is causing the fairy ring symptoms.

When considering fairy ring epidemiology, it is important to remember that all fairy ring species are not the same and there is likely to be some variation in response to environmental stimuli. Different species may be found in different environments and some species may have evolved to occupy ecological niches. Species with more robust hyphae, for example, may be better suited to life in heavy soils.

1.4 Controlling fairy rings as a disease of turf

1.4.1 Chemical control

For the majority of the twentieth century, the method of choice for controlling fairy ring was to fumigate the soil with a toxic chemical such as methyl bromide, thereby sterilising the rootzone and killing everything within, including the fairy ring fungus (Couch, 1986). Today, such methods are unacceptable in the UK due to the toxicity of the chemicals. An EU-wide ban on methyl bromide came into force in 2010 after adverse effects on human health and its role in ozone depletion became apparent (Foxall, 2010).

The continued prohibition of chemicals for use in disease control on amenity turf leaves greenkeepers with few options for effective fairy ring control. Particularly in the UK, there are now very few fungicides licensed for use on amenity turf and, currently, only azoxystrobin (e.g. Syngenta's Heritage and Heritage Maxx) includes fairy ring on the product label. The Heritage label claims 'control….of type 2 fairy rings', whereas Heritage Maxx claims 'reduction of type 2 fairy rings'.

The vast majority of existing literature on fairy ring control comes out of the United States of America, where they have several more fungicidal products available to them for amenity use than we do in the UK (Fidanza, 2009). In an information sheet by the TurfFiles Center of North Carolina State University (2014), for example, a review of products available for fairy ring control was carried out and reported 'excellent control' for both DMI (demethylation inhibitors) class fungicides tebuconazole and triadimefon, and for combinations of DMI and strobilurin fungicides fluoxastrobin and myclobutanil, and triadimefon and trifloxystrobin. None of these chemicals are available for amenity use in the UK. The same review rates azoxystrobin, the systemic strobilurin fungicide that is available in the UK, as providing 'good control'. Whilst the review does not provide details of the data on which these ratings are based, on what species of fairy ring fungus, or even on what type of fairy ring, there is mention of the DMI fungicides providing particularly effective control against puffball species (*Lycoperdon* spp., *Vascellum* spp, *Bovista* spp, or *Arachnion* spp) on golf putting greens.

In 2002, Fidanza *et al*. published results from a number of their field experiments carried out at five sites throughout the USA. In their results for the North Carolina site, they report that plots treated with the fungicide flutolanil, a carboxamide fungicide which, again, is not licenced for amenity use in the UK, contained a significantly lower number of basidiocarps. There is no evidence, however, that abundance of basidiocarps is linked to the severity of other fairy ring symptoms. The fungus implicated in this study remained 'unidentified', despite basidiocarps being present. Their data for the Florida site, however, was more convincing. Nine golf putting greens were divided into two, where half the green was untreated and half was treated preventatively over winter with flutolanil plus a soil surfactant. By March, no fairy rings had appeared on the treated greens, whereas an average of 23 type-1 fairy rings per green had appeared on the untreated. Some of their other experiments show that flutolanil is effective in isolation, as well as with a surfactant.

Work presented by Miller (2005) on his website (http://www4.ncsu.edu/~glmille2/research.html) shows that azoxystrobin and pyraclostrobin could be ineffective in suppressing fairy symptoms unless they are applied with a wetting agent. Flutolanil was effective on its own, but significantly more effective when applied with a wetting agent. The species of fairy ring involved in these experiments is, again, not specified.

Miller's *in vitro* work on fairy ring control (2010) did not identify any significant differences in the ability of flutolanil, propiconazole, tebuconazole, triadimefon, or triticonazole to inhibit the growth of 16 fairy ring isolates from five species.

The general consensus from the literature is to treat preventatively or curatively with flutolanil if chemical control is to be attempted (Miller and Tredway, 2009a, Fidanza *et al*.; 2000, Nelson, 2008), but this is largely due to flutolanil being the only fungicide in the USA that was labelled for fairy ring control around the time that the bulk of the existing research was carried out. As flutolanil is not licenced for amenity use in the UK, this does not provide a solution for fairy ring control in this country.

A word that surfaces regularly in the fairy ring control literature is 'inconsistent'. Also noticeable is that the experiments rarely make reference to the species of fungus causing the symptoms. Perhaps this is linked to the inconsistencies.

1.4.2 Non-fungicidal chemical control

There is brief mention in the literature of two other novel control methods that may provide an alternative to fungicides. One is potassium bicarbonate. An old article in Sports Turf Manager magazine from 1996 (http://archive.lib.msu.edu/tic/stnew/article/1996jun11.pdf) provides instructions on how to prepare and apply potassium carbonate as a promising solution to fairy ring control. A later Technical Fact Sheet, produced by the New Zealand Sports Turf Institute (undated) claims that potassium carbonate has '*been effective in reducing the severity of this disease and in some instances eliminating it'*, although they do not reference any data from experiments. At the International Turfgrass Society conference in Beijing in 2013, several personal communications were received relating to potassium carbonate for fairy ring control from academics and turf managers alike from Australia, New Zealand, and the United States of America. Again, no specific data could be traced.

The second potential novel treatment, again, came from the aforementioned Technical Fact Sheet from the New Zealand Sports Turf Institute (undated). Following on from potassium carbonate, they discuss sugar as a suppressant of fairy ring symptoms. They claim that applying sugar to the affected area provides a food source to other fungi, which then out-compete the fairy ring fungus. This seems like a dangerous tactic, however, as they mention in the fact sheet, sugar can feed other disease fungi and worsen symptoms of diseases such as brown patch and *Sclerotinia minor*.

1.4.3 Cultural control

Widely accepted as the most effective method to prevent fairy ring, and most other turf diseases, on the golf course is to implement a system of integrated turf management through good cultural practice (Mann, 2003). Cultural practices are golf course management techniques which aim to keep the turf in optimum condition. They include: -

- Aeration – to keep the rootzone aerobic and reduce thatch accumulation
- Fertilisation – to control nutrient status and pH
- Irrigation (and wetting agents) – to stop the turf from drying out
- Drainage – to stop waterlogging and associated disorders
- Mowing – using an appropriate cutting height to minimise plant stress

Various equipment, products, and methods are used with the aim of keeping turf in optimal health and the management programme for each golf course will vary according to environmental and climatic conditions, and also financial budget (Mann, 2003). Whilst no turf is immune to disease, generally, the less stressed and the more well-nourished it is, the less susceptible it will be to infection by pathogens (Nelson, 2008).

A relatively easy way to deal with type-2 fairy ring symptoms is to mask the difference in colour, where the fairy ring is usually darker than the surrounding turf, by applying nitrogen or iron in order to stimulate the growth of the surrounding turf to match that of the fairy ring. This is particularly effective on greens, where fairy ring can create a water-marked effect, as they are mown regularly and the problem is aesthetic rather than height of the turf becoming a problem for playability.

The most pertinent cultural practices relating to fairy ring prevention and control relate to water management. Smith (1957) observed that *M. oreades* appeared to be the species most commonly found causing type-1 (turf loss) symptoms. He also noted that they were usually found on fairways, very rarely on putting greens, and concluded that *M. oreades* prefers a nutrient-poor environment (Dernoeden, 2000).

The nature of putting greens, as the part of the golf course where contact between the golf ball and the turf is most integral to the game, is that they are intensively managed in order to provide a smooth and uniform playing surface. They are often the only part of the golf course that is artificially constructed and may have had the rootzone altered to be composed more of sand than the natural soils of other parts of the golf course. The turf is mown low, often, and sometimes mechanically rolled. They are regularly aerated, irrigated, and fertilised. If type-1 fairy ring occurs rarely on greens, it could be inferred that something about the construction and/or management of greens deters type-1 symptoms from developing.

As previously mentioned, the development of type-1 symptoms is largely a result of soil hydrophobicity, where the area colonised by the fungus repels water rather than absorbing it (Rillig, 2005). Employing cultural techniques, such as aeration to break up the hydrophobic soil mat and allow water to penetrate into the soil profile, wetting agent to aid the absorption of water, and intensive irrigation, it may be possible to alleviate fairy ring symptoms (Cisar *et al*., 2000). The more hydrophobic a fairy ring gets, however, the more difficult it becomes to rewet. Attempting any chemical treatment at this stage is futile, as the product fails to penetrate into the soil profile and make adequate contact with the causal fungus (Fidanza, 2009).

If attempts to rewet a type-1 fairy ring using aeration, wetting agent, and irrigation are unsuccessful, the ultimate option is to physically dig the affected area out and remove the infested soil from site (Couch, 1986). This is often an undesirable option, as it is labour-intensive, destructive, and not guaranteed to work. If the area is not dug out deep enough or wide enough, or if any of the infected material is left behind, then fairy ring is at risk of reoccurring (Couch, 1986).

1.4.4 Biological control

Other fairy ring control methods include biological treatments, such as compost teas, which are rich in the micro-organisms often found in compost, including bacteria, fungi, protozoa, and nematodes. The aim in applying biological treatments is to change the species dynamics in the edaphic environment and to boost the biodiversity and, hence, productivity of the rootzone. Do-it-yourself techniques are available, but such products are also available commercially, where the marketers claim that the species within the product will out-compete the fairy ring fungus being targeted.

A series of experiments by Smith in the late 1970s and 1980s reported on the ability of fairy ring fungus *M. oreades* to inhibit the growth of itself. This was termed mutual antagonism. *In vitro* tests showed that the fungus, when paired with another sample of itself in a Petri dish reached an equilibrium in growth with a mycelium-free inhibitory zone between the two (Smith and Rupps, 1978). The authors hypothesised that *M. oreades* produces some kind of inhibitory metabolite that prevents it from growing into or over itself. Field experiments appeared to support this theory. When a number of domestic lawns infected with *M. oreades* fairy rings were mechanically rotovated and re-turfed, no fairy rings reappeared during the several subsequent years that they were monitored (Smith, 1980b). No further progress appears to have been made in this field since these studies and no other fairy ring species have been investigated.

Overall, there is little available evidence of any of the control methods offering particular efficacy against fairy ring. Few impartial, academic studies have ever been carried out worldwide (and evidently none in the UK) and what data there are on the efficacy of control products lie in the commercial domain. With the amount of different species involved in causing fairy ring symptoms, however, it is likely that they will be dissimilar in their response to potential control methods. The expense and ongoing discontinuation of fungicidal products prompt a drive towards greener and more sustainable turfgrass practices and highlight a need to find alternative strategies for managing disease.

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2.0 Introduction

The need for this research is essentially driven by golfers, whom golf clubs aim to please by providing a visually attractive golf course and a high-quality playing surface, which leads to an enjoyable round of golf and an overall sense of well-being for the player.

The UK Golf Player Survey carried out by Syngenta in 2012 found that 'condition of the greens' was voted the most important feature overall by golfers when judging a golf club. The three most desirable characteristics of greens were voted as: -

1. Smooth ball roll
2. Free of weeds
3. Free of scars and disease

The occurrence of fairy ring on greens has potential to impact all three of these desirable characteristics (where type-1 fairy rings leave bare ground, it is often first recolonised by weeds rather than grasses), so it can be interpreted that control of fairy ring symptoms will lead to greater golfer satisfaction. In turn, greater golfer satisfaction can lead to more people participating in the game and players participating more frequently, which has clear economic benefits to the golf industry.

As the literature shows, fairy rings have been studied for over a hundred years and yet relatively little progress appears to have been made relating to their epidemiology and control. Unlike other fungal turf diseases, such as dollar spot (*Sclerotinia homoeocarpa*) or pink snow mould (*Michrodochium nivale*), which are generally caused by one species, the primary difficultly in studying fairy ring as a disease of turfgrasses is that it is well known to be caused by a multitude of different fungus species, spanning a wide range of the Class Agaricomycetes and, hence, with genetic variation that could see them respond dissimilarly to control treatments.

Fairy rings are ubiquitous, occurring in woodland, grassland, and arable ecosystems, and, from anecdotal reports, are found on a variety of golf courses all over the UK. Existing published research on fairy ring in relation to sports turf is lacking. During the 1970s and 1980s J.D. Smith worked intensively on experiments with the fairy ring former *Marasmius oreades* whilst he was based at the Sports Turf Research Institute (STRI), UK, which represents the bulk of the publications to date and few investigations appear to have been published since. As control of fairy ring is of commercial interest, data on the efficacy of control products are generally not available in the public domain or cannot be guaranteed to be impartial. This study, therefore, intended to stand as an unbiased academic, rather than commercial, investigation into the causes and control of fairy ring on UK golf courses.

The research objectives of this project were threefold: -

1. Identify the causal agents of fairy ring on UK golf courses
2. Investigate the epidemiology of the causal agents
3. Synthesise an integrated control strategy for fairy rings

As the existing understanding of fairy ring incidence, distribution, and severity in the UK was so limited, having never been formally studied, it was decided that a large-scale investigation should be carried out across the UK to gather information on the current status of the disease on golf courses. This was done via an online questionnaire, which was delivered to head greenkeepers or golf course managers at every golf course throughout the UK and Ireland. This investigation occupied the first year of the project and is discussed in Chapter 3.0.

With the aim of developing a geographical catalogue of the species involved in causing fairy ring on golf courses in the UK, samples were obtained from 154 fairy rings from 48 different sites across the UK and Ireland; 46 of which were golf courses, one was an amenity green, and one was a croquet lawn. These were collected either in person or via a large-scale appeal for greenkeepers to send in samples to STRI using pre-paid bags sent out to golf courses in fairy ring sampling kits.

Much of the second and third years of the project were spent in the laboratory, isolating fungi and extracting DNA from the fairy ring samples in order to genetically sequence causal, fairy ring forming species. Unfortunately, none of the DNA samples were successfully amplified using PCR to the necessary concentration and quality to be sequenced and this aspect of the project did not deliver any data. A commentary of the laboratory protocols performed over this period is included at Appendix I for reference.

With focus on the epidemiology and control of fairy rings, a series of experiments were carried out in years two and three of the project in order to investigate the efficacy of control products and techniques in treating fairy ring, and the relationship between fairy ring and soil moisture that appears to lead to the destructive loss of turf, which are covered in Chapters 4.0, 5.0, and 6.0.

3.0 Fairy Ring Questionnaire

3.1 Abstract

An online questionnaire delivered to every golf course in the UK and Ireland in spring 2012 aimed to evaluate the current status of fairy ring distribution and severity. A 5% response rate (n = 201 responses) was achieved from the 3,849 courses contacted. Most responses were from courses currently affected by fairy ring and 62% of these claimed that fairy ring caused a problem on their course; most often by negatively impacting aesthetics, but sometimes by affecting play. Greenkeepers reported that disease symptoms were at their worst in July and August. Type-2 fairy ring was the most common and also occurred frequently alongside type-1. The majority of courses were affected by more than one type of fairy ring. Although no one type of fairy ring was significantly more severe than the other types, they did differ in the effect they have on the course. As expected, fairy ring is considered most serious when occurring on greens. A significantly higher proportion of links courses were affected by fairy ring that was considered problematic, although severity index did not vary by course type. By geographically mapping severity using respondents' postcodes and comparing to climate data, the southeast region, where it is generally warmer and drier, has been shown to have double the proportion of courses with problematic fairy ring than that of the northwest of Great Britain, suggesting a relationship between climate and severity.

3.2 Introduction

As the first major investigation of the fairy ring research project, the questionnaire stood as an opportunity to ask industry professionals about the disease on their course; to gain an understanding of greenkeepers' perceptions of fairy ring and in what circumstances its occurrence could prove problematic.

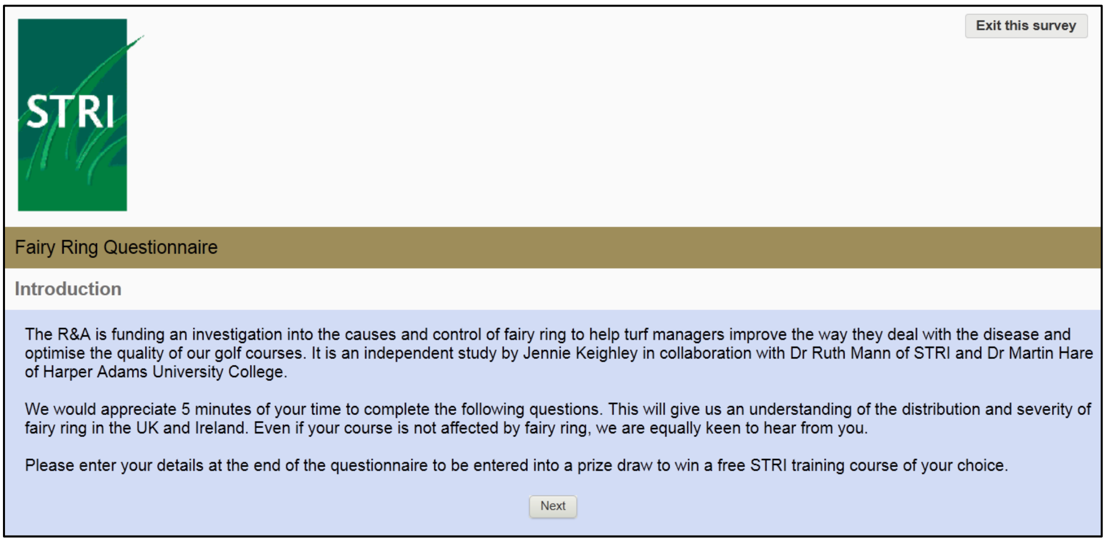
These questions aimed to gather an initial understanding of the severity of fairy ring and its temporal and spatial distribution across the golf course and across the UK and Ireland. Results promised to, not only give a much-needed review of the current status of fairy ring on golf courses, but also provide a data set with which to compare soil and climate records in the search for epidemiological relationships.

The other benefit of the questionnaire was to provide the foundation for subsequent stages of the project, by introducing the research outline to a wide audience and initiating contact with golf courses willing to participate in further research.

3.3 Methodology

A short, simple and concise questionnaire was developed, which aimed to maximise the number of responses. This consisted of ten closed questions (as shown in Figures 5 to 13), delivered in an online format, taking no more than five minutes for the respondent to complete. Care was taken to ensure that language and presentation were suited to the target audience of greenkeepers.

Fairy ring questionnaire



Fairy Ring Questionnaire

Introduction

The R&A is funding an investigation into the causes and control of fairy ring to help turf managers improve the way they deal with the disease and optimise the quality of our golf courses. It is an independent study by Jennie Keighley in collaboration with Dr Ruth Mann of STRI and Dr Martin Hare of Harper Adams University College.

We would appreciate 5 minutes of your time to complete the following questions. This will give us an understanding of the distribution and severity of fairy ring in the UK and Ireland. Even if your course is not affected by fairy ring, we are equally keen to hear from you.

Please enter your details at the end of the questionnaire to be entered into a prize draw to win a free STRI training course of your choice.

Figure 5: Screenshot of introductory page (page one of nine) from online Survey Monkey questionnaire

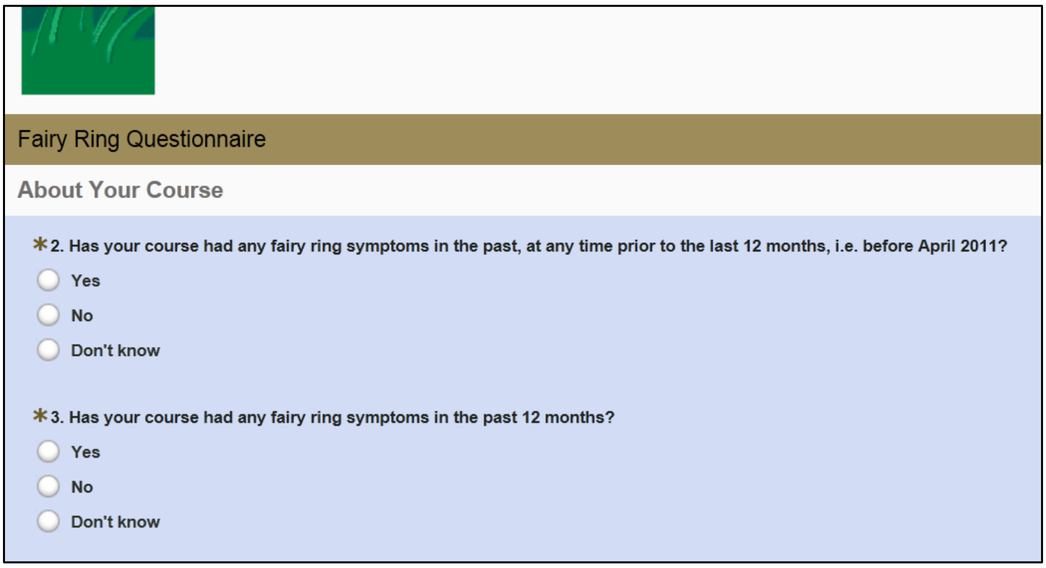
Fairy Ring Questionnaire

Course Location

✱1. Please enter the postcode or county in Ireland of your golf course.

Please note: this will only be used to map the geographical distribution of fairy ring.

Figure 6: Screenshot of Survey Monkey questionnaire (page two of nine).
Question 1: Please enter the postcode of your golf course



Fairy Ring Questionnaire

About Your Course

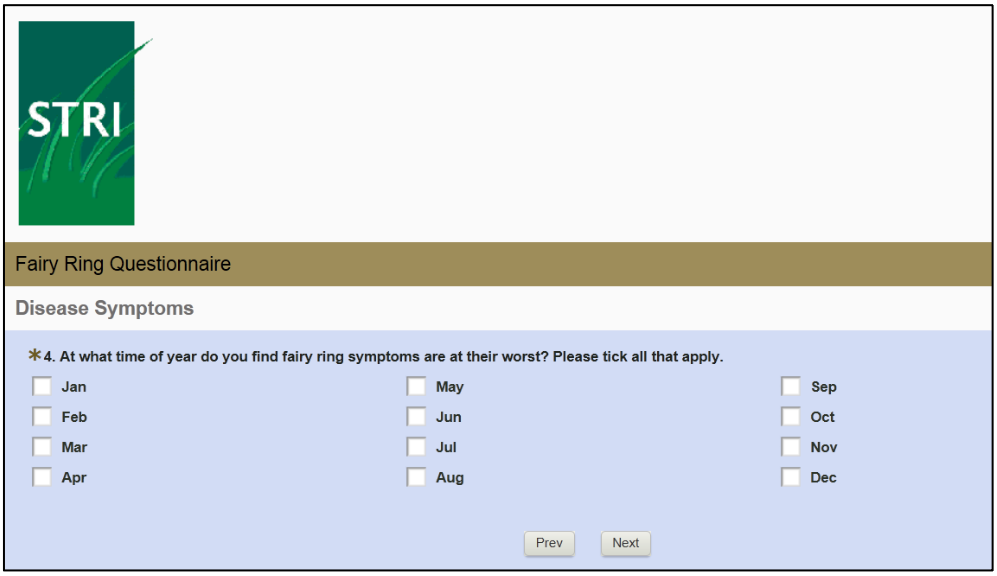
✱2. Has your course had any fairy ring symptoms in the past, at any time prior to the last 12 months, i.e. before April 2011?

○ Yes
○ No
○ Don't know

✱3. Has your course had any fairy ring symptoms in the past 12 months?

○ Yes
○ No
○ Don't know

Figure 7: Screenshot of Survey Monkey questionnaire (page three of nine).
Questions 2 and 3: Has your course had any fairy ring symptoms in the past, at any time prior to the last 12 months, i.e. before April 2011?
Has your course had any fairy ring symptoms in the past 12 months?



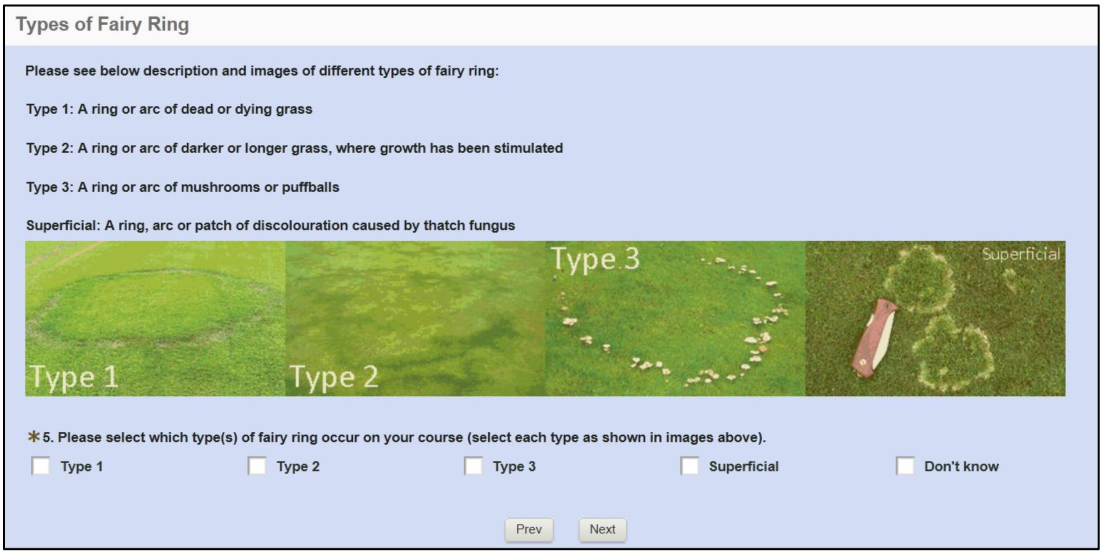
Fairy Ring Questionnaire

Disease Symptoms

*4. At what time of year do you find fairy ring symptoms are at their worst? Please tick all that apply.

☐ Jan
☐ Feb
☐ Mar
☐ Apr
☐ May
☐ Jun
☐ Jul
☐ Aug
☐ Sep
☐ Oct
☐ Nov
☐ Dec

Figure 8: Screenshot of Survey Monkey questionnaire (page four of nine). Question 4: At what time of year do you find fairy ring symptoms are at their worst? Please tick all that apply



Types of Fairy Ring

Please see below description and images of different types of fairy ring:

Type 1: A ring or arc of dead or dying grass

Type 2: A ring or arc of darker or longer grass, where growth has been stimulated

Type 3: A ring or arc of mushrooms or puffballs

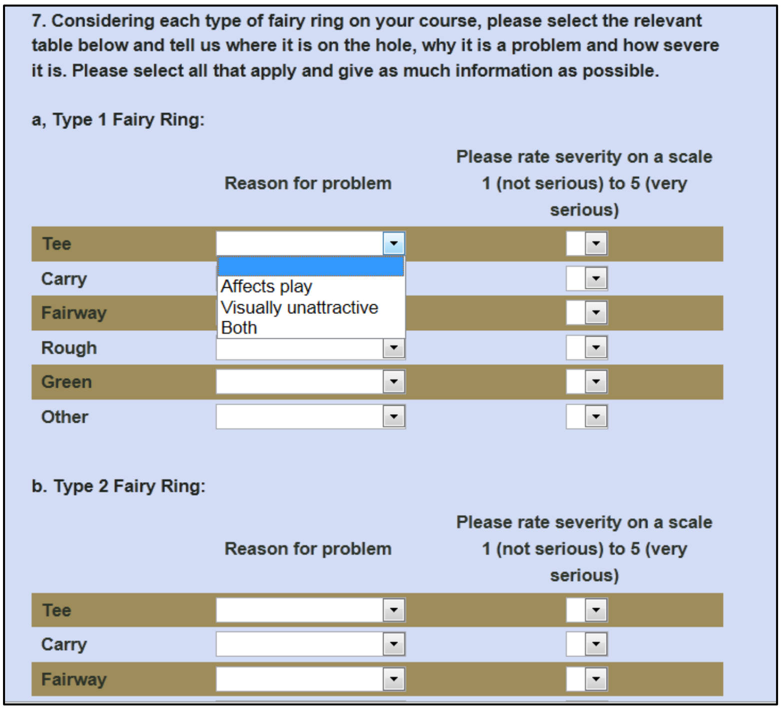
Superficial: A ring, arc or patch of discolouration caused by thatch fungus

*5. Please select which type(s) of fairy ring occur on your course (select each type as shown in images above).

☐ Type 1 ☐ Type 2 ☐ Type 3 ☐ Superficial ☐ Don't know

Figure 9: Screenshot of Survey Monkey questionnaire (page five of nine). Question 5: Please select which type(s) of fairy ring occur on your course (select each type as shown in images above).

Question 6 (not shown): Do any of these types cause a problem on your course? A fairy ring may be considered a problem if it affects game play or visual appearance of the course in a negative way.



7. Considering each type of fairy ring on your course, please select the relevant table below and tell us where it is on the hole, why it is a problem and how severe it is. Please select all that apply and give as much information as possible.

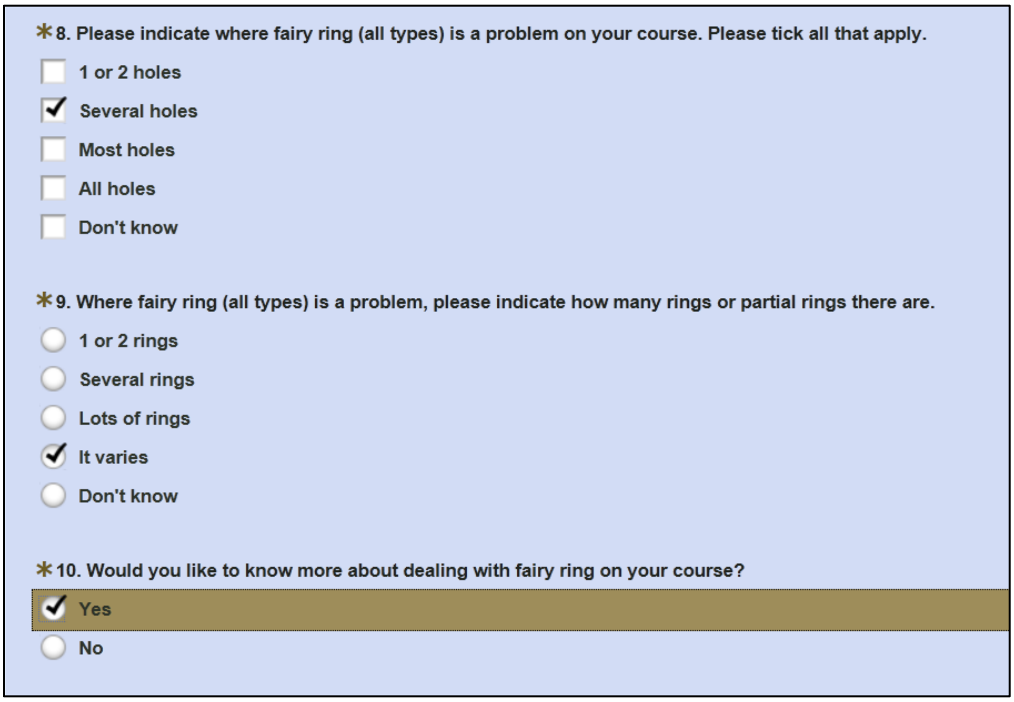
a, Type 1 Fairy Ring:

	Reason for problem	Please rate severity on a scale 1 (not serious) to 5 (very serious)
Tee		
Carry		
Fairway		
Rough		
Green		
Other		

b. Type 2 Fairy Ring:

	Reason for problem	Please rate severity on a scale 1 (not serious) to 5 (very serious)
Tee		
Carry		
Fairway		

Figure 10: Screenshot of Survey Monkey questionnaire (page six of nine). Question 7: Table with drop down boxes asking respondents to select where on the golf course each type of fairy ring was a problem and why, and to rate the severity of each occurrence from 1 (not serious) to 5 (very serious).



*8. Please indicate where fairy ring (all types) is a problem on your course. Please tick all that apply.

☐ 1 or 2 holes
☑ Several holes
☐ Most holes
☐ All holes
☐ Don't know

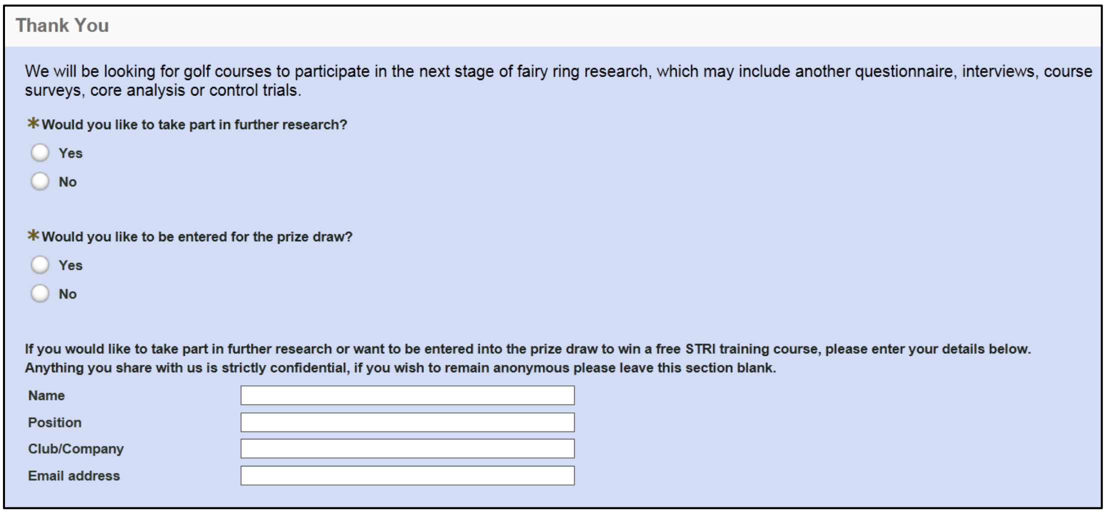
*9. Where fairy ring (all types) is a problem, please indicate how many rings or partial rings there are.

○ 1 or 2 rings
○ Several rings
○ Lots of rings
● It varies
○ Don't know

*10. Would you like to know more about dealing with fairy ring on your course?

● Yes
○ No

Figure 11: Screenshot of Survey Monkey questionnaire (page seven of nine). Questions 8, 9, and 10: Respondents asked how many golf holes affected, how many rings occur, and whether they would like to know more about dealing with fairy ring on their course



Thank You

We will be looking for golf courses to participate in the next stage of fairy ring research, which may include another questionnaire, interviews, course surveys, core analysis or control trials.

***Would you like to take part in further research?**

○ Yes
○ No

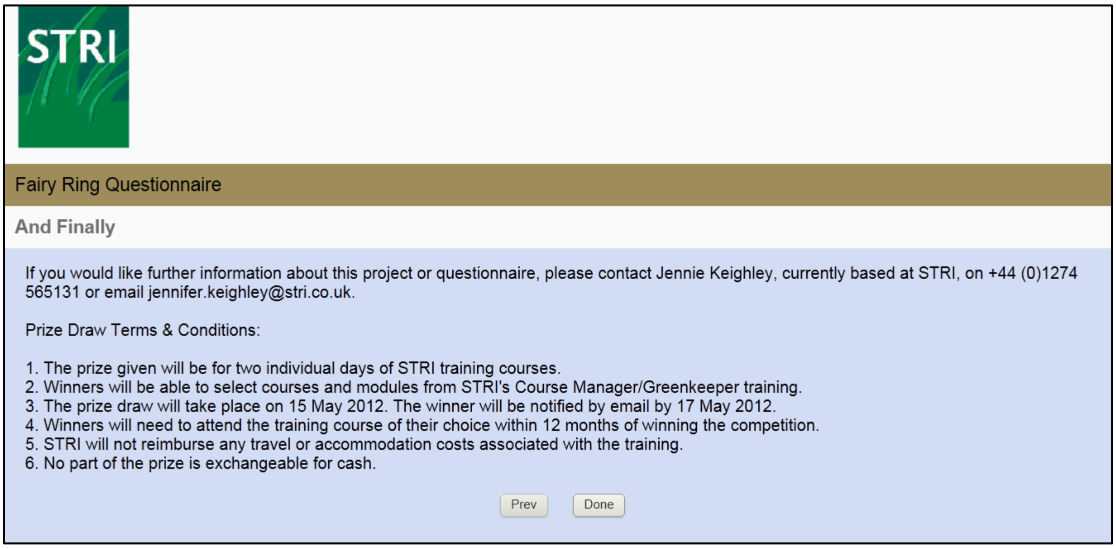
***Would you like to be entered for the prize draw?**

○ Yes
○ No

If you would like to take part in further research or want to be entered into the prize draw to win a free STRI training course, please enter your details below. Anything you share with us is strictly confidential, if you wish to remain anonymous please leave this section blank.

Name
Position
Club/Company
Email address

Figure 12: Screenshot of Survey Monkey questionnaire (page eight of nine). Respondents asked if they would like to participate in further research, whether they would like to be entered into the prize draw, and to leave their contact details if so.



Fairy Ring Questionnaire

And Finally

If you would like further information about this project or questionnaire, please contact Jennie Keighley, currently based at STRI, on +44 (0)1274 565131 or email jennifer.keighley@stri.co.uk.

Prize Draw Terms & Conditions:

1. The prize given will be for two individual days of STRI training courses.
2. Winners will be able to select courses and modules from STRI's Course Manager/Greenkeeper training.
3. The prize draw will take place on 15 May 2012. The winner will be notified by email by 17 May 2012.
4. Winners will need to attend the training course of their choice within 12 months of winning the competition.
5. STRI will not reimburse any travel or accommodation costs associated with the training.
6. No part of the prize is exchangeable for cash.

Figure 13: Screenshot of Survey Monkey questionnaire (page nine of nine). Contact details and prize draw terms and conditions.

To avoid any bias in selecting a sample of courses to survey, a census of every course in the UK and Ireland was conducted. This was made possible by using the free online survey software provided by SurveyMonkey® and STRI's comprehensive database of golf course email contacts. STRI's contact list comprised 3,849 recipients in the UK and Ireland. To ensure this was a realistic figure, it was compared to the number of courses listed by the European Golf Association (2012) and by Yell.com (searched 08/03/2012), which listed 2,991 (UK and Ireland) and

3,017 (UK only) golf courses, respectively. The STRI list contained somewhat more than this and was considered to be the most complete directory available.

Correspondence was marked for the attention of the Head Greenkeeper or Course Manager (generally the same role), in the expectation that they have the most proficient understanding of the turf and any associated management issues. A link to the online questionnaire was contained within a covering email, briefly explaining the nature of the study, and sent from STRI to the golf course contacts, which was the general course email address, the secretary (who would be expected to forward it on) or the course manager directly. As STRI confirmed that the vast majority of greenkeepers had internet access and were contactable via email, the questionnaire links were considered highly likely to have reached the desired recipients.

Following in-house testing by STRI agronomists, the online questionnaire went live on Thursday 5th April 2012, with the link being emailed to the courses at 1pm. This traditionally busy time proceeding Easter weekend would ordinarily have been undesirable but, as severe winter weather across the country saw many golf courses close, this was taken as an opportunity to increase response rates, whilst greenkeepers were more likely to be working inside.

As an incentive to reply, respondents could choose to enter a prize draw to win a complementary STRI training course of their choice, courtesy of the STRI's Sales and Marketing Department.

The questionnaire link stayed live for eight weeks, closing at 4pm on Friday 1st June 2012. Three reminder emails were sent within this time to encourage replies from those that had not already responded.

Data were downloaded from Survey Monkey into Microsoft Excel and then analysed statistically by chi-square tests, t-tests, Mann Whitney U-tests, and linear regression using IBM SPSS Statistics (version 19).

The data generated from the questionnaire responses were analysed geographically using Esri GIS software ArcMap (version 9.2) and Ordnance Survey map data downloaded from EDINA Digimap. During the mapping process, XY coordinates for the centre point of each postcode were found using the conversion tool: UK Grid Reference Finder (http://gridreferencefinder.com/#).

3.4 Results

3.4.1 Response rate

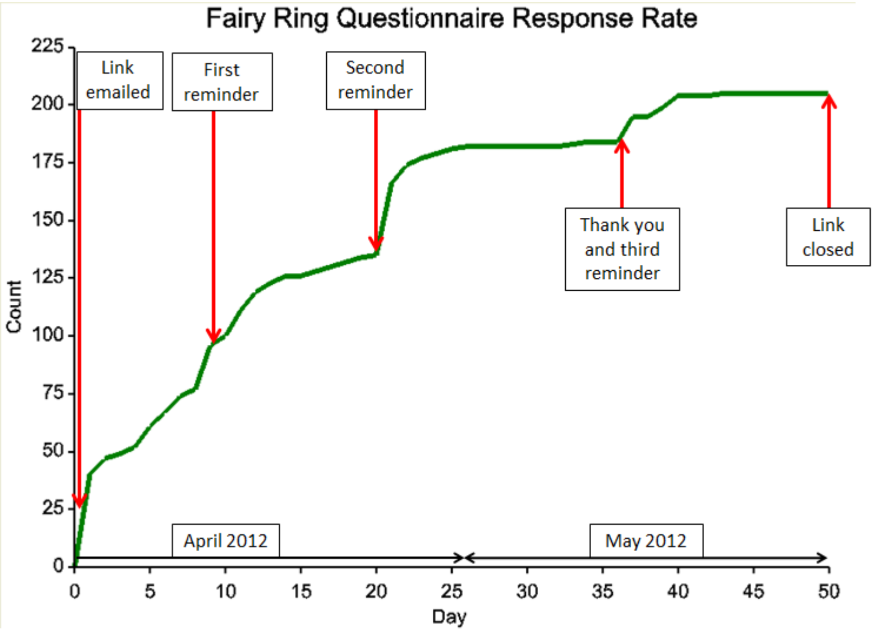


Figure 14: Responses accumulated during fairy ring online questionnaire duration

Figure 14 shows the number of responses accumulated over the duration that the online questionnaire was active. The second reminder prompted the most notable response, which, interestingly, was the only one sent on a Wednesday rather than a Friday.

From the 3,849 courses contacted, 201 responses were received; a 5% response rate. Of those that started the questionnaire, 79% continued to finish it, suggesting that the audience had no particular issues in answering any of the questions.

3.4.2 Distribution of respondents

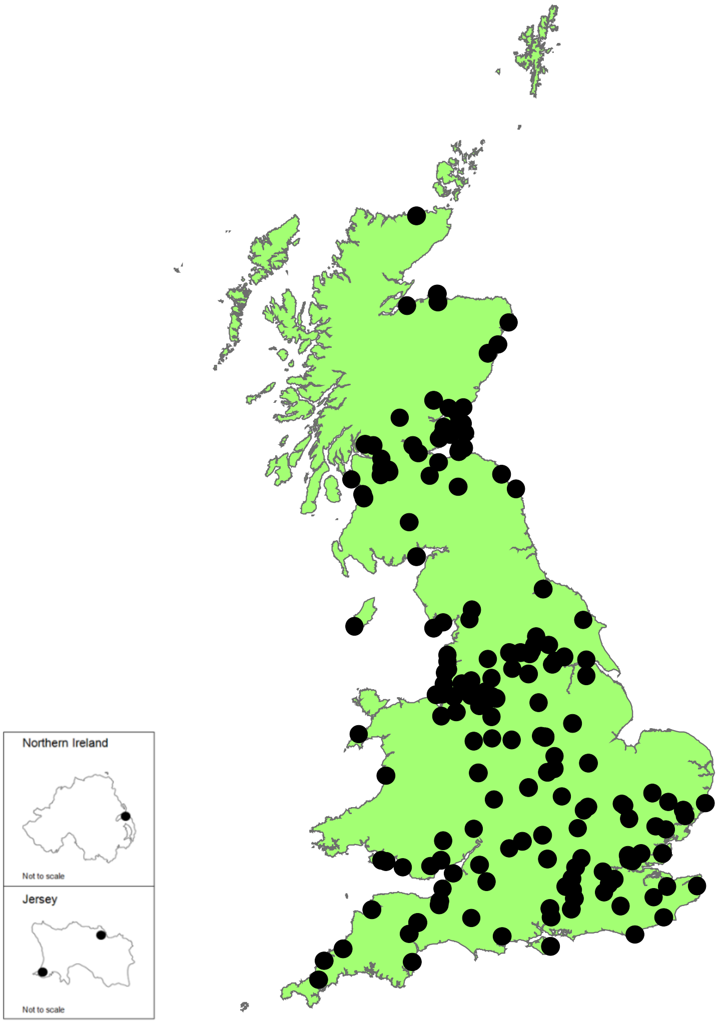


Figure 15: Distribution of UK golf courses that responded to the fairy ring questionnaire

The primary identifier for each response was post code. As the Republic of Ireland does not operate a postal code system, Irish courses were asked to provide their county instead. Figure 15 shows the distribution of the 176 valid post codes provided by respondents in Great Britain, Northern Ireland and Jersey. Republic of Ireland contributed 21 responses, but, as they can only be located by county, they cannot be accurately mapped.

Figure 15 shows a wide distribution of respondents across the UK, with clusters around the Wirral and East Scotland. The lack of golf courses on the rugged uplands of Wales, Northern England and the Scottish highlands is likely to account for the lack of response from these areas.

3.4.3 Fairy ring status

Asking respondents *when* their course has been affected by fairy ring aimed to identify any temporal trends by distinguishing which courses have had fairy ring in the past and which have it now (with ‘now’ being classed as the past 12 months, to encompass last year’s peak fungus season). This aimed to show whether the number of affected courses was increasing or decreasing. Results showed that 82% of courses had had fairy ring in the past, whereas 68% of courses had been affected more recently. Figure 16 shows the categorisation of courses according to fairy ring occurrence. Approximately one third of courses have either never suffered from fairy ring or have had it before, but not now. Only one course had acquired fairy ring in the past 12 months, having never had it before. If fairy ring was an increasing problem, we might expect there to be more than this. Whilst skewed due to the heightened response from courses currently affected by fairy ring, the data would suggest that fairy ring occurrence is not becoming more prominent.

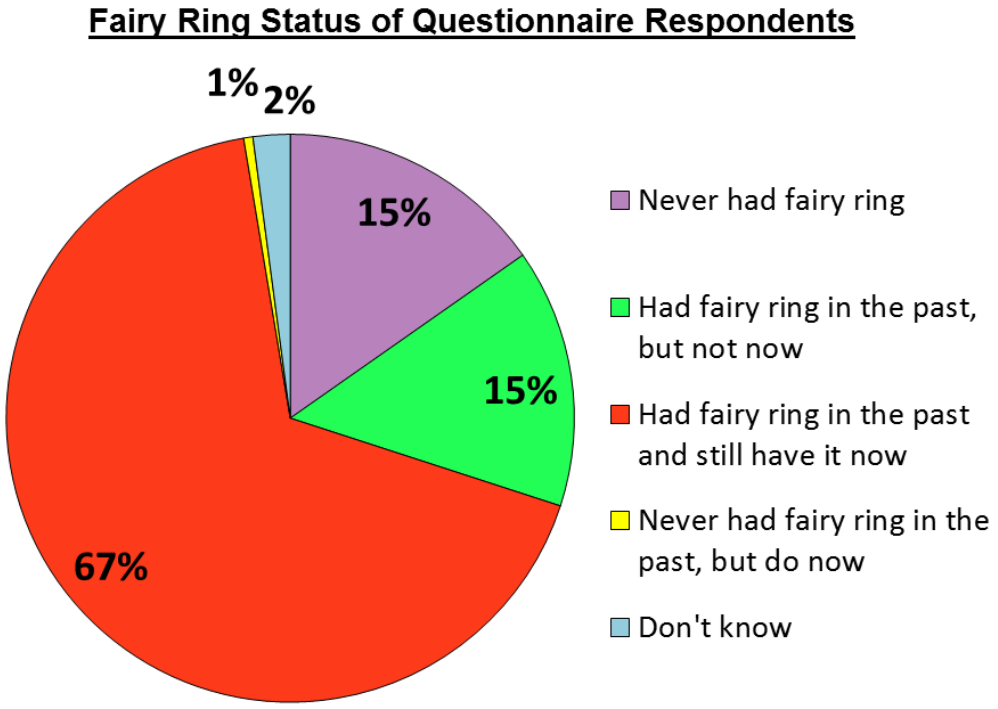


Figure 16: History of fairy ring incidence on the golf courses of the respondents (n = 190)

Only courses reporting that they had been affected by fairy ring in the past 12 months were allowed to proceed onto subsequent questions. Those that had not had fairy ring recently or that didn’t know were diverted to the end of the questionnaire.

3.4.4 Time of year

Figure 17 shows that greenkeepers reported fairy ring symptoms to be significantly worse in the summer months, particularly July and August (one sample t-test, t = 3.544, df = 11, $p = 0.005$). Respondents could select as many months as applied.

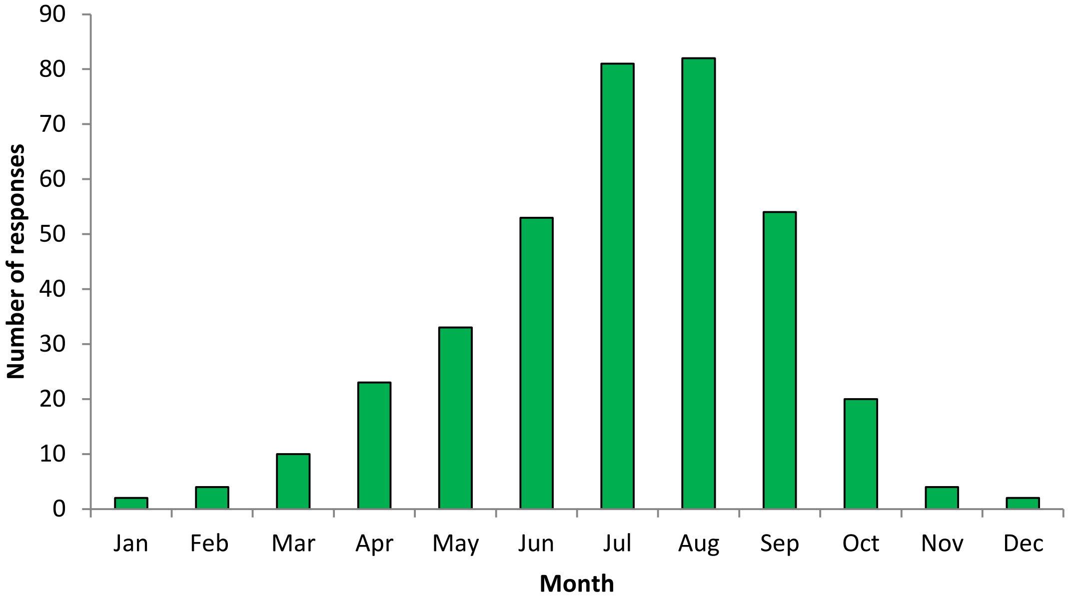


Figure 17: Time of year when fairy ring symptoms were at their worst, according to questionnaire respondents (n = 368; multiple months could be selected)

3.4.5 Fairy ring types

Next, respondents were shown photographs, with descriptions, of the four types of fairy ring and asked to select any which were present on their course. Figure 18 shows how frequently each type was selected. Overall, type-2 was the most commonly selected (one sample t-test, t = 34.717, df = 252, p = <0.001), with 90% of respondents reporting that it was present on their course. This was followed by type-1 – 55% of respondents; type-3 – 33%; and superficial – 30%. Respondents were allowed to select as many types as applied. Figure 19 shows the breakdown of combinations selected. The most respondents said that *only* type-2 was present on their course; followed by combinations ‘types-1 & -2’, ‘types-1, -2, -3 and superficial fairy ring’ and ‘types-1, -2, & -3’ (one sample t-test, t = 3.079, df = 14, p = 0.008).

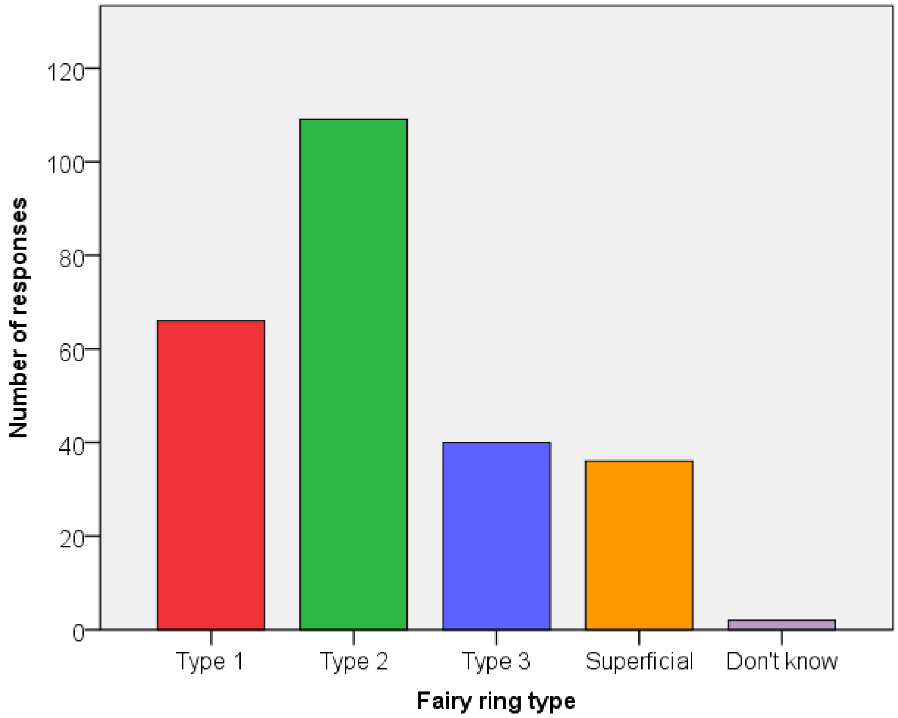


Figure 18: Frequency of fairy ring types on golf courses
(n = 253; multiple types could be selected)

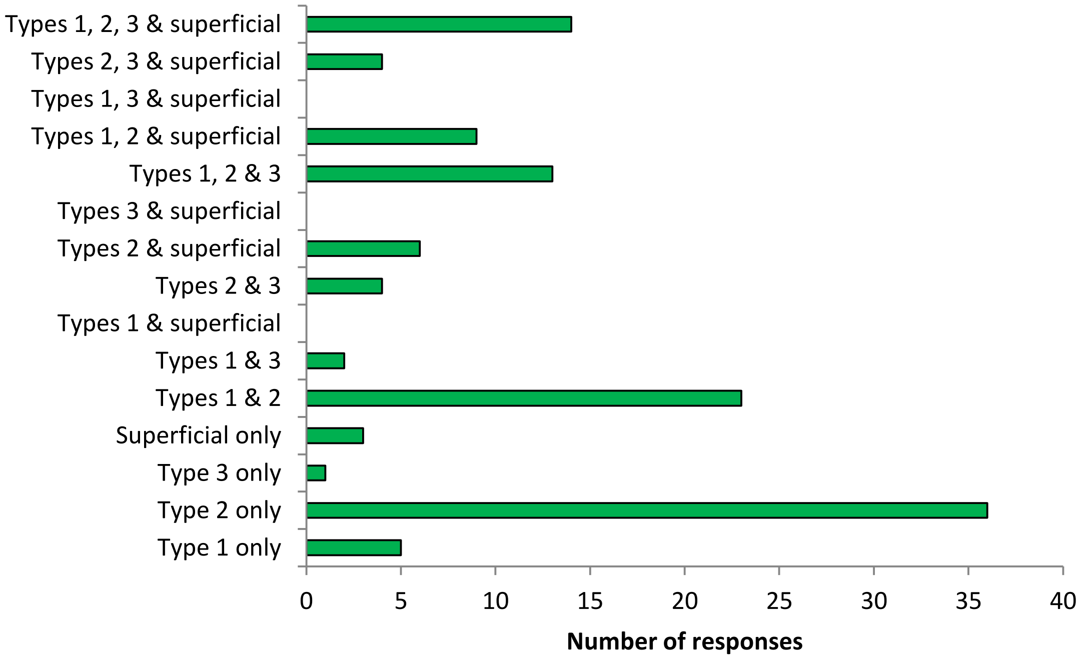


Figure 19: Combinations of fairy ring types present on golf courses

3.4.6 Time of year and type

To avoid complexity in the questionnaire, respondents were not asked to specify if the symptoms of different types were worse at different times of year. It was anticipated that this could be identified from analysing the data set as a whole, but too few responses were received from courses affected solely by types-1, -3 and superficial for valid comparisons to be made. However, the configuration of the four type combination categories with the highest responses allowed for some examination of time of year according to fairy ring type.

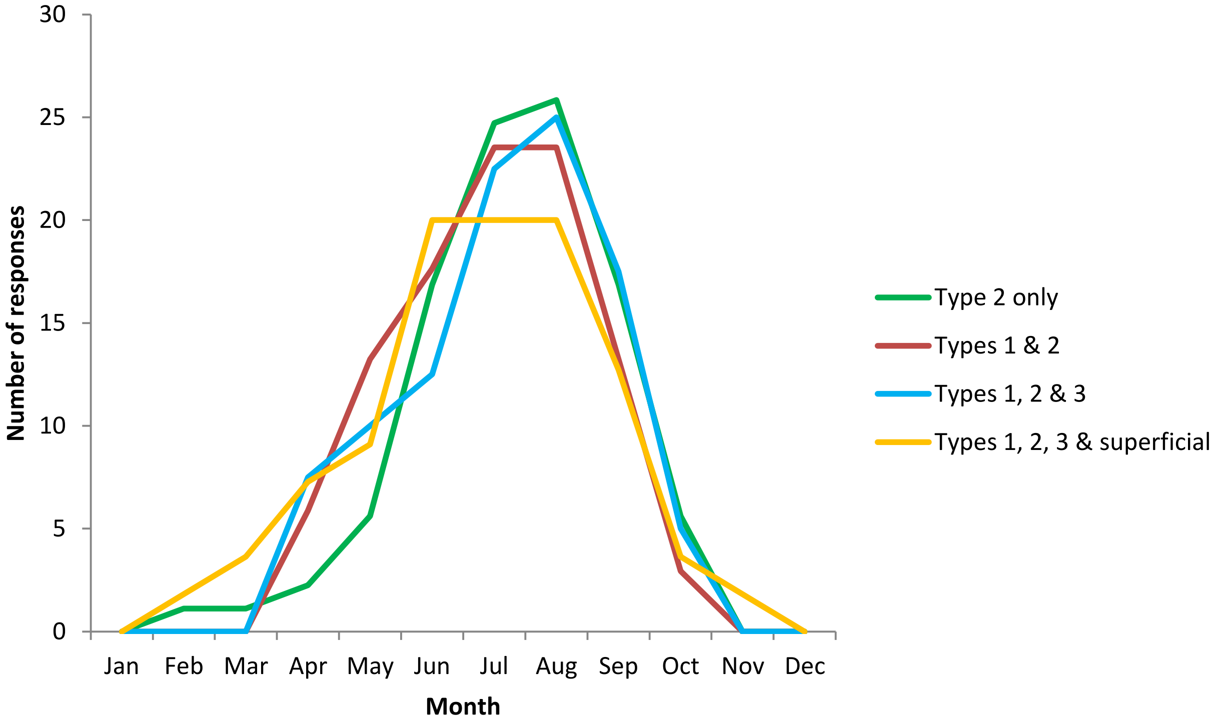


Figure 20: Time of year fairy ring symptoms at their worst for varying type combinations

Categories in Figure 20 range from one type to all four types of fairy ring. Differences in the distribution between categories may represent a distinct characteristic expressed by the type not present in the previous category. Here, we see little variation in the time of year different type combinations are displaying the worst symptoms. The graph suggests that superficial fairy ring may persist for a greater proportion of the year and that type-2 may flare up slightly later, as combinations with additional types have higher responses during April and May than type-2 alone. However, these observations are not supported by the statistics, as ANOVA confirms that there is no significant difference between groups (F <0.001, df = 3, *p* = 1.000).

3.4.7 Is fairy ring a problem?

When asked if any of the fairy ring types caused a problem on their course, 62% of respondents claimed that it does cause a problem by negatively affecting game play or visual appearance. Type-2 fairy ring appears less problematic than the all-types average, with only 44% considering it a problem. Data were too few for courses affected solely by type-1, -3 or superficial to draw any firm conclusions on the other types at this stage.

Table 2 shows that, the more different types of fairy ring present on a course, the more it is considered problematic.

Table 2: Percentage of respondents considering fairy ring a problem

Fairy ring types present	**Problem?**	
	Yes	**No**
Type 2 only	44%	56%
Types 1 & 2	68%	32%
Types 1, 2 & 3	77%	23%
Types 1, 2, 3 & superficial	93%	7%

3.4.8 Problematic fairy ring

Only respondents that considered fairy ring to be a problem were diverted to the remaining questions, which aimed to explore this further. They were asked: on which part(s) of the golf hole each type of fairy ring is a problem, the reason for it being a problem, and how severe it is on a scale on 1-5; with 1 being ‘not serious’ and 5 being ‘very serious’.

Firstly, Figures 21 to 25 show why respondents found fairy ring to be a problem, by type and then on the various parts of the golf hole. For each affected part of their course, respondents could choose from: ‘visually unattractive’, ‘affects play’, or ‘both’. We see that fairy ring is more commonly found to be visually unattractive than affecting play (X^2 = 120.554, df = 1, p <0.001), with the only exception being type-1 on greens. Reason does vary significantly between types (X^2 = 12.909, df = 3, p = 0.005). Type-2, in particular, is predominantly problematic due to aesthetics; whereas type-1 has the worst effect on play overall. Reason also varies according to part of the hole (X^2 = 30.723, df = 4, p <0.001); with effect on play mainly being an issue on greens and visual impact important on greens and fairways. Superficial fairy ring only affects play on the green. Interestingly, type-1 was found to be more problematic on the fairway than on the green. This may show that symptoms are worse where the turf is less intensively managed and may be worth investigating further. Another point to note is the highest value for type-3; showing that greenkeepers find mushrooms/puffballs unsightly, even when occurring in the rough.

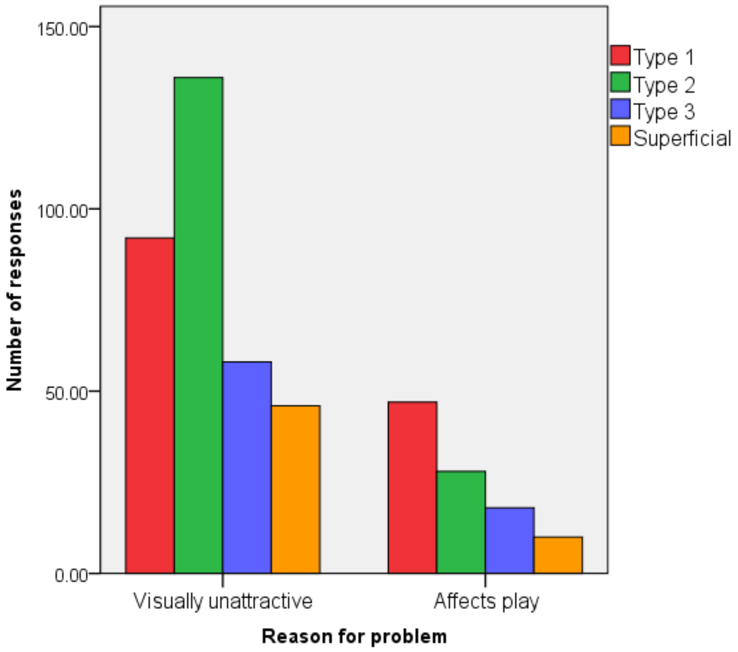


Figure 21: Reason respondents consider fairy ring to be problematic (n = 389)

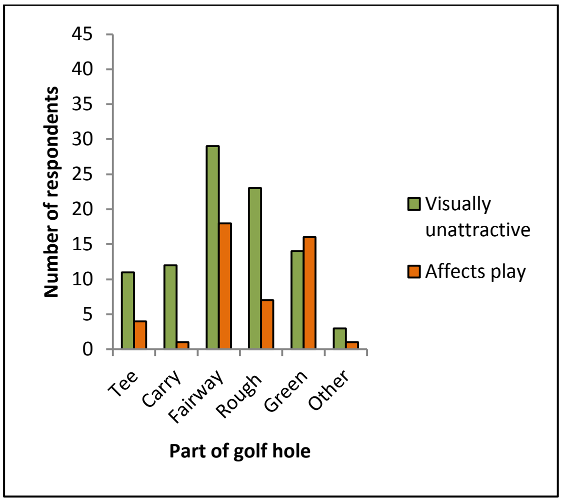


Figure 22: Reason type-1 fairy ring is considered problematic

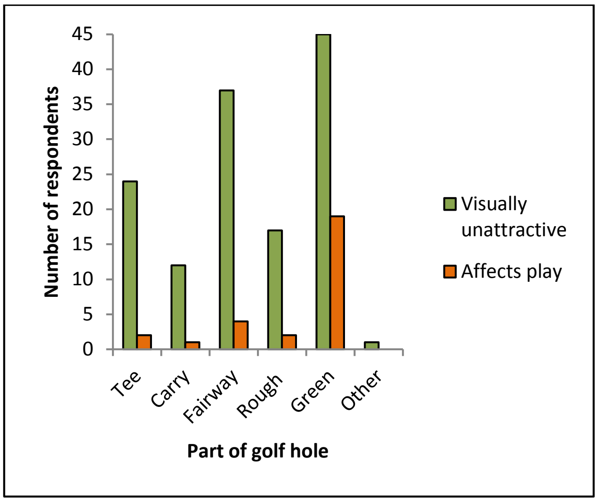


Figure 23: Reason type-2 fairy ring is considered problematic

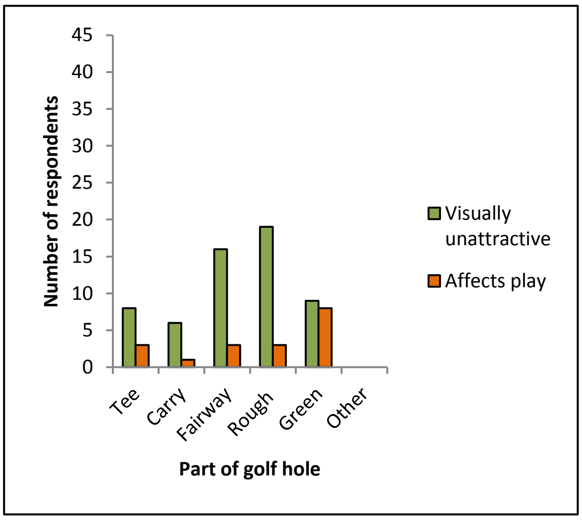


Figure 24: Reason type-3 fairy ring is considered problematic

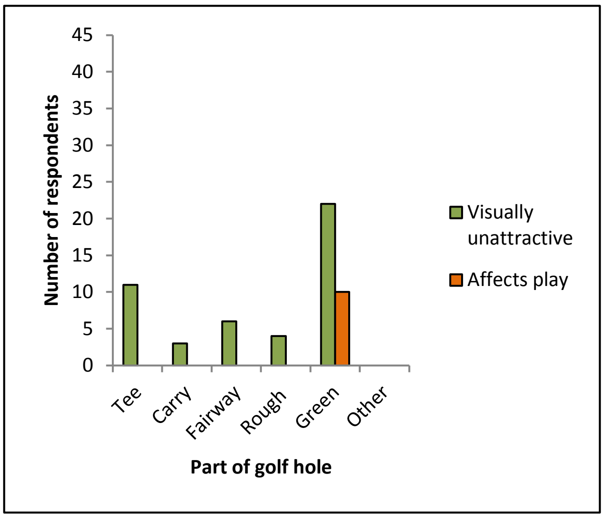


Figure 25: Reason superficial fairy ring is considered problematic

Respondents were asked to assign a severity score (1-5) to each occurrence of fairy ring. As Table 3 shows, the negative impact of fairy ring is most commonly solely visual, the severity of which is considered less serious (severity score = 1.83, SD 0.06) than when it affects play (severity score = 3.09, SD 0.19) or both of the above (severity score = 3.52, SD 0.12). However, means cannot be compared statistically as severity scores are not continuous data, and this difference is not reinforced by chi-square analysis ($X^2 = 18.427$, df = 16, $p = 0.299$; $X^2 = 6.410$, df = 16, $p = 0.983$; $X^2 = 16.452$, df = 16, $p = 0.422$).

Table 3: Severity of reasons fairy ring is problematic

	Visually unattractive	**Affects play**	**Both**
No. of responses	244	22	83
Mean severity score	1.83 (SD 0.06)	3.09 (SD 0.19)	3.52 (SD 0.12)
Median severity score	2	3	3

The scores were then used to compare the severity of the different types of fairy ring on the different parts of a golf hole. Although Figure 26 shows that type-1 has the most severe impact on the course, the difference in severity between fairy ring types, is not statistically significant, albeit marginal ($X^2 = 20.874$, df = 12, $p = 0.052$).

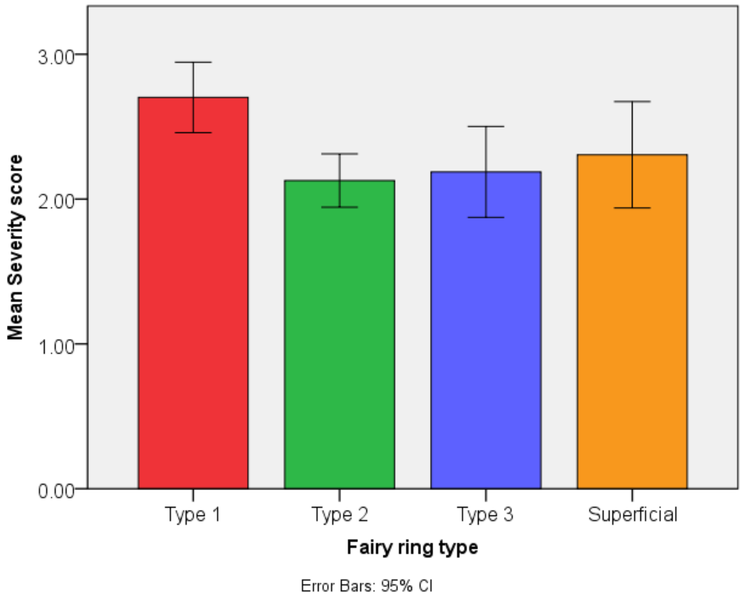


Figure 26: Mean severity score for each fairy ring type (error bars represent SEM)

As seen in Figure 27, fairy ring severity is perceived as significantly more serious when occurring on greens ($X^2 = 107.116$, df = 20, $p < 0.001$). The error bar showing low confidence in the ‘other’ category is representative of low sample size and variable severity scores of those with fairy ring on ‘other’ parts of the golf course, such as practice areas. The ‘other’ category was removed from all statistical tests so that it did not produce misleading results when compared to definitive parts of the hole.

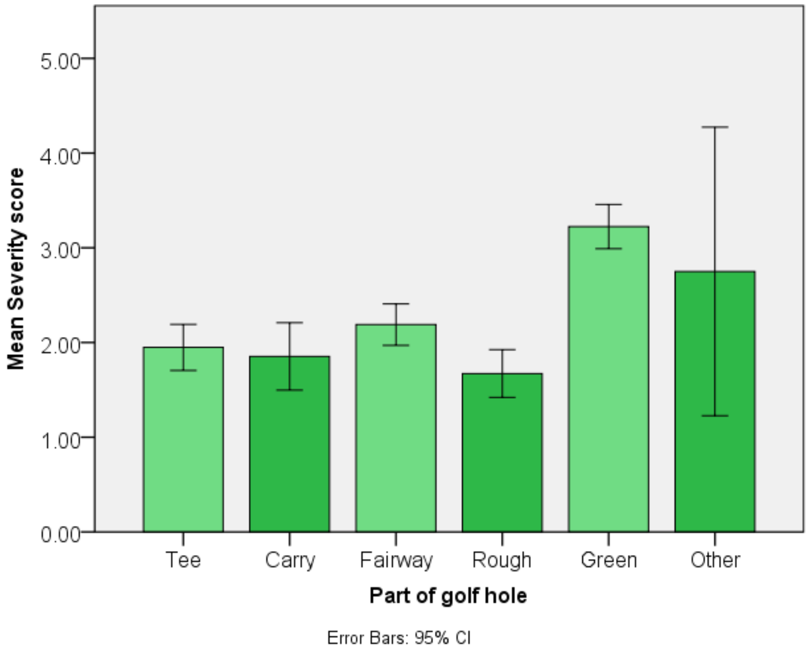


Figure 27: Mean severity score for each part of the golf hole
(error bars represent SEM)

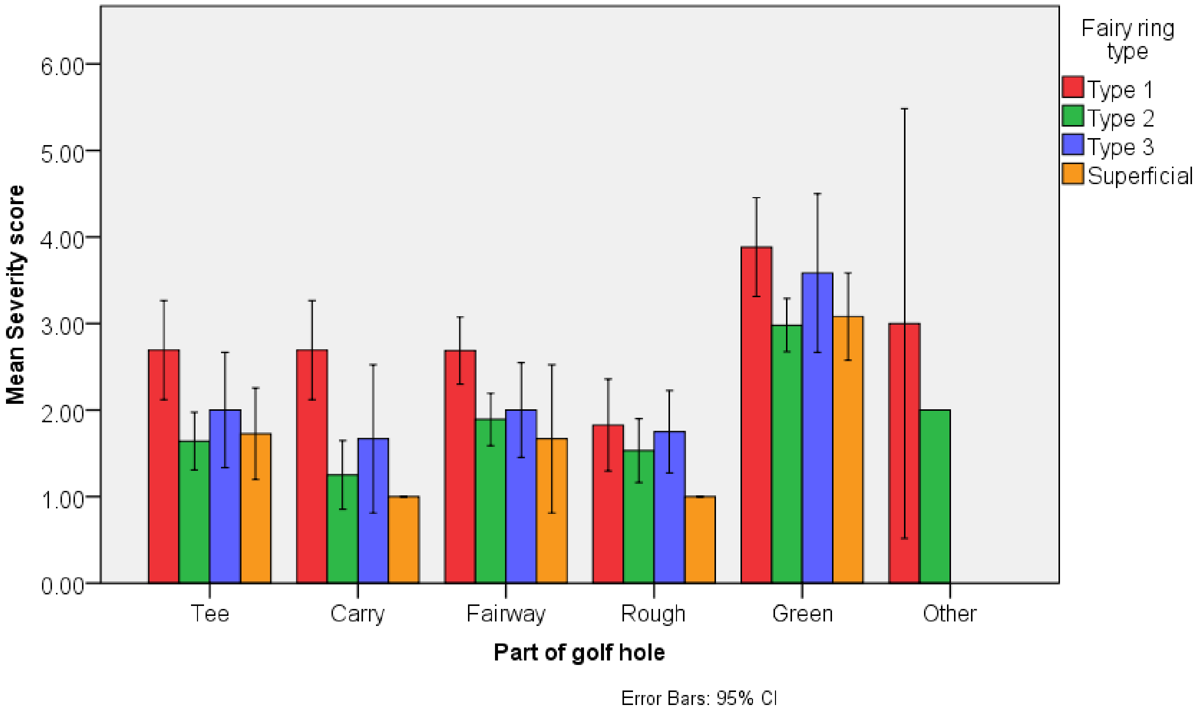


Figure 28: Mean severity score for each type of fairy ring on each part of the golf hole (error bars represent SEM)

As Figure 28 shows, severity of the different types of fairy ring follows a similar pattern for each part of the hole, with every type being most serious when occurring on greens. Greenkeepers consider type-1 fairy ring to be the most serious problem on every part of the golf hole. This is followed by type-3; the appearance of mushrooms or puffballs. Superficial fairy ring causes the least problems on the carry, fairway and rough, but is considered more serious than type-2 where it occurs on the more intensively managed areas of greens and tees.

Of the respondents that reported having problematic fairy ring, 91% said they would like to know more about dealing with fairy ring on their course. This suggests that there is a lack of confidence and/or success in managing the disease on golf courses at present.

3.4.9 Number of holes and rings

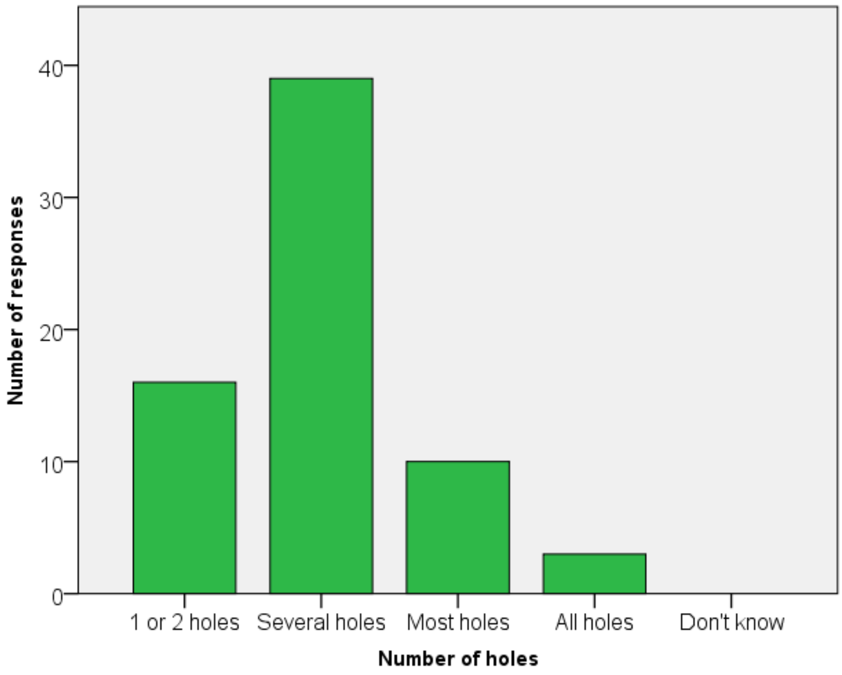


Figure 29: Proportion of holes on the golf course affected by fairy ring

As seen in Figure 29, most courses with problematic fairy ring reported that several holes were affected (one sample chi square test: $p < 0.001$). Categories could not state specific numbers of holes here, as golf courses responding could have had different numbers of holes; anywhere from 9 to 54.

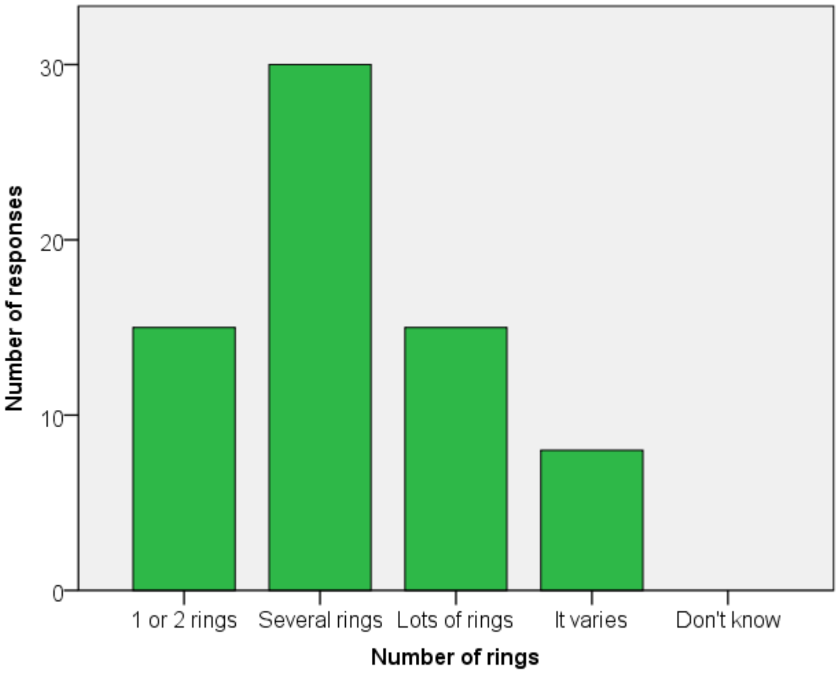


Figure 30: Number of fairy rings present in each affected area (n = 68)

A similar pattern is seen with the number of fairy rings in each affected area (Figure 30), where most courses have said ‘several rings’ (one sample chi square test: *p* = 0.002).

Table 4: Comparison of questionnaire responses – number of holes against number of rings

Number of holes	Number of fairy rings			
	1 or 2 rings	**Several fairy rings**	**Lots of fairy rings**	**It varies**
1 or 2 holes	8	8	0	0
Several holes	7	20	8	4
Most holes	0	2	4	4
All holes	0	0	3	0

Chi-square contingency table analysis shows a significant difference when comparing the number of holes affected on each course with the number of rings present in each affected area (X^2 = 34.430, df = 9, *p* <0.001). Over one quarter of respondents had several fairy rings on several holes. As Table 4 shows, courses with only 1 or 2 holes affected only had 1 or 2 to several rings in each case, rather than lots of rings. Courses with most or all holes affected were more likely to suffer from lots of rings.

3.4.10 Severity Index

Obtaining a measure of severity with which to perform analyses can be difficult, especially when data is gathered from a subjective research method like a questionnaire. In the respect of turf disease, the term ‘severity’ can encompass numerous factors: -

- Size of the rings
- Frequency of rings
- Type or turf condition
- Turf colour
- Persistence
- Difficulty of treatment
- Expense of treatment
- Effect on ball roll

Asking about these aspects in detail at this stage was expected to inhibit the questionnaire response rate. So, rather than assigning units (such as area covered in m^2), severity was defined as the greenkeeper’s opinion of the seriousness of fairy ring as a problem which negatively impacts play and/or aesthetics of the course. This way, the severity score (a 1-5 Likert scale from ‘not serious’ to ‘very serious’) could encompass as many factors as the respondent felt were important.

With the questionnaire completed, next, a measure was needed that amalgamated all severity-related responses given by each respondent into one value for each course; a severity index (SI). To develop a SI for each course, the severity scores given in Question 7 for each type of fairy ring present on each part of the golf hole were first pooled. A course suffering from all four types of fairy ring on every part of the golf hole (tee, carry, fairway, rough, green and other), scoring severity in each case as 5 for ‘very serious’, would achieve the maximum possible total severity score (TSS) of 120 (4x6x5).

Answers given to Question 8, indicating the proportion of holes on the golf course that were affected, were then incorporated by adding the following assigned category values to the TSS: -

- ‘1 or 2 holes’ = 10
- ‘Several holes’ = 40
- ‘Most holes’ = 70
- ‘All holes’ = 100

These values were chosen due to their even distribution and they added enough value to the TSS to be statistically viable. The new TSS was then converted into SI by dividing by 220, the maximum possible score, and multiplying by 100.

$$\frac{\text{TSS}}{220} \quad \text{X } 100 = \text{SI}$$

The answers to Question 9 – the number of rings in each problematic area – could not be included due to the ‘it varies’ option, which could not be assigned a value and would, therefore, invalidate the other responses.

SI could be calculated for 68 courses. This created a new variable with which to carry out comparative analysis and also, when used alongside corresponding postcodes, could be used to map the geographic distribution of fairy ring severity in the UK.

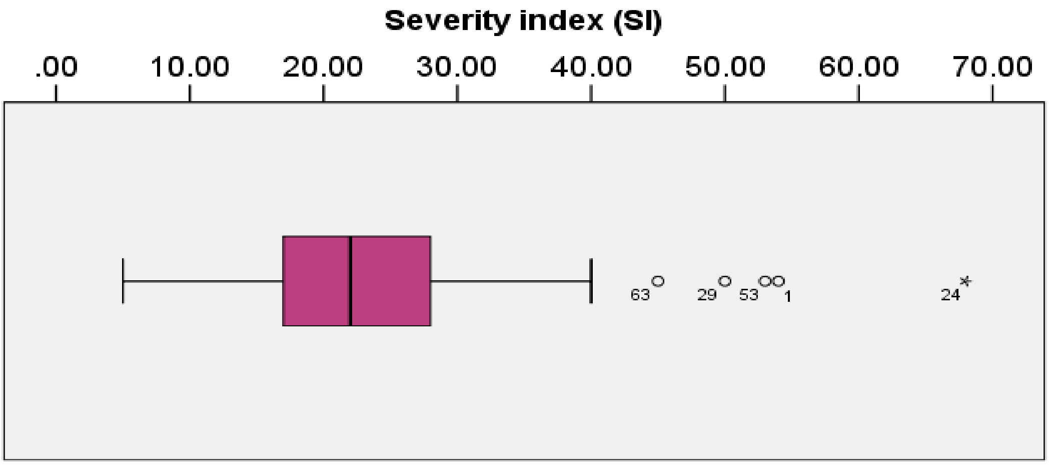


Figure 31: Distribution of fairy ring severity indices for the 68 golf courses

Results ranged from 5-68 SI, with a mean of 23.57 (SD 1.58). As seen in Figure 31, the SI data set is not normally distributed (Kolmogorov-Smirnov $p = 0.005$) and the highest value of 68 SI sits as a particularly high outlier, unfitting with the rest of the data set. This shows that there are five golf courses with an unusually high fairy ring severity.

3.4.11 Mapping severity

In order to map fairy ring severity, SI values had to be split into low (0-19 SI), medium (20-39 SI) and high (40+ SI) categories and assigned various colours. When assigned categories, these courses with problematic fairy ring were compared to the number of those that had never had fairy ring, used to have fairy ring and have non-problematic fairy ring. The difference is statistically significant (one sample chi-square: $p < 0.001$), with most courses having non-problematic fairy ring and very few courses having high severity fairy ring (Figure 32).

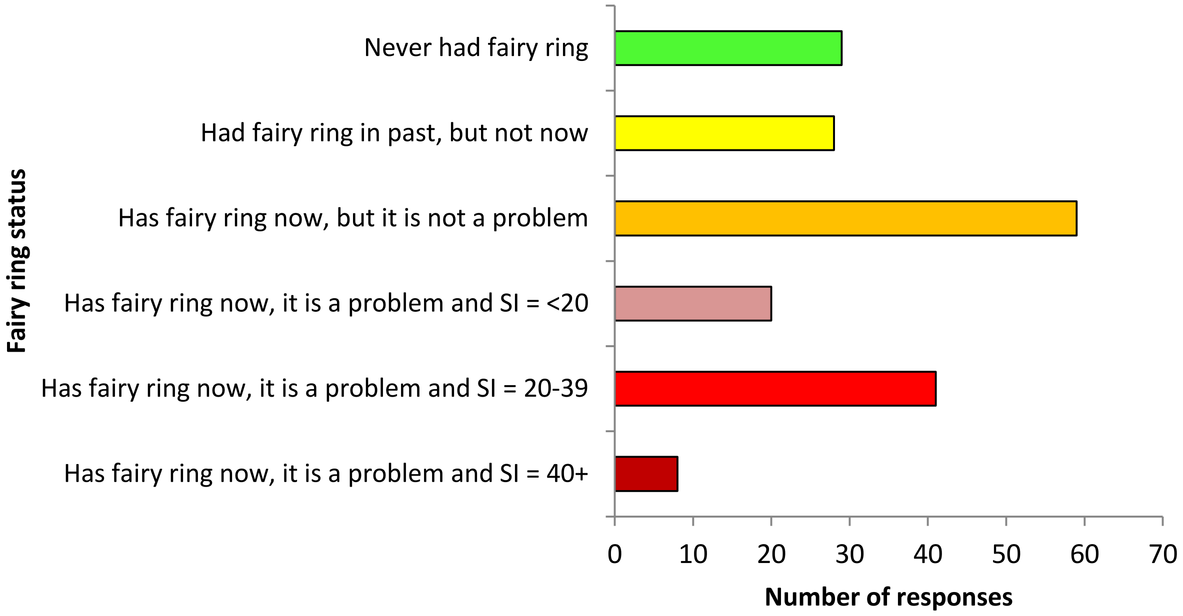


Figure 32: Comparison of fairy ring status of all courses (n = 185)

In Figure 33, the map of Great Britain has been divided into 10 km^2 squares and, using the fairy ring questionnaire respondents' post codes, the average fairy ring status of courses within each square has determined its colour, as indicated in Figure 19. No incidences of conflicting status within the same square occurred at this scale. This was done for Great Britain rather than the UK due to limitations in availability of map data for use in GIS and to protect the identity of courses from smaller areas that may be easily identified. As aforementioned, Irish courses could not be mapped as there is no postcode system there.

On inspection of Figure 33, there appears to be no link between fairy ring status and geographical location, as the categories are largely intermingled. Courses with high severity occur both inland and on the coast; north, south, east and west.

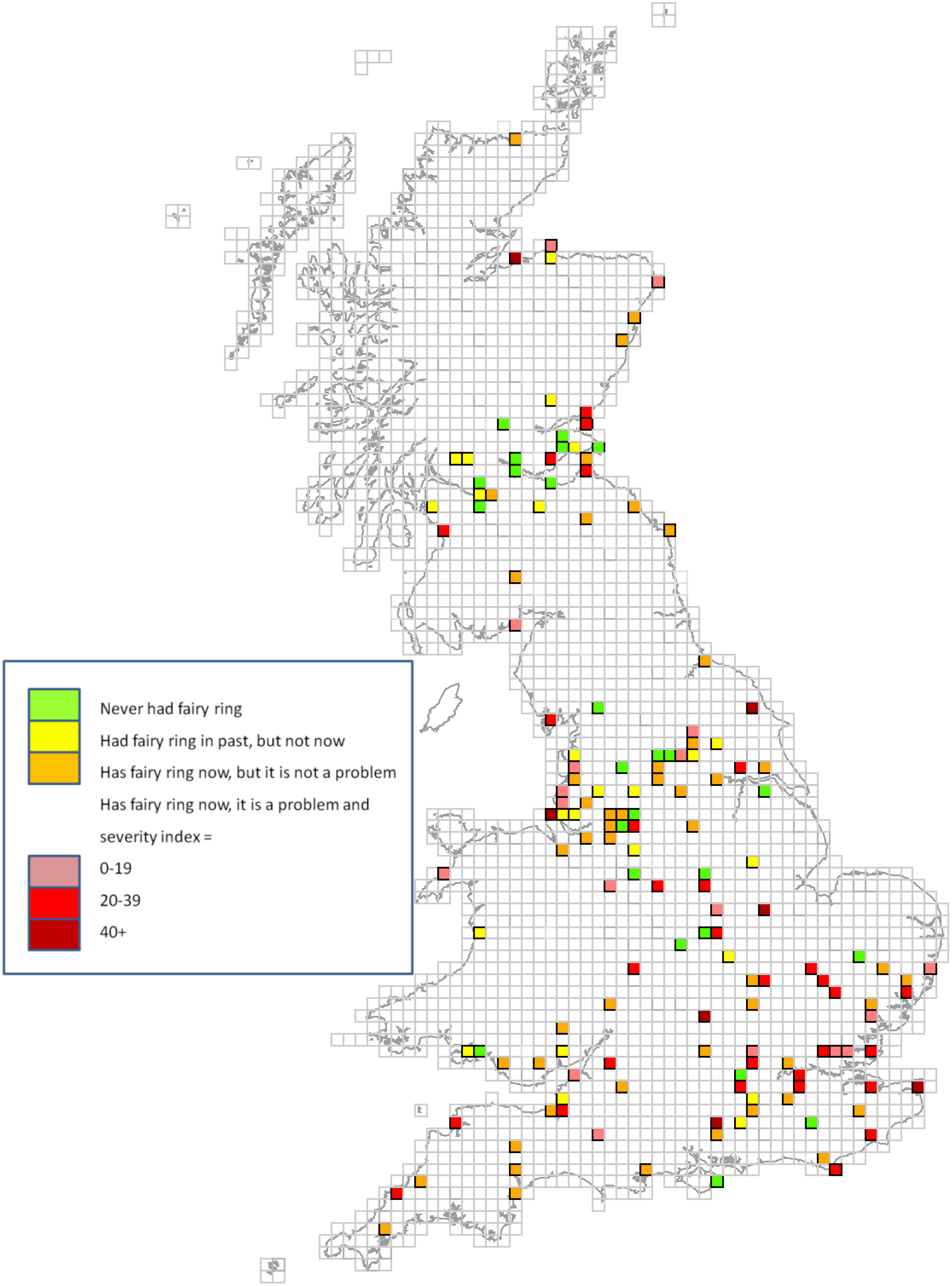


Figure 33: Fairy ring severity and distribution in Great Britain

3.4.12 Climate

The severity map in Figure 33 presents the first step in analysing the potential impact of climatic effects on fairy ring incidence and severity.

Met Office maps (http://www.metoffice.gov.uk/climate/uk/anomacts/) show how the climate in Great Britain varies between the southeast and northwest; with the southeast being hotter and drier (example shown by rainfall data in Figure 34). This contrast was applied to the severity map using a line of best fit (Figure 34) and the proportions of coloured cells in each section were compared.

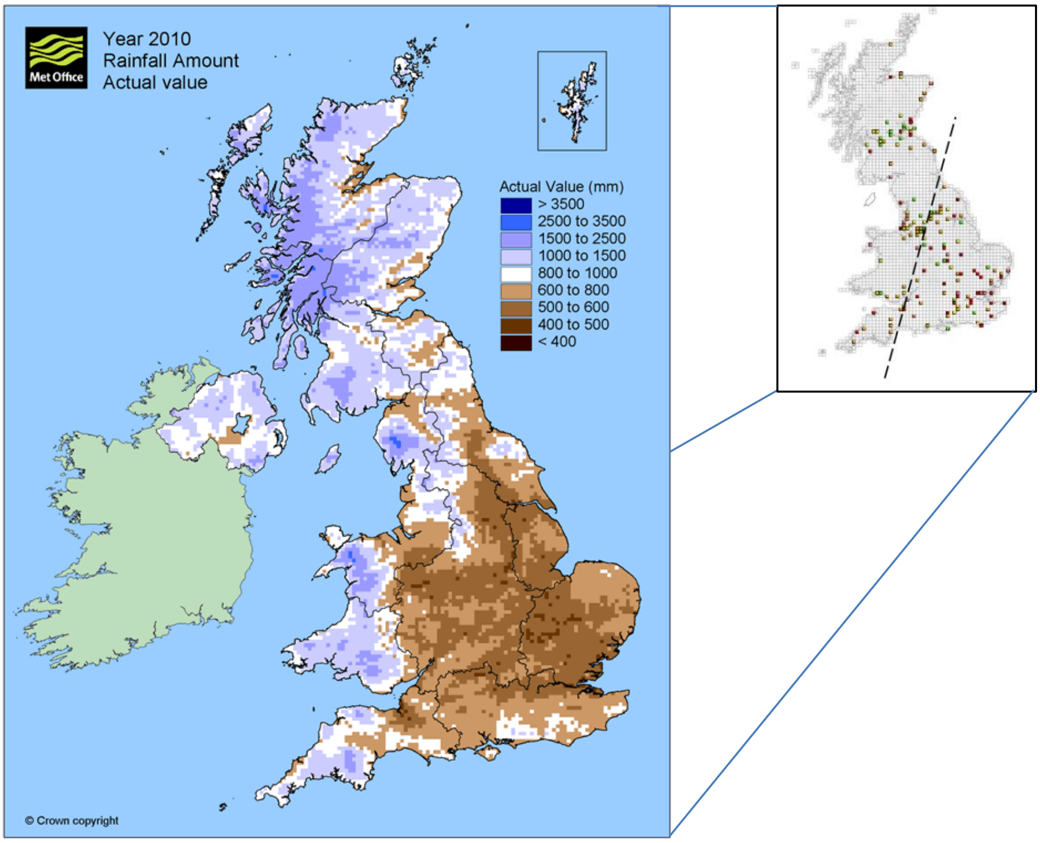


Figure 34: Met Office map showing actual annual UK rainfall for 2010 (reproduced with permission) provides a good example of the UK NW-SE split seen in many of the climate maps, including: temperature, days of rain, and sunshine. Inset shows the way this was used to divide the severity map.

Figure 35: Comparison of fairy ring status of golf courses in northwest and southeast Great Britain showing the southeast has a greater proportion of courses with mid- and high-level SI

The north-west and south-east sections contained coloured cells of similar sample size: 74 and 78, respectively. Composition of the fairy ring status categories varies significantly between the pair ($X^2 = 169.830$, df = 25, $p < 0.001$). As Figure 35 shows, the yellow slice for the north-west, representing courses that used to have fairy ring, but do not anymore, is double that of its southeast counterpart. This may indicate that courses in the northwest have greater success in treating fairy ring or that the disease is less persistent. Also, the proportion of courses suffering from mid- (20-39 SI) and high-level (40+ SI) SI in the south-east are twice that of the northwest; showing the south-east is significantly more problematic. Fairy ring could be exacerbated by low rainfall or periods of drought in the south-east, especially type-1s, where successful treatment seems dependent on re-wetting of the rootzone.

To further explore climate, the 20 most northerly courses with fairy ring were compared to the 20 most southerly. There were no significant differences between the north and south UK in time of year (Mann-Whitney U test $p = 0.657$) or fairy ring type (Mann-Whitney U test $p = 0.309$).

This early analysis suggests that aspects of climate may be correlated with fairy ring incidence and severity.

3.4.13 Course type

Golf courses can be categorised by type, which provides information about the soil and environment in which they reside. Respondents that provided their course's name or post code on the questionnaire were searched online using Google to find the course's website and determine, from reading course information and looking at photographs, what type of course it was. Courses were all either: links, parkland, meadowland, heathland or moorland. Although heathland and moorland are each distinct habitats, their similarities are such that, for the purpose of this investigation, they are categorised together as the golf course type 'heathland'. The same can be said for meadowland courses, which have been pooled with 'parkland'. The variable 'course type' was then used to look for relationships with the existing questionnaire data.

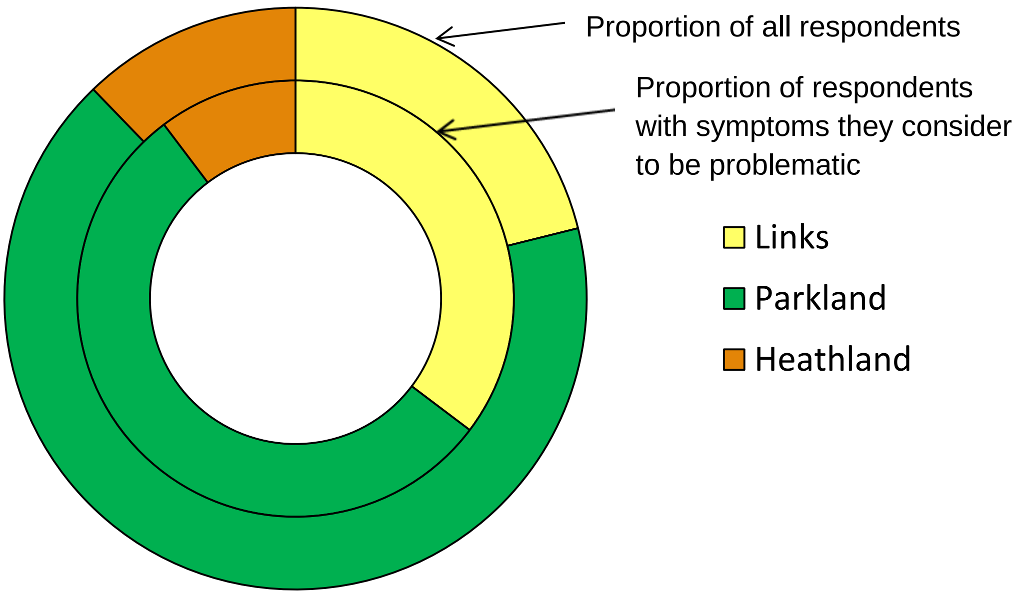


Figure 36: Course types of all respondents (outer ring) compared to courses with problematic fairy ring (inner ring) showing greater proportion of links courses affected

Figure 36 shows analysis of the course types of all questionnaire respondents compared to course type of those that reported problematic fairy ring. The breakdown shows that a greater proportion of respondents reporting problematic fairy ring were reporting it on links golf courses.

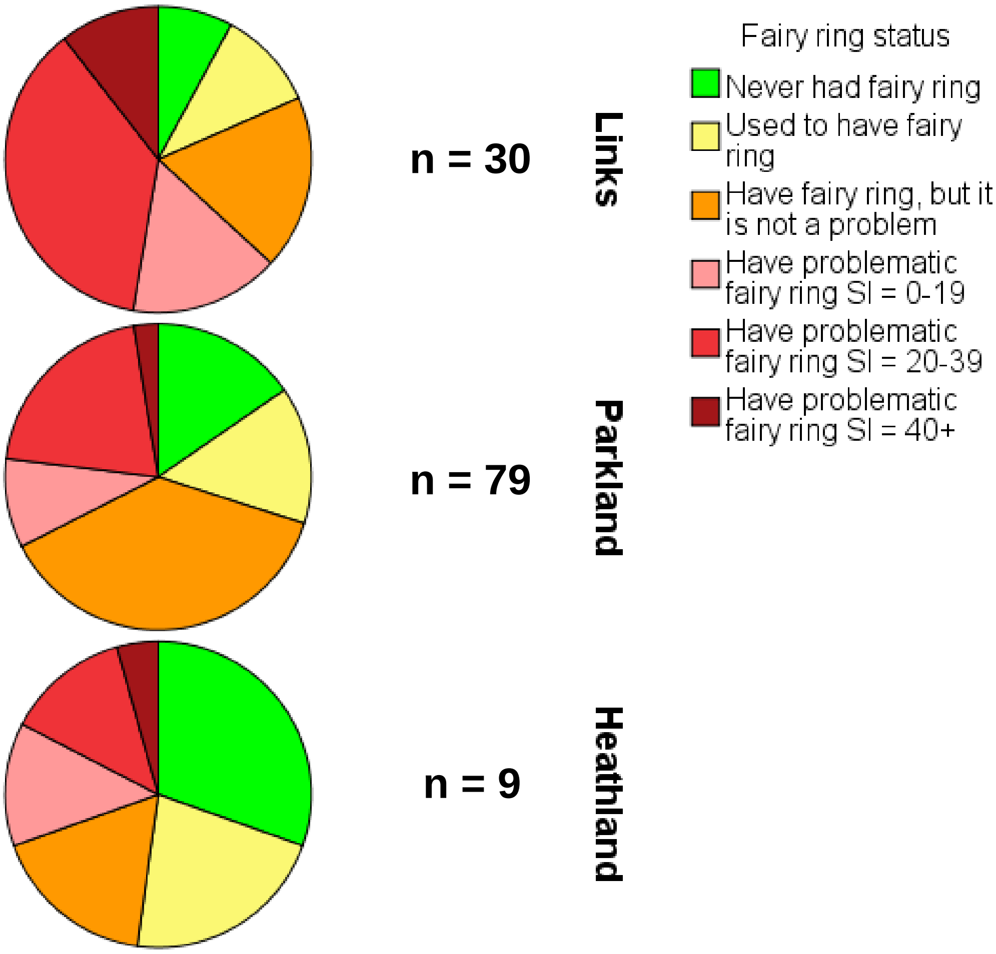


Figure 37: Fairy ring status by golf course type
(links n = 30, parkland n = 79, heathland n = 9)

Comparing course type with the categorical fairy ring status data showed significant differences between course types ($X^2 = 14.486$, df = 6, $p = 0.025$). As Figure 37 shows, a higher proportion of links courses have a problem with fairy ring, especially with high-level severity. Heathland courses are the most likely to have never had fairy ring and parkland courses find it less of a problem.

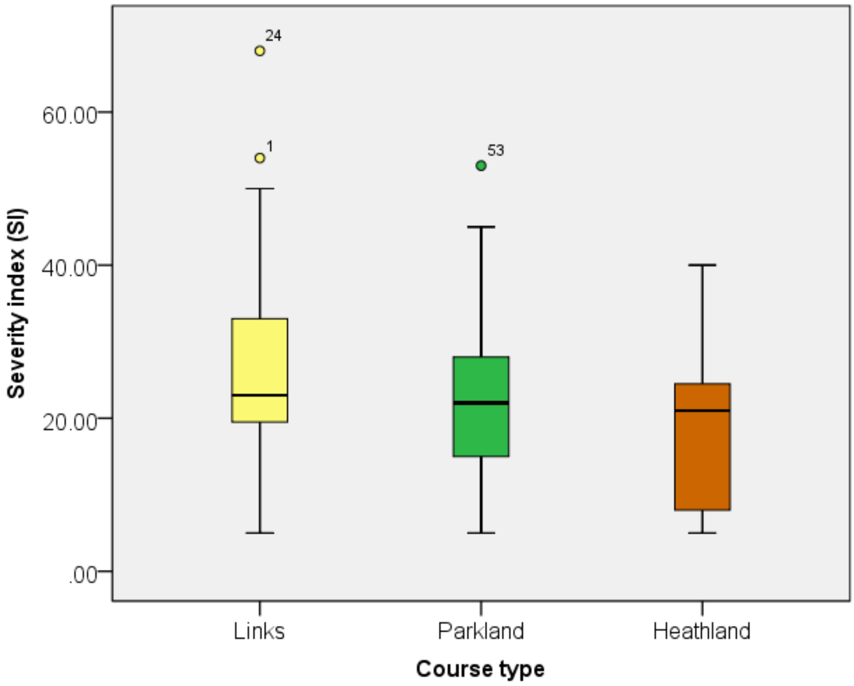


Figure 38: Severity index by golf course type

Comparing SI between course types shows considerably overlapping ranges and similar medians (Figure 38). Links SI is the only category that is not normally distributed (Shapiro-Wilk *p* = 0.020) and has particularly high outliers at 54 and 68 SI. Links courses show the highest mean SI (26.75 +/- 3.02), followed by parkland (22.43 +/- 1.93), and then heathland (18.71 +/- 4.77), but this is not a statistically significant difference (Kruskal-Wallis *p* = 0.507).

There were no statistically significant differences found between types of fairy ring occurring on links, parkland and heathland courses, showing that each type of fairy ring occurs in approximately equal proportions on each type of course (X^2 tests: *p* =

0.748; p = 0.606; p = 0.814; p = 0.699). The number of different fairy ring types present also did not differ by course type (X^2 = 2.234, df = 6, p = 0.897).

3.4.14 Course age

It was noticed that the top ten courses with the highest SI seemed to be some of the longest-established, so internet research investigated the year of construction for each of the identified courses with a pre-calculated SI in order to search for a correlation. The theory was that basidiomycetes materialising as fairy rings may be larger, more numerous, and cover a larger proportion of holes on older courses that have been environmentally stable since the disturbance event marked by construction of the course.

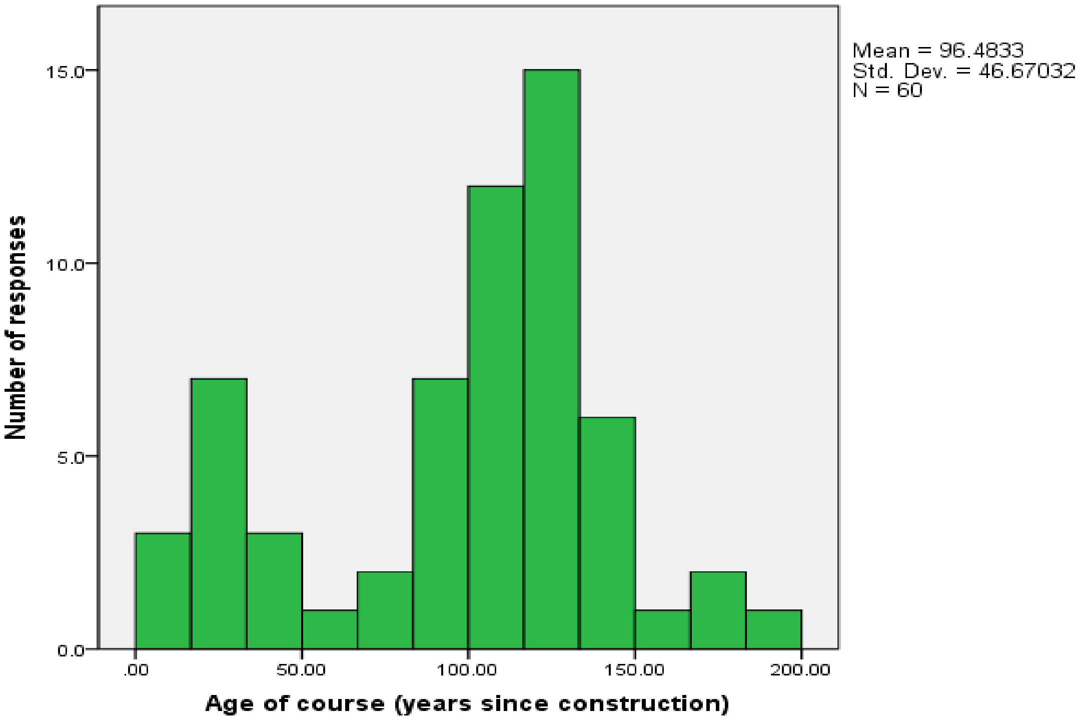


Figure 39: Age of golf courses with problematic fairy

As seen in Figure 39, age of course (years since construction), with a mean of 96.48 years (+/- 6.03) and ranging from 4 to 195 years old, is not normally distributed

(Kolmogorov-Smirnov *p* <0.001). Parkland courses were found to be significantly younger than links and heathland (Kruskal-Wallis *p* = 0.006) (Figure 40), which is fitting of the evolution of the game.

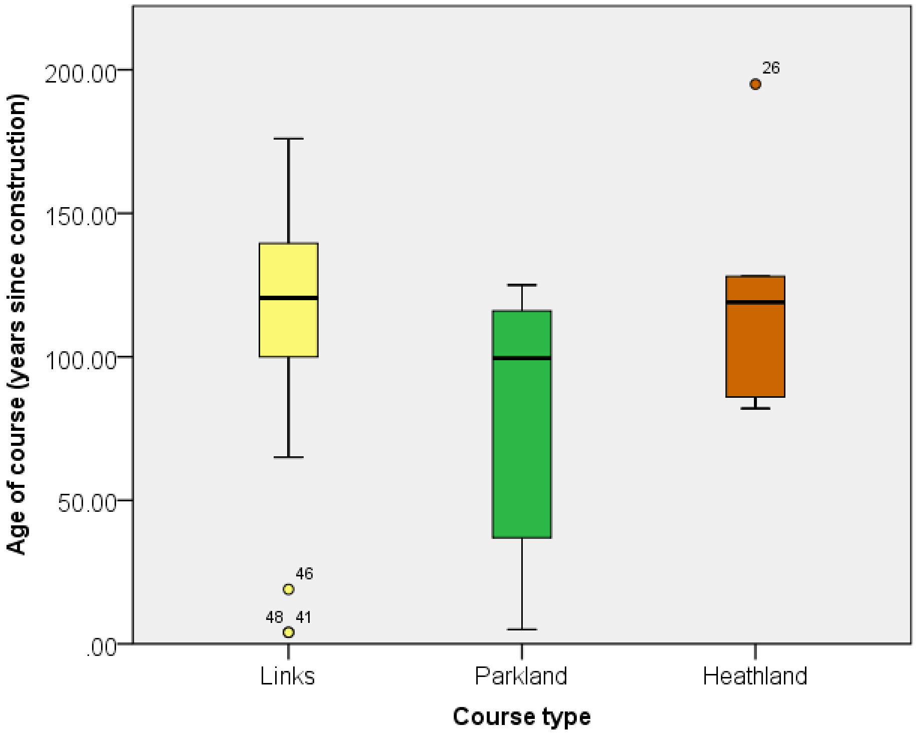


Figure 40: Age of course by course type

Figure 41 shows a very weak positive correlation between SI and age of course (R^2 = 0.011), which is not statistically significant, according to the nonparametric Spearman's test (correlation coefficient = 0.184, *p* = 0.159).

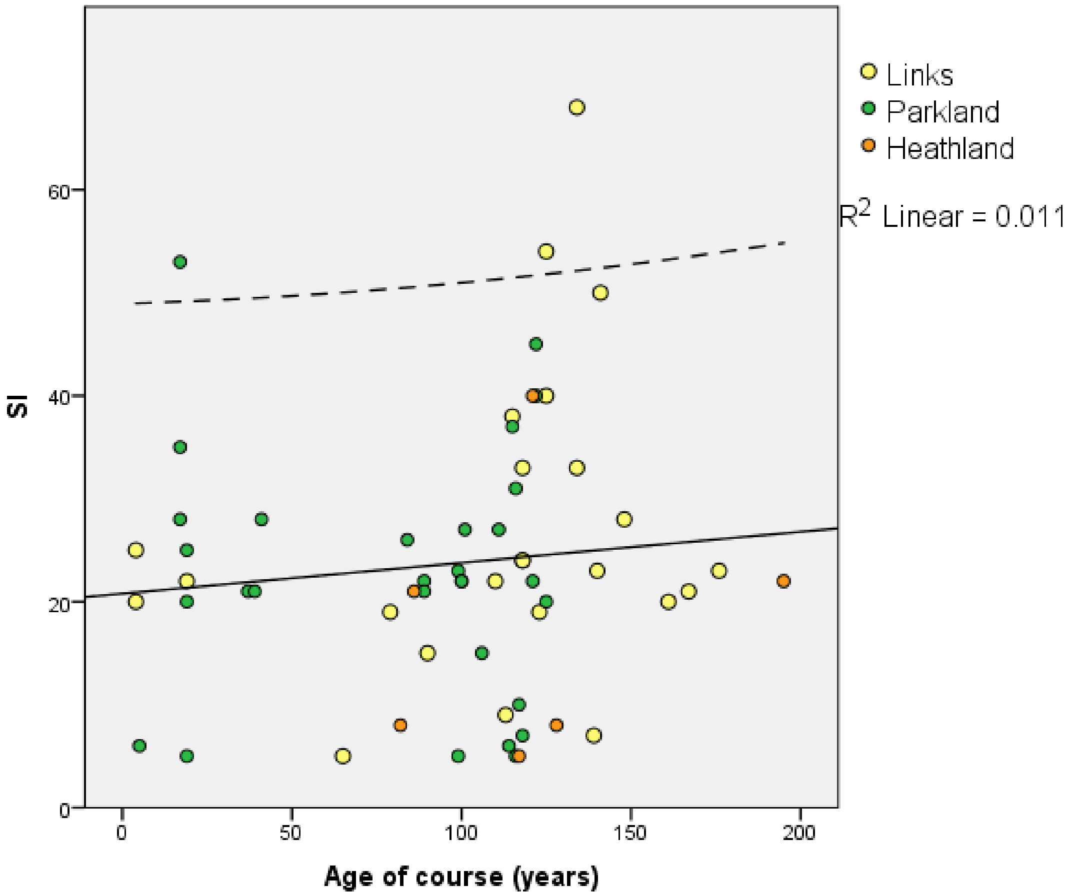


Figure 41: Severity Index (SI) by age of course, categorised by course type, with line of best fit and dashed line representing 95% confidence interval

Older courses did not have more types of fairy ring present (ANOVA F = 0.973, df = 3, *p* = 0.412), and fairy ring type did not vary by age of the course (Kruskal-Wallis *p* = 0.337).

3.5 Discussion

Although the questionnaire response rate was lower than anticipated (5%), the figure was fitting with a similar questionnaire undertaken by Mann and Newell (2005), which had a 7% response, suggesting that this may be typical of the industry. Responses were highly variable, with courses that had never had fairy ring, to those that had lots of rings and numerous types on every hole. Two thirds of respondents did have fairy ring at present and the majority of these described it as problematic on their course.

As the first UK-based fairy ring study, there is little existing literature with which to compare these results, so the predominant purpose of the data will be to help guide and tailor subsequent stages of the project.

As Fidanza (2009) stipulates, fairy ring persists year-round and some courses reported symptoms to be at their worst throughout the year. Contrary to the expectation that fungal activity would be highest in the autumn months (the traditional fruiting time for many basidiomycete species), results have shown the peak months for fairy ring symptoms to be July and August.

Type-2 fairy ring was considerably more prominent, but less than half of respondents considered it a problem and, when it was, the impact was predominantly visual. Although less common, type-1 was found to have the greatest effect on playability. Greens are the most important part of the course on which to concentrate potential control methods, as they were voted a significantly more serious problem.

Results have shown that type-2 fairy ring frequently occurs with type-1. As discussed earlier, type-2 symptoms can develop into type-1. It would be worth investigating the ‘tipping point’ at which a type-2 becomes a type-1. Many courses only had type-2. It would be interesting to see if there is something about these courses that inhibits the progression to type-1 or whether the transition is characteristic of some type-2-associated species, but not others.

Fairy ring symptoms are known to fluctuate according to weather conditions, becoming particularly active after extended periods of drought followed by warm, wet periods (Mann, 2004; Nelson, 2008). The mapping process has shown that fairy ring occurs at varying severities throughout the UK and that the drier and warmer south-east of the country appears to be worse-affected. This may be due to lower rainfall and extended periods of drought exacerbating the hydrophobicity associated with type-1 fairy ring.

Links courses have been shown to be the worst-affected. It may be that fairy ring *is* more prominent on these courses, as fungal mycelium advances more readily through sandy soils (York, 1998), or, it is possible that links courses are more likely to *perceive* fairy ring as a problem as, being some of the most ancient and prestigious courses, they are under more pressure to provide immaculate playing surfaces. Socio-economic factors, involving location and clientele, are likely to have had some effect on the response given by courses. These aspects can be elaborated upon during one-to-one contact with the courses involved.

Statistical tests have shown that there is no relationship between golf course age (years since construction) and fairy ring severity. It must also be noted that the evolution of a golf course is such that holes may have been added, amended, or reconstructed during its history meaning the date of construction given on the website is not always an accurate representation of the age of affected parts of the course.

The fact that the vast majority of respondents want to know more about dealing with fairy ring on their course shows that the current level of uncertainty about fairy ring management is very high. This project, therefore, aimed to, not only develop effective methods for controlling fairy ring, but also communicate this knowledge to greenkeepers.

3.6 Limitations and recommendations

Numerous limitations have been considered during the questionnaire process. Firstly, the relatively low response rate triggered questions about whether the greenkeepers/course managers actually received the communication. Possible reasons for non-response included:

- Invalid email address
- Email diverted to recipients ‘junk’ email folder
- Email did not reach desired recipient
- Desired recipient does not have access to email
- Recipient did not wish to/forgot to respond
- Course was not on STRI mailing list

If another questionnaire was carried out, it would be worth considering delivering a postal questionnaire alongside the online format to encourage response. Although this would incur a cost, it may be a preferable alternative to those not keen or able to access the internet.

Marketing advice suggested that recipients receiving a questionnaire after lunch on a Friday afternoon were more likely to reply, as it is a quick and easy job to get done before the end of the working week (personal communication, Dr Keith Walley, Harper Adams University). This advice was followed, but what was not considered is that greenkeepers do not necessarily follow a normal working week. They usually start early and finish early, perhaps working 6am-3pm Mon-Fri, meaning that they may not have received the communication at the expect time. This may have impacted response rate.

The most important limitation to consider here is, as with any social data, results are subjective; a product of greenkeeper perception. The symptoms that one greenkeeper finds a mild annoyance may be a serious issue for another greenkeeper, which may also be influenced by the prestige of the golf club, particularly for those under scrutiny as hosts of championship competitions.

The results may further have been influenced by the role of the person completing the questionnaire. Whilst the link was emailed for the attention of the Course Manager or Head Greenkeeper, many courses have a single email address, whereby the link may not have made it to the desired respondent. In at least one case, the course secretary had answered the questionnaire. A course secretary may well have a different perception of turf management than the course greenkeeper. Furthermore, duration of employment at the club is likely to limit staff’s knowledge of the course’s history.

Questionnaire design was restricted by the SurveyMonkey® format, so the ordering of questions 2 and 3 was not ideal. Including course type as an additional question would have saved time during data analysis and proven minimally disadvantageous to the respondent. The questionnaire design was slightly over-simplified in this respect.

The photographs of the fairy ring types shown in the questionnaire aimed to make clear distinctions between the symptoms that the disease can express. Whilst many courses may have found it easy to identify which rings were on their course, in some cases symptoms can occur together, even in the same ring, which may have caused confusion for some respondents. Also, some symptoms are less obvious, such as mushrooms/puffballs of type-3 fairy ring, which may not fully emerge or go unnoticed on areas regularly mown.

3.7 Conclusion

The questionnaire acted as an effective tool in gathering sufficient data to offer an understanding of the current state of UK fairy ring on golf courses. This basis of knowledge has helped direct the progression of the project and has already provided a series of points upon which to build with further research. By understanding that type-2s are most frequent, links courses are worst-affected and greens need particular attention, investigations can be tailored to meet the golf industry’s current needs. Possibly the most notable finding is the south-east-north-west severity divide, which suggests that geographical distribution of fairy ring severity is influenced by climate.

3.8 Further research

At the end of the questionnaire, all respondents were asked if they would be willing to participate in further research. Eighty per cent answered ‘yes’, although 5% of these then failed to leave their contact details.

A total of 89 courses in the UK and Ireland that opted into further research had suffered from fairy ring recently (Figure 42). These made up the shortlist of courses involved in subsequent stages of the study. A number of them were visited over the subsequent year, in order to inspect their fairy rings, take samples, and gather information from the greenkeepers on their experiences with fairy ring and its management. These anecdotal reports of what greenkeepers found to be effective and ineffective in managing fairy ring and the background information about when and where the rings appear helped to tailor the later stages of the project.

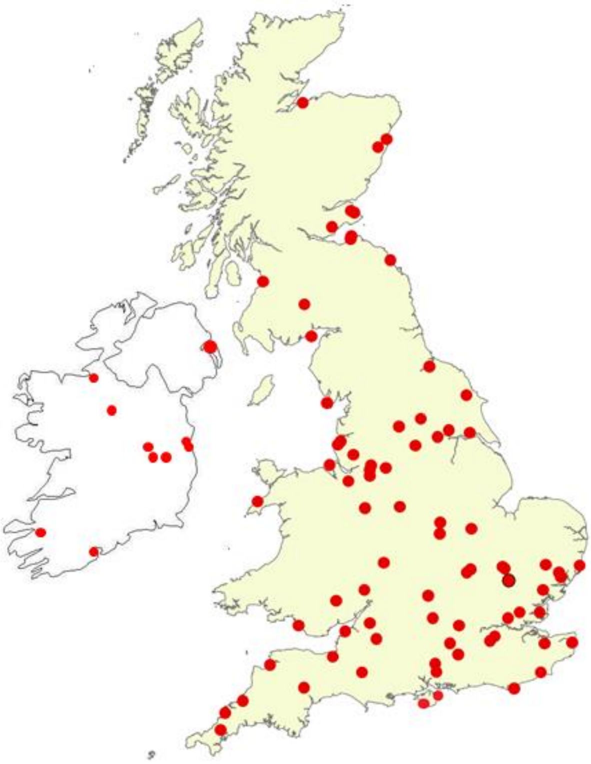


Figure 42: Locations of golf courses willing to participate in further research

3.9 Acknowledgements

For this part of the fairy ring project, thanks are offered to the project supervisors: Dr Martin Hare, Dr Ruth Mann, and Prof. Simon Edwards, for their feedback and advice during the questionnaire development process. Thanks to Dr Keith Walley of Harper Adams University and Carolyn Beadsmore of STRI for providing their marketing expertise. The STRI agronomists, particularly Henry Bechelet, and soil scientist Dr Christian Spring helped test and adjust the final drafts of the questionnaire. Finally, thanks to Helen Waite of STRI for constructing the online questionnaire on Survey Monkey and sending the link and subsequent reminders to the golf courses on my behalf.

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4.0 Controlling Fairy Ring Fungi *In Vitro*

4.1 Abstract

To observe how some common fairy ring fungi responded to chemical control treatments *in vitro*, Petri dishes of Leonian agar amended with varying concentrations of four fungicides (flutolanil, azoxystrobin, propiconazole, and pyraclostrobin) and one simple salt (potassium bicarbonate) were inoculated with samples of eight fairy ring isolates representing four different species (*Marasmius oreades, Agaricus campestris, Bovista plumbea*, and *Handkea utriformis*) and incubated at 23°C.

Their growth was measured after 14 days and the relative growth for each sample calculated in relation to the growth of their equivalent untreated controls. Relative growth measurements showed that propiconazole was significantly more effective at inhibiting growth of all eight isolates tested compared to the other chemicals. Flutolanil and potassium bicarbonate were the least effective at inhibiting growth overall.

Another *in vitro* experiment saw three *Marasmius oreades* and *Agaricus campestris* isolates paired in Petri dishes of potato dextrose agar with either themselves, an isolate of the same species, or an isolate of another species in order to test for self, intraspecific and interspecific antagonism, respectively. For each pair, the nature of the interaction between the two mycelia was categorised.

Both of the *Marasmius oreades* isolates and the *Agaricus campestris* isolate exhibited a mycelium-free inhibition zone between the isolates when paired with themselves. When paired with each other, *M. oreades* isolate M1 was dominant

over M2 in 50% of cases, suggesting greater vigour. Both of the *M. oreades* isolates were dominant over *A. campestris* in at least 50% of cases. In the remaining cases, the pairs existed side-by-side without any apparent interaction.

However, existing literature suggests that the inhibition zone observed between self-paired isolates only occurs on the surface of the agar medium and that submerged hyphae interact, rather than antagonise each other. As this was not inspected in more detail at the time of the experiment, the inhibition zones observed cannot be construed as evidence of mutual antagonism.

4.2 Introduction

Fairy ring symptoms can cause damage to turf that negatively affects the aesthetics and playability of the golf course. As a disease, it is notoriously difficult to treat, partially due to the number of different species that can cause the symptoms and partially due to a lack of knowledge and understanding about how to deal with the disease.

From the numerous personal communications received during this project, it is evident that greenkeepers in the UK are largely reliant on anecdotal reports on how to treat the symptoms but, as there have been no detailed, academic studies carried out on fairy ring in the UK to date, there is no point of reference to either support or dismiss the anecdotal evidence.

The majority of information available on fairy ring control originates in the USA, where they are generally using fungicides, such as flutolanil, that are not available for amenity use here in the UK (Fidanza, 2002; Fidanza, 2009; Miller, 2010; NCSU, 2014). The following parts of the project, therefore, aimed to generate impartial data on fairy ring control, specific to UK needs, where it is currently lacking.

The *in vitro* experiments aimed to: -

1. Test the efficacy of products currently recommended for fairy ring control in the UK compared to products reported in USA studies

2. Investigate novel control methods, where little or no data on their efficacy currently exist

3. Look for differences in response between fairy ring isolates and species to attempted control methods

4.3 Experiments

4.3.1 Mycelial growth assay

4.3.1.1 Introduction

Chemical treatment of fairy rings often yields inconsistent results. This is likely to be down to several factors. Firstly, as fairy ring symptoms can be caused by a number of different basidiomycete species, the chemical chosen must be suitable for the target species. Product labels, however, do not specify which fairy ring species they claim to be effective against. Secondly, maximal contact between the product and target fungus must be ensured, meaning success of the treatment is often reliant on sufficient aeration of the ring beforehand and application of a wetting agent to aid delivery of the product into the rootzone. With fairy rings that are severely hydrophobic and/or particularly deep-growing, which is often associated with *Marasmius oreades* (Smith *et al.*, 1989), the product is unlikely to penetrate sufficiently into the soil profile to take effect. Lastly, there is the problem of fungicide resistance, whereby chemicals may become less effective on the target, the more they are applied (Mann, 2003).

In several experiments by Miller (2010), including field experiments on areas affected by the puffball *Vascellum curtisii* , the fungicide triticonazole was found to provide excellent preventative control. Triticonazole is one of the demethylation inhibitor (DMI) class of fungicides, which work by disrupting ergosterol synthesis (Mann, 2011a) and, whilst triticonazole is not available for amenity use in the UK, a related DMI fungicide, propiconazole, is available. Propiconazole, in the form of Banner Maxx by Syngenta was, hence, included in this assay. Banner Maxx is not labelled, however, for control of fairy ring.

Another fungicide included in the assay was azoxystrobin, in the form of Heritage Maxx by Syngenta, which is the only fungicide currently labelled for fairy ring control in the UK. This is one of the strobilurin fungicides, which works by preventing electron transfer in mitochondria and, hence, reducing energy available for fungal growth (Mann, 2011a). Another available strobilurin fungicide, pyraclostrobin, was also included, in the form of Insignia by BASF. This product is also not labelled for fairy ring control.

The carboxamide fungicide, flutolanil, frequently recommended in the American literature (Fidanza, 2002a; Miller, 2010; Nelson, 2008), was also included in the experiment as a comparison, despite it being unavailable for amenity use in the UK. Carboxamide fungicides inhibit mitochondrial respiration by blocking electron transport at the succinate dehydrogenase stage in the Krebs cycle (Hayes and Kruger, 2014).

The final chemical evaluated was a novel treatment introduced by the New Zealand Sports Turf Institute in their fact sheet on fairy ring as a potential solution. Potassium bicarbonate is a simple salt that aims to make the pH of the medium unfavourable for basidiomycetous fungal growth (New Zealand Sports Turf Institute, undated).

Besides observing for differences in efficacy of the control treatments, testing a number of different common fairy ring forming species and different isolates from each species aims to identify whether they respond dissimilarly to the treatments.

4.3.1.2 Methodology

An *in vitro* mycelial growth assay was used to determine the efficacy of four fungicides and a simple salt in inhibiting the growth of eight fairy ring fungus isolates representing four different species commonly found on golf courses.

A 5mm cork borer was used to extract circular samples from the edges of active fungal cultures, which were established from spores dropped from gill sections of fresh basidiocarps (identified using Phillips, 2006 guide) and maintained on Leonian agar (Leonian, 1924). Samples taken from three *Marasmius oreades* isolates, three *Agaricus campestris* isolates, one *Bovista plumbea* isolate and one *Handkea utriformis* isolate (Table 5) were used to inoculate a series of Petri dishes containing Leonian agar (1924) amended with a range of concentrations of fungicides Heritage Maxx (Syngenta UK Ltd, Cambridge), Prostar 70 WG (Bayer Environmental Science, NC, USA), Banner Maxx (Syngenta UK Ltd, Cambridge), Insignia (BASF Corporation, NJ, USA) and the simple salt potassium bicarbonate (Sigma-Aldrich Company Ltd, Gillingham, UK), as shown in Table 6, by placing the fungal plug in the centre of the Petri dish. The chemical dilution series, which were determined through a set of preliminary growth tests, of the same nature as herein, to find a range between total inhibition and normal growth, were prepared in acetone and added to autoclaved Leonian agar, cooled to 53°C, so that the total concentration of acetone in each preparation was 0.1% (v/v). A preparation of unamended Leonian agar was similarly prepared with acetone and a second unamended agar preparation was made with sterile distilled water in the place of acetone in order to act as untreated controls. Each isolate-chemical dilution combination was replicated twice. Each isolate had two untreated acetone controls and one untreated sterile distilled water control. The Petri dishes were fully sealed with Parafilm and stored in a dark environment cabinet at 23°C for 14 days.

Table 5: Fairy ring isolates used in mycelial growth assay, the origins of the fairy rings from which the basidiocarps were collected, and their average radial mycelial growth rate (mm/day) on Petri dishes of unamended Leonian agar stored in a dark environment cabinet at 23°C for 14 days

Isolate no.	**Species**	**Origin**	**Turfgrass**	**Soil type**	**Radial growth rate (mm/day)**	**Year of isolation**
M1	*Marasmius oreades*	Rugby pitch, Harper Adams University, Shropshire	*Poa annua* L., *Lolium* sp.	Loam	2.23	2014
M2	*Marasmius oreades*	Practice area, Royal Liverpool Golf Club, Hoylake	*Festuca* spp.	Sand	0.32	2013
M3	*Marasmius oreades*	Rugby pitch, Harper Adams University, Shropshire	*Poa annua* L., *Lolium* sp.	Loam	0.26	2014
A1	*Agaricus campestris*	Football pitch, Harper Adams University, Shropshire	*Poa annua* L., *Lolium* sp.	Loam	1.56	2014
A2	*Agaricus campestris*	Rugby pitch, Harper Adams University, Shropshire	*Poa annua* L., *Lolium* sp.	Loam	1.35	2014
A3	*Agaricus campestris*	9th tee, Shropshire Golf Centre, Telford	*Unknown*	Loamy clay	1.59	2014
BP	*Bovista plumbea*	Football pitch, Harper Adams University, Shropshire	*Poa annua* L., *Lolium* sp.	Loam	0.16	2014
HU	*Handkea utriformis*	5th tee, Shropshire Golf Centre, Telford	*Unknown*	Loamy clay	0.19	2014

Table 6: Products used in mycelial growth assay, their active ingredients, advised application rate from the product label, and dilution series used, which was determined through a set of preliminary mycelial growth tests

Product	Manufacturer	Active ingredient	Application rate	Concentration of product (µl/ml)
Heritage Maxx	Syngenta	95 g/L Azoxystrobin	25 ml product in 8-10 L water for spot treatment	0.0001, 0.001, 0.01, 0.1, 1, 10, 100
Prostar 70 WG	Bayer	70% Flutolanil	2.2 oz. / 1,000 sq. ft. for preventative treatment or 4.5 oz. / 1,000 sq. ft. for curative treatment	0.0001, 0.001, 0.01, 0.1, 1, 10, 100
Banner Maxx	Syngenta	156 g/L Propiconazole	30 ml product in 4-10 L water for spot treatment	0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10
Insignia	BASF	20% Pyraclostrobin	0.9 oz. / 1,000 sq. ft.	0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10, 100
				Concentration of product (µg/ml)
Potassium bicarbonate	Sigma-Aldrich	-	-	0.0001, 0.001, 0.01, 0.1, 1, 10, 100

The diameter of mycelial growth of each sample was measured from edge to edge along the centre line at which the plug was placed and again at a right angle to it. In order to calculate a representative area of mycelial growth for each sample, an average radius was calculated as half the average diameter, which was then squared and multiplied by pi to give an area for each isolate in the assumption that it was a circle. The area of the inoculation plug was deducted from the calculated area of growth before generating relative growth values for the treated samples in comparison to their untreated counterparts as follows:

$$\text{Relative Growth} = \left(\frac{\text{average area of chemical-treated sample with plug area deducted}}{\text{average area of untreated sample with plug deducted}}\right) \text{X } 100$$

As growth of the control isolates did not vary between the untreated agar with acetone and the untreated agar with sterile distilled water, the untreated average was calculated as a result of all three controls for each isolate.

The relative growth values for each sample were analysed using IBM SPSS Statistics (version 23) and were compared using ANOVA followed by *post-hoc* analyses using a LSD Test.

4.3.1.3 Results

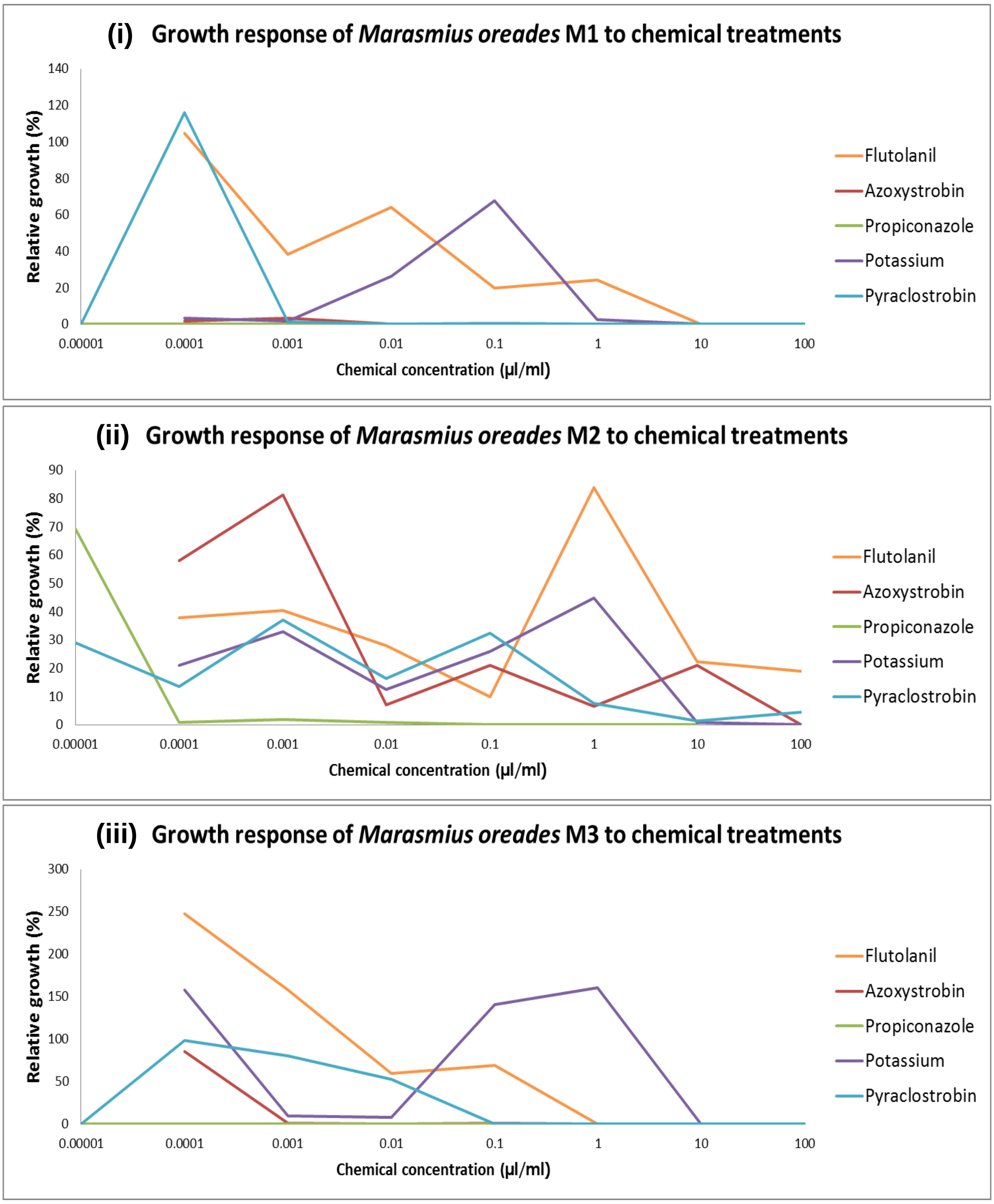
Examples of fungal growth from one of the preliminary experiments to find the optimal chemical concentration ranges for the main experiment is shown in Figures 43 and 44.

Figure 43: Growth of isolate M1 (*M. oreades*), on amended Leonian agar after 14 days in a dark environment cabinet at 23°C, during a preliminary experiment that included only five chemical dilutions. Columns represent dilutions, left to right: 1, 0.1, 0.01, 0.001, 0.0001 µl/ml. Rows represent chemicals, top to bottom: potassium bicarbonate, flutolanil, propiconazole, azoxystrobin, pyraclostrobin. The three plates on the bottom row are controls growing on unamended media.

Figure 44: Growth of isolate A2 (*A. campestris*), on amended Leonian agar after 14 days in a dark environment cabinet at 23°C, during a preliminary experiment that included only five chemical dilutions. Columns represent dilutions, left to right: 1, 0.1, 0.01, 0.001, 0.0001 µl/ml. Rows represent chemicals, top to bottom: potassium bicarbonate, flutolanil, propiconazole, azoxystrobin, pyraclostrobin. The three plates on the bottom row are controls growing on unamended media.

The three *M. oreades* isolates all responded differently to the treatments. As seen in Figure 45, the results for relative growth were sometimes erratic and few followed the curvilinear relationship that would usually be expected.

Isolates M1 and M3 failed to grow at all on propiconazole, suggesting that more dilute concentrations would need to be tested in order to witness uninhibited growth. Propiconazole was the most effective treatment of M2, where it had inhibited growth completely at a concentration of 0.0001 µl/ml. M1 and M3 also grew little on azoxystrobin, suggesting some efficacy in control, reinforced by the result seen for M2. Flutolanil followed a generally downward trend, but was not particularly effective at inhibiting growth in the *M. oreades* samples. All three samples saw a rise in growth between 0.1 µl/ml and 1 µl/ml when treated with potassium bicarbonate, suggesting that this concentration may favour growth. All three *M. oreades* results suggest that pyraclostrobin may be less effective than propiconazole and azoxystrobin at inhibiting growth, but more effective than flutolanil and potassium bicarbonate.

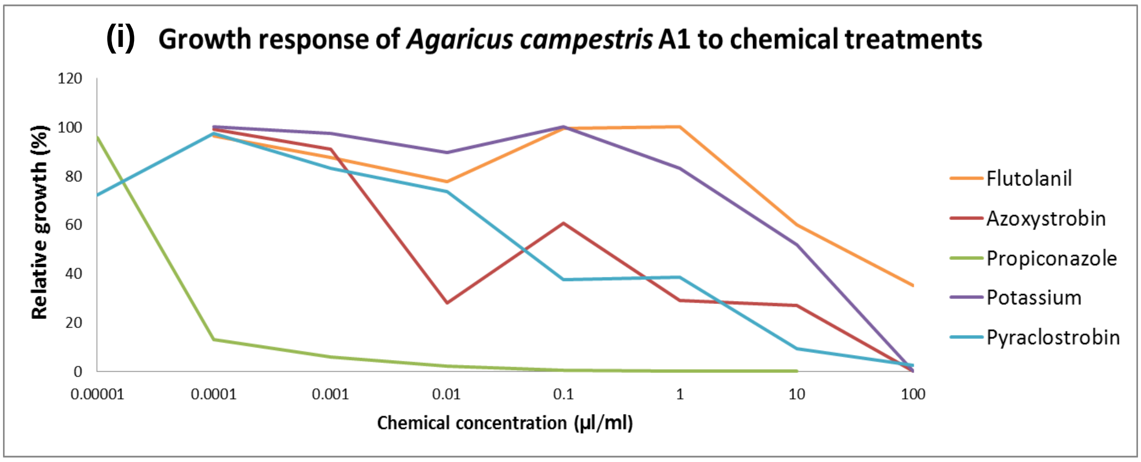


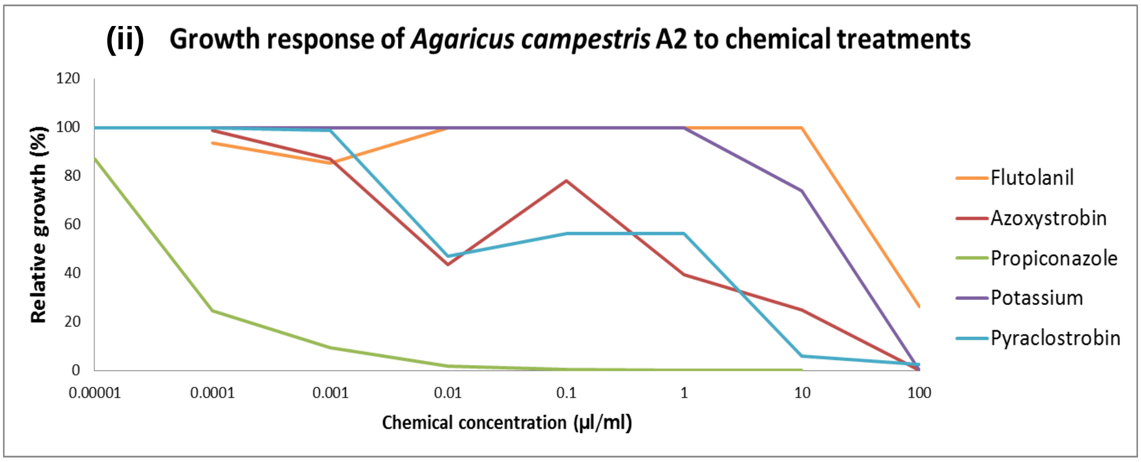
(i) Growth response of *Marasmius oreades* M1 to chemical treatments

(ii) Growth response of *Marasmius oreades* M2 to chemical treatments

(iii) Growth response of *Marasmius oreades* M3 to chemical treatments

Figure 45: Relative growth of *Maramius oreades* isolates M1 (i), M2 (ii) and M3 (iii) on Leonian agar amended with varying concentrations of five chemical treatments in comparison to unamended controls (mean of two replicates for each treatment shown)





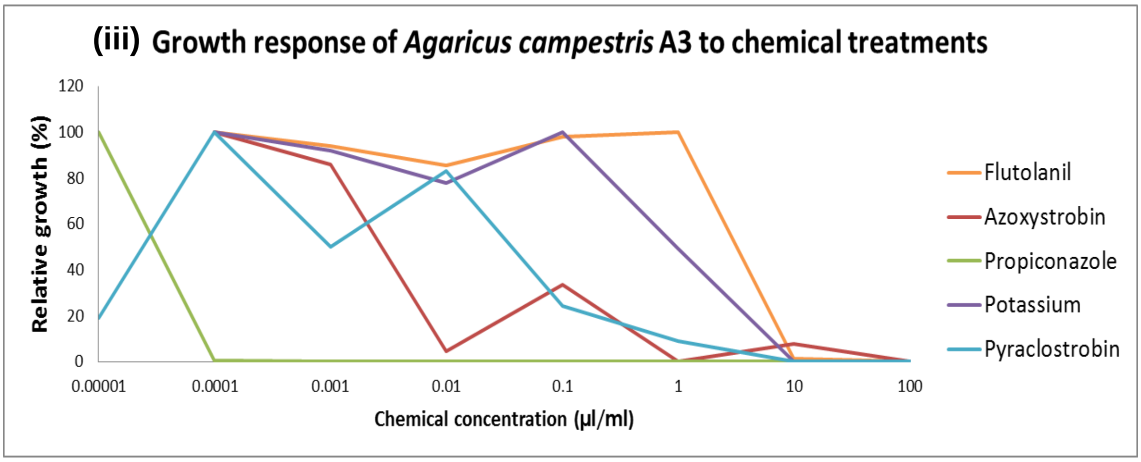


Figure 46: Relative growth of *Agaricus campestris* isolates A1 (i), A2 (ii) and A3 (iii) on Leonian agar amended with varying concentrations of five chemical treatments in comparison to unamended controls (mean of two replicates for each treatment shown)

The *A. campestris* isolates all responded similarly to the five chemicals, with propiconazole inhibiting growth at the lowest concentration and flutolanil having the highest concentration required to inhibit growth. Figure 46 would suggest that propiconazole is particularly effective at controlling *A. campestris*, whilst flutolanil and potassium bicarbonate are particularly ineffective.

As Figure 47 shows, potassium bicarbonate needed the highest concentration in order to achieve full control of *B. plumbea*, however there were some fluctuations at low concentrations, particularly 0.0001 µl/ml, where control was better than at 0.01 µl/ml. Flutolanil, azoxystrobin, and pyraclostrobin followed a similar trend to each other, proving less effective at inhibiting growth than propiconazole.

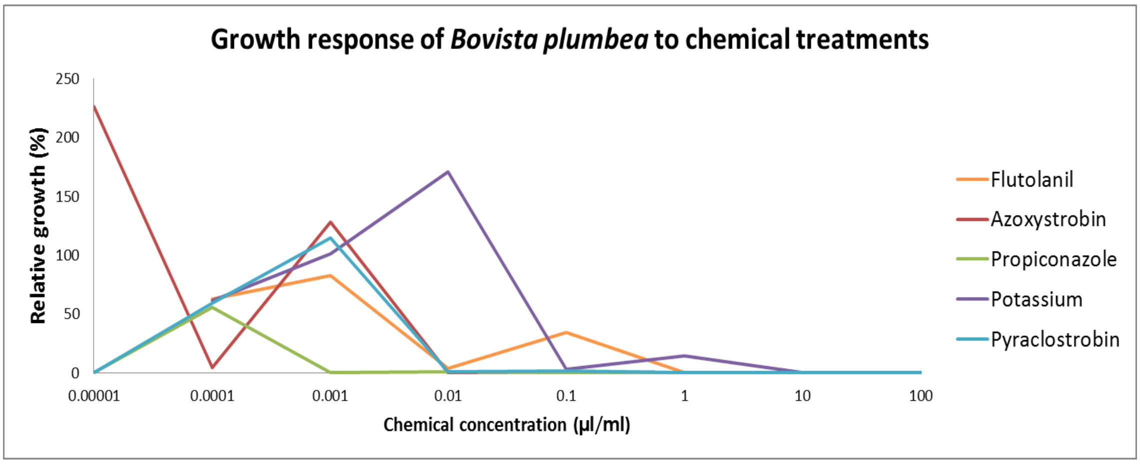


Figure 47: Relative growth of *Bovista plumbea* isolates on Leonian agar amended with varying concentrations of five chemical treatments in comparison to unamended controls (mean of two replicates for each treatment shown)

Hankea utriformis failed to grow at all on propiconazole, again suggesting that lower concentrations would need to be tested. Azoxystrobin and potassium bicarbonate provided consistent control from lower concentrations, but did not fully inhibit growth unless higher concentrations were used (1 µl/ml and 10 µl/ml, respectively). Both flutolanil and pyraclostrobin showed some efficacy in inhibiting growth, but, as seen previously, some anomalies were experienced, as shown in Figure 48 by the absence of growth on the 0.00001 µl/ml pyraclostrobin concentration.

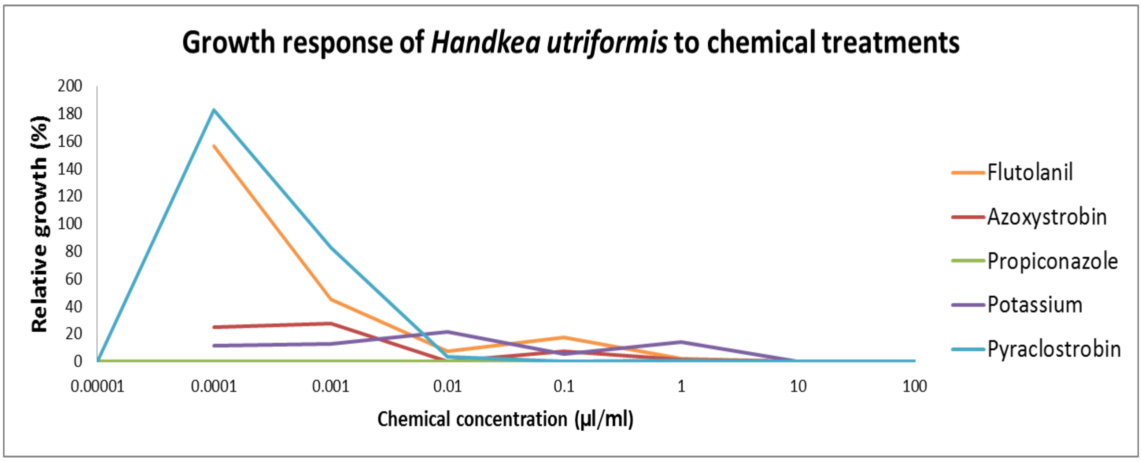


Figure 48: Relative growth of *Handkea utriformis* isolates on Leonian agar amended with varying concentrations of five chemical treatments in comparison to unamended controls (mean of two replicates for each treatment shown)

In a comparison of mycelial growth of all isolates across dilutions 0.0001 to 10 µl/ml, which were used for all chemicals, propiconazole inhibited growth of the fungal isolates significantly more than any of the other chemicals (ANOVA, df = 4, F = 20.82, *p* <0.001). As seen in Table 7, propiconazole was the most effective chemical at inhibiting growth in all of the eight isolates tested. Flutolanil was generally the least effective at inhibiting growth, followed by potassium bicarbonate. As shown by Table 8, *post-hoc* LSD analysis revealed that the chemicals all varied significantly from each other in the extent to which they inhibited fungal growth, with the exception of flutolanil and potassium bicarbonate, and azoxystrobin and pyraclostrobin. Azoxystrobin and pyraclostrobin could be expected to yield similar results, as they are from the same group of strobilurin fungicides and, hence, have the same mode of action.

Table 7: Mean relative growth of fungal isolates on chemical amended Leonian agar (concentrations 0.0001 to 10 µl/ml pooled for each isolate) in comparison to unamended controls (mean is of two replicates for each treatment; SE = standard error of the mean; values not sharing the same superscript letter are significantly different at $p \leq 0.05$)

Isolate	Chemical Mean relative growth (%)				
	Flutolanil	Azoxystrobin	Propiconazole	Potassium bicarbonate	Pyraclostrobin
M1	36.00 ^{a} (SE 10.41)	0.79 ^{b} (SE 0.39)	0.00 ^{b} (SE 0.00)	14.64 $^{a, b}$ (SE 9.80)	14.75 $^{a, b}$ (SE 10.00)
M2	34.57 ^{a} (SE 7.07)	27.86 ^{a} (SE 9.31)	10.50 ^{b} (SE 7.41)	19.79 $^{a, b}$ (SE 5.23)	17.75 $^{a, b}$ (SE 4.46)
M3	76.43 ^{a} (SE 31.47)	12.57 ^{a} (SE 8.68)	0.00 ^{a} (SE 0.00)	68.43 ^{a} (SE 33.21)	29.06 ^{a} (SE 15.83)
A1	83.43 $^{a, d, e}$ (SE 6.81)	54.86 $^{a, b}$ (SE 9.61)	18.36 ^{c} (SE 9.46)	81.71 ^{d} (SE 9.5)	61.26 $^{b, e}$ (SE 9.97)
A2	86.50 ^{a} (SE 7.39)	53.14 ^{b} (SE 9.46)	17.64 ^{c} (SE 8.34)	82.00 ^{a} (SE 9.61)	58.44 ^{b} (SE 10.46)
A3	68.43 ^{a} (SE 12.06)	35.69 $^{a, b}$ (SE 11.66)	14.36 ^{b} (SE 9.70)	59.93 ^{a} (SE 11.89)	35.69 ^{a} (SE 10.47)
BP	26.29 ^{a} (SE 12.58)	45.00 ^{a} (SE 20.98)	8.07 ^{a} (SE 7.84)	46.23 ^{a} (SE 18.89)	22.06 ^{a} (SE 15.38)
HU	32.79 ^{a} (SE 14.82)	8.93 ^{a} (SE 4.13)	0.07 ^{a} (SE 0.07)	9.64 ^{a} (SE 3.56)	33.94 ^{a} (SE 21.05)

Table 8: Mean relative growth and significance values for each chemical tested, as determined by LSD analysis *pot-hoc* to ANOVA

	Treatment				
	Flutolanil	Azoxystrobin	Propiconazole	Potassium bicarbonate	Pyraclostrobin
Mean relative growth (%)	55.55	30.07	8.70	47.81	34.12
Standard Error	5.57	4.31	2.45	5.92	4.72
Flutolanil	-	-	-	-	-
Azoxystrobin	<0.001*	-	-	-	-
Propiconazole	<0.001*	0.002*	-	-	-
Potassium bicarbonate	0.258	0.010*	<0.001*	-	-
Pyraclostrobin	0.001*	0.539	<0.001*	0.039*	-

*significant at $p \leq 0.05$

Relative growth differed significantly by species (Kruskal-Wallis test *p* <0.001), which *post-hoc* analysis, using the Dunnett's T3 Test for uneven sample sizes, revealed was due to *A. campestris* growing significantly more than *M. oreades* (*p* <0.001), *B. plumbea* (*p* = 0.013), and *H. utriformis* (*p* <0.001) overall.

As listed in Table 9, relative growth also differed significantly by isolate (ANOVA, df = 7, F = 9.01, *p* <0.001). LSD *post-hoc* analysis showed that isolates M1 and M3 were significantly different, despite being from rings located relatively close to each other geographically. M1 and M3 were not significantly different, however, from M2, which was isolated from a ring located some 60 miles away. A1 and A3, and A2 and A3 were significantly different, but only marginally.

Table 9: Significance values for difference in relative growth for each isolate tested, as determined by LSD analysis *pot-hoc* to ANOVA

	Isolate							
	M1	M2	M3	A1	A2	A3	BP	HU
M1	-	-	-	-	-	-	-	-
M2	0.306	-	-	-	-	-	-	-
M3	0.004*	0.067	-	-	-	-	-	-
A1	<0.001*	<0.001*	0.009*	-	-	-	-	-
A2	<0.001*	<0.001*	0.010*	0.958	-	-	-	-
A3	0.001*	0.015*	0.548	0.043*	0.049*	-	-	-
BP	0.055	0.372	0.342	<0.001*	<0.001*	0.120	-	-
HU	0.615	0.601	0.019*	<0.001*	<0.001*	0.003*	0.157	-

*significant at $p \leq 0.05$

4.3.1.4 Discussion

The most unexpected outcome of this experiment was the clear efficacy of propiconazole in inhibiting growth of the fairy ring fungi tested. Propiconazole is the sole active ingredient of the product tested, Banner Maxx, which is not labelled for control of fairy ring. The Banner Maxx product label claims control of dollar spot (*Sclerotinia homoeocarpa*), Fusarium patch (*Microdochium nivale*), anthracnose (*Colletotrichum graminicola*), and brown patch (*Rhizoctonia solani*) on managed amenity turf and amenity grassland (http://www.greencast.co.uk/media/114586/banner%20maxx%20label%20-%20joint%20aug09.pdf).

Few previous studies appear to have investigated propiconazole as a treatment for fairy ring, but Miller (2010) did include it in his *in vitro* mycelial growth assay. Miller (2010) tested an unspecified *Marasmius* species in his control experiment and, whilst triticonazole was the significantly most effective chemical, the results for propiconazole were also relatively effective and statistically comparable with those of tebuconazole and triadimefon.

Miller (2010) also tested seven *Bovista dermoxantha* isolates, which, although their responses were significantly different at times, on average, showed the least growth when treated with tebuconazole. Propiconazole was second most-effective, behind tebuconazole, on a par with triticonazole in treating *B. dermoxantha*, and flutolanil was one of the least effective and most variable treatments when it came to growth of *B. dermoxantha*. The success of propiconazole in treating BP (*Bovista plumbea)*, as a close relative of *B. dermoxantha*, in this investigation appears to be in agreement with Miller's study.

North Carolina State University's (2014) review of fungicide efficacy in treating fairy ring rates propiconazole as having 'good control when disease pressure is high, or excellent control when disease pressure is moderate'. Azoxystrobin and pyraclostrobin receive the same 'good' rating, both independently and when used together. Flutolanil is given a lower efficacy rating of 'good control when disease pressure is moderate, excellent control when disease pressure is low'. This publication does not, however, explain how these ratings were attributed, on what data they are based, on what species they have been tested, or what type of fairy ring symptom.

Moderately effective inhibition was provided by azoxystrobin, the only fungicide with fairy ring on the product label in the UK, with the exception of isolates M2 and BP. Azoxystrobin was significantly more effective overall than flutolanil (p <0.001) and potassium bicarbonate (p = 0.010), but significantly less effective than propiconazole (p = 0.002).

Isolate M2, from Royal Liverpool Golf Club, was the only isolate that was known to have come into contact with one of the chemicals in the past. The Head Greenkeeper had stated that the golf practice area from which the sample was isolated had been treated in the past with azoxystrobin, but not in the last year or so. M2 was isolated from a fairly large ring that was probably several years old, so was likely to have experienced the azoxystrobin treatment. During this experiment, the growth of M2 on azoxystrobin was significantly greater than that of M1 and M3 (p <0.001). As M1 and M3 were both from Harper Adams University and were not expected to have come into contact with any of the chemicals, this could possibly be evidence that M2 has developed some resistance as a result of previous exposure to azoxystrobin.

4.3.1.5 Conclusion

Propiconazole was the most effective chemical in controlling growth of fairy ring isolates *in vitro* at the lowest dilution rates in every case, despite the product, Banner Maxx, not being labelled for treatment of fairy ring. Preliminary tests leading up to this experiment were giving similar indications, but further investigations would still be advisable in order to confirm these significant results.

Flutolanil, the fungicide often recommended in the American literature for control of fairy ring, was surprisingly ineffective and results showed significantly better efficacy in control of the isolates tested was offered by azoxystrobin, which is the only available product in the UK that is currently labelled for control of fairy ring.

Whilst not amongst the best performers, potassium bicarbonate did show some efficacy in controlling fairy ring isolate growth, suggesting that it may have some potential as a cost-effective alternative to fungicides. This could be particularly useful for golf courses with lower maintenance budgets, for whom fungicides may not be affordable.

4.3.2 Mutual antagonism

4.3.2.1 Introduction

Many species of fungi produce antibiotic metabolites in order to antagonise and, hence, gain a competitive advantage over other organisms. Since early investigations, such as those by Coville (1897) and Bayliss (1911), there has been suggestion that some fairy ring species also produce self-inhibiting metabolites that can lead to them eliminating themselves or each other. This may explain why rings on slopes usually lack a lower half, as these metabolites may be washed downhill by rainwater for example, eliminating the bottom half, and why, when two rings meet, they can partially or even fully disappear (Smith, 1980). Following comprehensive investigations into fairy ring biology, both Coville (1897) and Bayliss (1911) put forward theories that the action of fairy ring fungi in the soil resulted in the secretion of some kind of toxic product; the exact nature and action of any such self-inhibitory metabolites, however, remain unidentified.

Several studies have demonstrated the ability of the common fairy ring-forming fungus *Marasmius oreades* (Bolton) Fr. to inhibit the growth of itself and other fungal species, both *in vitro* and in the field (Smith and Rupps, 1978; Smith, 1978; Smith, 1980b). In *in vitro* tests, Smith and Rupps (1978) were the first to report ‘mutual antagonism’ between *M. oreades* isolates paired in Petri dishes. They claimed this was indicated by the clear inhibition zone that occurred between the two isolates where mycelium failed to grow. From the isolates obtained from three different lawns in or near Saskatoon, Canada, they reported that most isolates from different localities, from the same ring, and from the same culture did not grow in contact with each other when paired in Petri dishes and displayed this characteristic clear inhibition zone.

Around this time, Lebeau (1975) reported similar results after carrying out the same experiment on an unidentified low temperature basidiomycete responsible for causing a snow mould on turfgrass. In contrast, Smith and Arsvoll (1975) noted that this phenomenon is not observed in some turf diseases caused by ascomycetes, whereby they reported that both *Fusarium nivale* (now recognised as *Microdochium nivale* – www.mycobank.org) and *Sclerotinia borealis* grow into or over each other in culture.

A later study by Mallett and Harrison (1988) investigated the genetic relationship between *M. oreades* fairy rings by pairing samples *in vitro*. They reported that the clear inhibition zone between self-paired isolates only occurred at the surface of the medium and that the submerged hyphae were in fact interacting and compatible. They described a clear ‘line of demarcation’ between intraspecific isolates of incompatible genotype, which was not previously recognised by Smith and Rupps (1978).

Whilst testing mutual antagonism *in situ*, studies by Smith (1980) on domestic lawns during the 1970s showed that, following the mixing of soil in areas infested with *M. oreades* fairy rings through rotovation, fairy rings did not reoccur on the lawns in several years after treatment. This prompted interest at the time as a potential chemical-free solution to fairy rings as a disease of turf, yet in the past 30+ years, no further research appears to have been undertaken on fairy ring antagonism and effective treatment techniques are not well-established in the field.

Smith and his team focussed predominantly on *M. oreades*, but there is potential for other fairy ring species to act similarly antagonistically. In this study, another common fairy ring species, *Agaricus campestris* L., was also tested to see if it exhibited antagonistic behaviour *in vitro* similar to that described by Smith and Rupps (1978) with *M. oreades.*

4.3.2.2 Methodology

Potential interspecific, intraspecific and self-antagonism of fairy ring-forming fungus isolates was investigated using an *in vitro* mycelial growth assay from Marx (1969) and results were recorded using a variation on the classification system described by Holdenrieder (1984).

Fungal cultures were created using the spore drop method, whereby a section of the gills from a fresh basidiocarp from each fairy ring shown in Table 10 was suspended over a Petri dish of potato dextrose agar (39 g PDA in 1 L distilled water) for several hours to allow the spores to drop onto the agar surface. Spore samples were left on a laboratory bench at room temperature (≈20°C), where they germinated within 48 hours. As single spores were not isolated from the cultures, they were likely to be dikaryotic. This was confirmed through microscopic inspection of the cultures, which revealed that they all had clamp connections.

Pairs of 5 mm plugs taken from the outer edge of the fungal cultures were placed 3 cm apart, with each plug 1.5 cm from the centre line, in Petri dishes containing PDA and then sealed with Parafilm. Two *M. oreades* isolates and one *A. campestris* isolate were tested against themselves (M1+M1; M2+M2; A+A – see Table 10), against a different isolate of the same species (M1+M2), and against isolates of a different species (M1+A; M2+A). Ten replicates of each isolate combination were incubated in the dark at 25°C for 14 days. The diameter of mycelial growth of each isolate on each plate was measured from edge to edge along the centre line at which the plug was placed and again at a right angle to it. In order to calculate a representative area of mycelial growth for each isolate, an average radius was calculated as half the average diameter, minus the 5 mm plug, which was then squared and multiplied by pi to give an area for each isolate in the assumption that it was a circle. The interaction between the two isolates on each plate was observed and categorised as either:

1 – the two isolates exist side by side, evidently without interacting;
2 – a mycelium-free inhibition zone forms between the two isolates, which stop expanding; or
3 – one isolate grows around or over the other, suggesting one is dominant over the other.

Table 10: Isolates used for the antagonism mycelial growth assay

Isolate no.	**Species**	**Origin**	**Grass**	**Soil type**	**Year of isolation**
M1	*Marasmius oreades*	Golf course, Hoylake, Merseyside, UK	*Festuca* spp.	Sand	2014
M2	*Marasmius oreades*	Rugby pitch, Newport, Shropshire, UK	*Poa annua* L., *Lolium* sp.	Loam	2014
A	*Agaricus campestris*	Rugby pitch, Newport, Shropshire, UK	*Poa annua* L., *Lolium* sp.	Loam	2014

4.3.2.3 Results

As shown in Figure 49, isolates M1, M2, and A all displayed a mycelium-free inhibition zone when paired with themselves in culture. In almost all of these cases (Table 11), each mycelium occupied approximately 50% of the plate and a mycelium-free inhibition zone could be seen between the individuals. The pair appeared to reach an equilibrium, where they existed side-by-side and neither achieved a competitive advantage over the other. Although the width of the inhibition zones was not formally measured, it was informally observed to vary within species, with the zone between M1 isolates being far wider and more distinct than that of the M2 isolates. The width of the inhibition zone between A isolates was comparable with that of the M1 isolates.

For all other isolate combinations, the mycelia either existed side-by-side with no apparent interaction or one mycelium was clearly dominant over the other. Where M2 was paired with A, M2 surrounded A and occupied from three to five times more of the plate in every replicate. Where M1 was paired with M2 or A, mycelia existed side-by-side without interaction in 50% of the replicates and M1 overgrew the other isolate in the other 50%. Overall, from the visual observations of interactions between pairs, M1 grew the most vigorously in culture and was the most dominant isolate when paired with others.

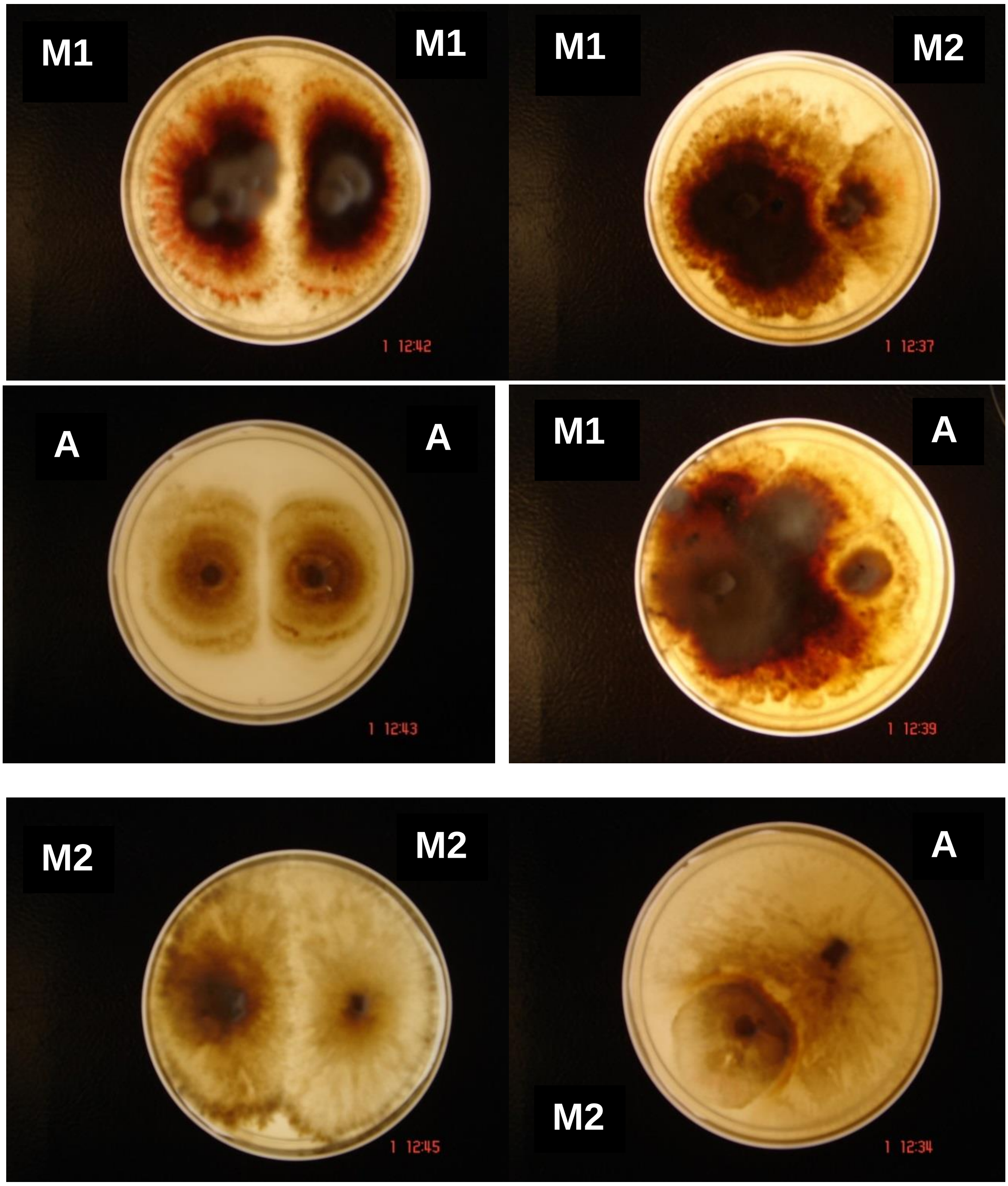


Figure 49: Examples of interactions of each isolate combination tested, including self-pairing (M1+M1, top left; A+A, middle left; and M2+M2, bottom left), intraspecific (M1+M2, top right), and interspecific (M1+A, middle right; and M2+A, bottom right). Note: photographs were taken seven days after the date of final measurement, during which time the samples were stored at room temperature.

Table 11: Interaction categorisation of fairy ring isolate combinations, where 1) the two isolates exist side by side, without interacting; 2) a mycelium-free inhibition zone forms between the two isolates, which stop expanding; or 3) one isolate grows around or over the other, suggesting one is dominant over the other.

Isolate 1	Isolate 2	Interaction number
M1	M1	**2** (80%); 1 (20%)
M2	M2	**2** (100%)
A	A	**2** (100%)
M1	M2	**1** (50%); **3** (50%)
M1	A	**1** (50%); **3** (50%)
M2	A	**3** (100%)

4.3.2.4 Discussion

The results have shown that a clear mycelium-free inhibition zone only forms when an isolate is paired with itself. When paired with a different species or another of the same species, they either exist side-by-side without an inhibition zone or one is dominant over the other.

The inhibition zones seen between *M. oreades* mycelia from the same isolate were similarly described by Smith and Rupps (1978). Smith and Rupps (1978) also reported that this interaction occurred between their *M. oreades* isolates taken from three different rings at different locations, although this was not the case in this study. Here, there was a notable difference in vigour between the *M. oreades* isolates M1 and M2, with M1 either engulfing M2 or existing next to it without an apparent inhibitory interaction in all cases. When growing in culture, M1 would always form a denser and more deeply pigmented mycelium and would generally grow more quickly than M2.

The fact that only two *M. oreades* isolates from different localities were used in this experiment is obviously a major limiting factor in drawing conclusions on this, but worth noting is that Smith and Rupps (1978) did not give any details about the sites from which their isolates were obtained and only stated that they were from fairy rings from three lawns in or near Saskatoon, Canada. In this experiment, the two *M. oreades* rings sampled were from different soil types, different turf species, under differing usage and management, and were from localities >60 miles apart. It is reasonable to assume that they have greater potential to be genetically more diverse and dissimilar in their response to environmental stimuli than those sampled by Smith and Rupps (1978) and, therefore, are more likely to have varying responses when cultured *in vitro*.

Whilst Smith and Rupps (1978) suggested that the inhibition zones between self-paired isolates were evidence of self-antagonism, Mallett and Harrison (1988) interpreted their *in vitro M. oreades* isolate pairing results differently. Mallet and Harrison (1988) reported that their dikaryotic *M. oreades* isolates ‘grew into each other’ when paired with themselves. They claimed that, whilst they did observe zones devoid of hyphae on the surface of the medium, submerged hyphae from the two isolates were intermingling freely (Figure 50). This raises questions as to whether the mycelium-free inhibition zones observed herein were in fact devoid of mycelium through the depth of the media or whether they appeared superficially devoid of mycelium from the surface (microscopic inspections would have been necessary in order to determine this, which was not carried out at the time). Mallett and Harrison (1988), therefore, proffer that self-paired isolates are compatible (rather than antagonistic), yet offer no explanation as to the cause of the inhibition zone at the surface of the culture.

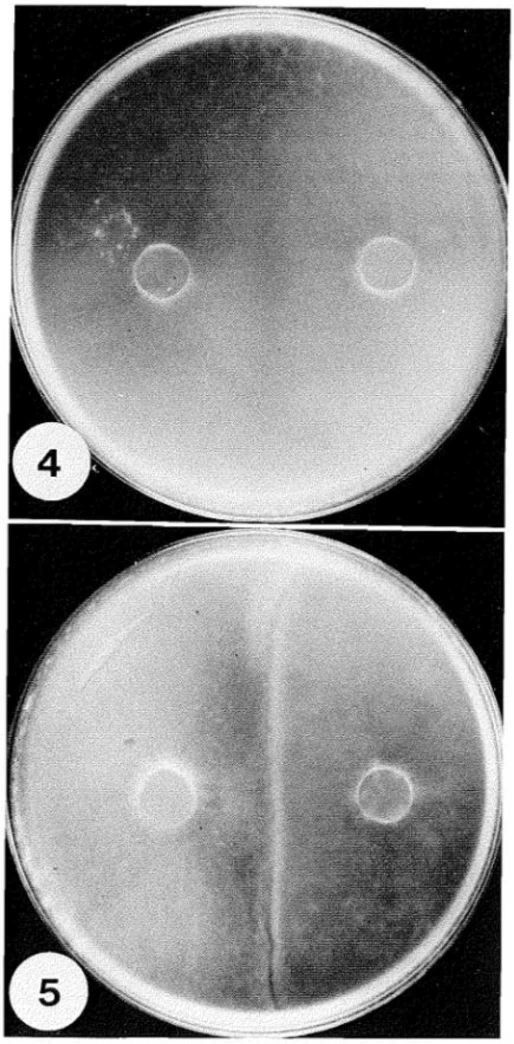


FIG. 4. A dikaryotic isolate of *M. oreades* paired with itself on malt agar. No line of demarcation. FIG. 5. Two vegetatively incompatible isolates of *M. oreades* paired on malt agar. A line of demarcation forms at the confronting margins.

Figure 50: Images from Mallett and Harrison (1988) where they distinguish between dikaryotic self-paired isolates, with no line of demarcation (top), and two vegetatively incompatible isolates, with a line of demarcation formed at the confronting margin (bottom)

In contrast to compatible self-pairings, Mallett and Harrison (1988) reported that intraspecific pairings of *M. oreades* isolates with different mating-type genotypes produced a ‘visible line of demarcation at the confronting margins’ (Figure 50). This line of demarcation, which they say was comprised of knots of fungal hyphae, they claim is the indicator that two isolates are vegetatively incompatible. From their 23 *M. oreades* samples, they identified 13 unique genotypes by distinguishing compatible and incompatible samples through pairing experiments and presence or absence of the line of demarcation. As an indicator of incompatibility between isolates, it could be argued that Mallett and Harrison's (1988) line of demarcation is the true indicator of fungal antagonism, rather than Holdenreider's (1984) mycelium-free inhibition zone.

On review of the isolate combinations tested, M2+A, *M. oreades* 2 and *A. campestris* (Figure 49, bottom right) exhibited a line of demarcation as described by Mallett and Harrison (1988), indicating interspecific antagonism between this pair. It is difficult to confirm a distinct line of demarcation between intraspecific combination M1+M2 and interspecific combination M1+A, as the growth of M1 is so vigorous and pigmented that it masks any such feature.

Since the past literature has focussed on *M. oreades*, a notable observation of this study was that *A. campestris* exhibits similar behaviour to that of *M. oreades*. This is the first recorded incidence of this. Whilst the fairy ring symptoms expressed in the field by *A. campestris* are generally less severe and less persistent than those of *M. oreades* (see Chapter 3.0 - The Questionnaire), both are common species that produce aesthetic problems on golf courses that often warrant treatment. If *A. campestris* responds similarly to *M. oreades* in field studies like those conducted by Smith and Rupps (1978), then the initiation of self-antagonism in the field through mixing of fairy ring soils may be a potential solution to disease symptoms.

This study has shown that *M. oreades* is dominant over *A. campestris in vitro*, but a far greater number of species combinations would need to be tested in order to draw any further conclusions on interspecific antagonism between fairy ring species. As with any method of biocontrol, the danger of using one species to control another is of exacerbating the problem by introducing a more damaging pathogen than the one trying to be controlled and the potential of a species to eliminate itself through self-antagonism is perhaps a more appealing option.

In further experiments, isolate combinations would also need to be tested on a variety of nutrient media, as Ayer and Craw (1989) found that the production of secondary metabolites by *M. oreades*, some of which may be linked to antagonistic behaviour, varied in isolates grown on PDA in comparison to those grown on malt agar. Whilst Ayer and Craw (1989) described the chemical structure and properties of several metabolites produced by *M. oreades* in great detail, there is still no certainty as to their roles.

4.3.2.5 Conclusion

Besides being the basis of the development of many fungicides, fungal antagonism as a means of controlling target species is a well-established technique in many industries; from forestry to medicine, and is certainly a concept that should be explored further with regard to fairy ring control.

Smith's aforementioned work showed evidence that common fairy ring fungus *M. oreades* may exhibit mutual antagonism and inhibit its own growth under certain circumstances. Results of this experiment were comparable with those seen by Smith and Rupps (1978), as isolates paired with themselves appeared to have a mycelium-free inhibition zone between them.

However, findings by Mallett and Harrison (1988) suggest that the perceived inhibition zones observed between isolates paired with themselves may only be at the surface of the culture and that hyphae submerged in the medium are in fact interacting and compatible. This would contradict the theory that isolates are antagonistic when paired with themselves. As the inhibition zones were not inspected microscopically to confirm the absence of mycelium in this investigation, evidence of mutual antagonism cannot be concluded.

4.4 Acknowledgements

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5.0 Controlling Fairy Rings *In Situ*

5.1 Abstract

In the first of two control experiments carried out on fairy rings *in situ*, the efficacy of the only three products currently marketed for fairy ring control in the UK (the fungicide Heritage Maxx, the surfactant Clearing, and the biocontrol AquaCept) was tested. Plots on a large *Marasmius oreades* fairy ring at two sites were treated with the products in isolation and in combination with aeration and/or wetting agent.

Plots were assessed for visual turf quality and soil moisture content directly before the first treatment and six weeks after the second treatment. The change in turf quality before and after treatment did not vary by treatment. Soil moisture increased significantly in plots that had received aeration, but none of the three products tested or wetting agent had any significant effect on soil moisture content. The hydrophobicity of the rings tested, however, is likely to have affected the extent to which the products could penetrate into the ring profile.

In the second year, three non-hydrophobic type-2 *Agaricus campestris* fairy rings by the side of a football pitch at Harper Adams University, Shropshire were divided into plots and treated with either aeration and wetting agent alone or plus Heritage Maxx (azoxystrobin), Banner Maxx (propiconazole), or potassium bicarbonate. Plots were assessed for visual turf quality, percentage symptom cover, and soil moisture content directly before the first treatment and four weeks after the second treatment.

At the time of final assessment, the configuration of the football pitch had been changed so that the rings were within the playing area and the turf had been damaged to the point that turf quality and percentage symptom cover could not be

reliably assessed. The change in soil moisture before and after application did not vary by treatment.

5.2 Introduction

The majority of information available on fairy ring control originates in the USA, where they are generally using fungicides, such as flutolanil, that are not available for amenity use here in the UK (Fidanza, 2002; Fidanza, 2009; Miller, 2010; NCSU, 2014). This part of the project, therefore, aimed to generate impartial data on fairy ring control, specific to UK needs, where it is currently lacking.

The *in situ* experiments aimed to: -

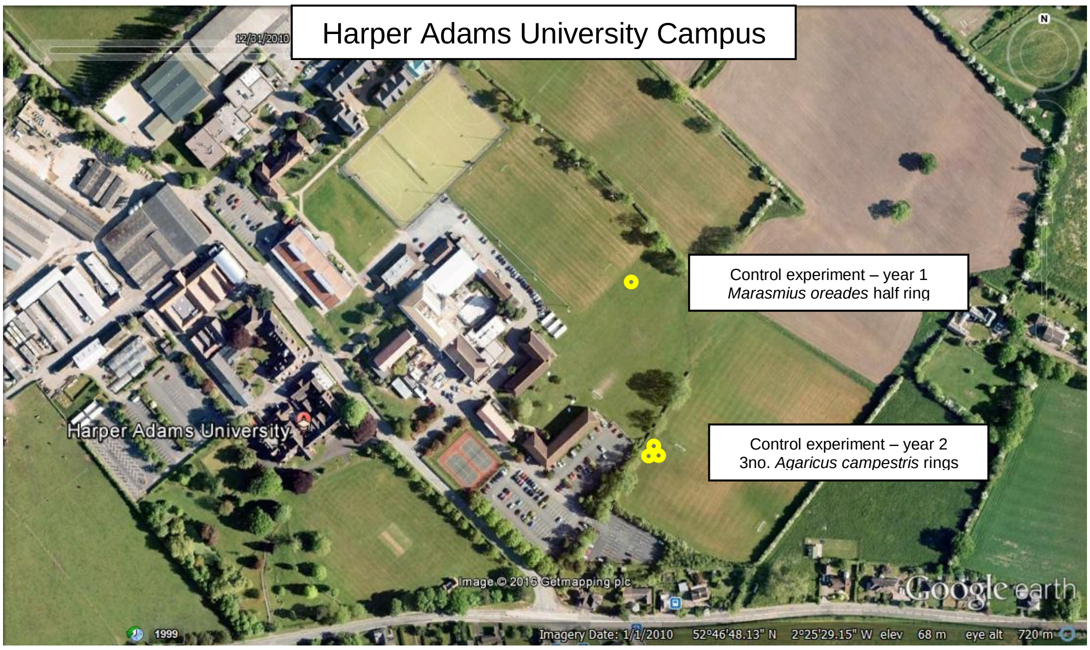
1. Test the efficacy of the products currently marketed for fairy ring control in the UK compared to products reported in USA studies

2. Measure the necessity of cultural practices, such as aeration and use of wetting agent

3 Investigate novel control methods, where little or no data on their efficacy currently exist

4. Look for differences in response between fairy ring isolates and species to attempted control methods

This chapter is divided into two parts, for each of the fairy ring seasons studied. Year one focused on two *Marasmius oreades* fairy rings – one at Harper Adams University, Edgmond, Shropshire and one at Royal Liverpool Golf Club, Hoylake, Merseyside, and year two was based on three *Agaricus campestris* rings at Harper Adams University. Locations of the fairy rings studied at each site are shown in Figures 51 and 52.


Harper Adams University Campus

Control experiment – year 1
Marasmius oreades half ring

Control experiment – year 2
3no. *Agaricus campestris* rings

Figure 51: Locations of the *M. oreades* fairy ring and three *A. campestris* fairy rings at Harper Adams University used for the control experiments, shown in yellow


Royal Liverpool Golf Club

Control experiment – year 1
Marasmius oreades oval ring

Figure 52: Location of the *M. oreades* fairy ring at Royal Liverpool Golf Club used for the year 1 control experiment, shown in yellow

5.3 Experiments

5.3.1 Year one

5.3.1.1 Introduction

Control treatments that are effective in laboratory experiments may yield completely different results when they are tested in the field. The amount of variables that the natural environment introduces has potential to significantly alter results. In order to test the efficacy of fairy ring control treatments *in situ*, experiments were set up on fairy rings growing in sports turf over two consecutive growing seasons.

As suitably infected areas could not be identified in advance within the limited timescale of this project, field control experiments for both years one and two involved curative applications once fairy rings had already appeared, rather than investigating preventative treatment.

As no impartial data relating to control of fairy rings in the UK was available, the first year of the investigation aimed to generate data to either support or question the efficacy of products currently marketed for fairy ring control in the UK. It also aimed to identify whether aeration and the use of a wetting agent are indeed a worthwhile preparatory treatment to use in advance of chemical application.

5.3.1.2 Methodology

In late summer to autumn of 2013, the first curative control experiment was carried out on fairy rings on sports turf *in situ*. The experiment aimed to test the efficacy of what were evidently the only three products licenced in the UK at that time that were marketed specifically for fairy ring control, as indicated on the product labels. These products were as follows:-

1. Heritage Maxx by Syngenta
   A systemic strobilurin fungicide containing 95 g/l of the active ingredient azoxystrobin. The label states that the product is approved for control of type-2 fairy rings only (http://www.greencast.co.uk/uk/products-offers/fungicides/heritage-maxx.aspx).

2. Clearing by Vitax
   A blend of surfactants developed to assist the penetration of water in dry soil, claiming '*effective treatment of fairy rings*' through tackling the soil hydrophobicity they may induce (http://www.vitax.co.uk/amenity/clearing/).

3. AquaCept by Symbio
   A biological treatment containing up to 10% *Bacillus* sp. and *Pseudomonas putida* bacteria, which are claimed to outcompete fairy ring fungi for nutrients and produce enzymes that break down the hydrophobins produced by fairy ring fungi that induce soil hydrophobicity (http://www.symbio.co.uk/files/datasheets/sports_turf_datasheets/Sports%20turf%20datasheets%202014/Symbio%20AquaCept.pdf and http://www.symbio.co.uk/files/2015%20sds/Symbio%20AquaCept%20SDS.pdf).

These three treatments were incorporated into a factorial design that tested each on its own and in combination with the wetting agent 'Revolution' by Aquatrols, which is labelled for the improvement of water distribution within the rootzone rather than to treat disease, and/or manual soil aeration through forking to a depth of approximately 25 cm. The use of wetting agents and aeration techniques are both considered good cultural practice on the golf course in promoting turf health regardless of disease incidence, but are also widely accepted, anecdotally, as tools to help combat the soil hydrophobicity associated with fairy rings by helping to rewet the soil and physically break up the fairy ring mycelium, respectively. Besides this, they are also thought to assist in delivering control products into the rootzone and increasing the level of contact with the fungus, although there are no existing data to support this theory. Hence, the application of Heritage Maxx, Clearing, and AquaCept was expected to be more effective in suppressing fairy ring disease symptoms when applied with wetting agent or aeration than when applied alone.

Furthermore, the treatments were expected to be at their most effective when applied with both wetting agent and aeration.

In order to avoid any potential variation in response between different fairy rings, each ring tested needed to be large enough to accommodate the necessary number of replicates for each treatment, plus untreated control plots, within its own circumference. The full set of treatments would then be replicated again by applying them to several different fairy rings.

The fairy rings sampled needed to be large in size, accessible, in a suitable location to be experimented on, easy to visibly distinguish by eye, and needed to continue to be visibly distinguishable for long enough to facilitate the repeated application of the control treatments and subsequent evaluation. It was also desirable that the appearance of the disease symptoms was approximately constant all the way around the ring, which would aid in assessing any change.

Whilst it was difficult to find fairy rings that met these criteria on which to experiment, two useable rings were identified; one growing by the side of the golf practice area at Royal Liverpool Golf Club, a links golf course at Hoylake, Merseyside, and the other growing by the side of the rugby pitch at Harper Adams University, Shropshire. Both were identified as being caused by the fungus *Marasmius oreades*, from examination of the fruiting bodies, which had started growing around the edges of the rings earlier on in the season.

Both rings selected exhibited type-1, type-2, and type-3 symptoms. The size of the rings, in both overall diameter and width of the symptomatic zone, particularly the type-1 area, suggested that they were well-established and mature.

The ring at Royal Liverpool Golf Club, Hoylake was a large, complete oval (possibly as a result of two rings joining together) and located in an area where it could be worked on without disrupting play for the golfers. The grass here was a fescue mix, growing on a sandy soil, which was regularly mowed to a height of 40 mm and also irrigated as and when required as part of a maintenance regime. The Head Greenkeeper stated that, although there had been various attempts in the past to control fairy rings in this area (which were numerous in the immediate locality), there had been no chemical, fertiliser, or surfactant treatments applied during the experiment, from August 2013 to November 2013, or in the months preceding it. There had also been no irrigation applied in that area during the period of the experiment or in the several weeks preceding it, as rain water had kept the turf sufficiently wet.

The ring by the rugby pitch at Harper Adams University, Shropshire was a large half ring located off the pitch, approximately 10 m east of the eastern goal posts. The turf here was a mix of grasses, including ryegrass and *Poa annua*, and also other vegetation, such as clover, growing on a loamy soil. The Grounds Manager stated that this area was not subject to any formal maintenance regime, other than mowing as and when required, according to weather conditions. No chemical, fertiliser, or surfactant treatments had ever been applied to this area, to the Grounds Manager's knowledge.

In August 2013, twelve 1 m^2 plots were marked out around the circumference of each fairy ring with twine and plastic pegs, so that the active zone of the ring ran across the middle of each plot (Figures 53 and 54). Each of the twelve plots was numbered and randomly assigned to one of the treatment numbers shown in Table 12.

Figure 53: Twelve 1 m^2 plots marked out around the active zone of the Harper Adams *M. oreades* ring ready for the first set of treatments

Figure 54: Twelve 1 m^2 plots marked out around the active zone of the Hoylake (Royal Liverpool Golf Club) *M. oreades* ring ready for the first set of treatments

Table 12: The range of treatments used and their abbreviations

No.	Treatment	Abbreviation
1	Heritage Maxx, Revolution and aeration	HWA
2	Heritage Maxx and aeration	HOA
3	Heritage Maxx only	HOO
4	Clearing, Revolution and aeration	CWA
5	Clearing and aeration	COA
6	Clearing only	COO
7	AquaCept, Revolution and aeration	AqWA
8	AquaCept and aeration	AqOA
9	AquaCept only	AqOO
10	Revolution and aeration	OWA
11	Aeration only	OOA
12	Untreated	OOO

Whilst a full factorial design would have been desirable, fairy rings of sufficient size to accommodate the full range of treatments could not be sourced prior to the start of the experiment, hence, as can be seen from Table 12, a Revolution only treatment and Revolution plus chemical treatment were not included.

Fairy ring symptoms were to be measured before, during and after treatment. A subjective visual assessment of turf quality (in line with STRI's Standard Operating Procedure No. 1B0712 in Appendix II), soil moisture content, and quantification of fungal biomass within the soil were all used to measure the effect of the treatments. The first set of measurements was taken immediately prior to the first application of the treatments.

Visual assessment, as outlined in Appendix II, scored the turf quality of each plot qualitatively on a scale of 1 to 10, with 1 representing '*turf in very poor condition, largely dead grass or bare ground*' and 10 indicating '*turf perfect*'. As both rings were fairly wide, they generally occupied most of each plot. Turf quality scores were, therefore, assigned as an average for each plot as a whole rather than for each plot at its worst point.

Soil moisture content (%) was measured at a depth of 7.5 cm using the Fieldscout TDR 100 soil moisture meter (Spectrum Technologies, Inc.). This device works on the principle of time-domain reflectometry, whereby percentage volumetric water content is measured between the full depth of the two rods that are inserted into the ground. For each plot, three measurements were taken at equal intervals in the outer zone, centre of the active zone, and inner zone of each fairy ring, equating to nine soil moisture measurements per plot.

Following initial assessment, the treatments were applied. Plots due to be aerated were done so manually to a depth of approximately 25 cm with a fork, firstly due to the impracticalities associated with treating small and interspersed plots mechanically and, secondly, because the tines of machines are not generally long enough to reach the depths associated with *M. oreades*, which is thought to grow up to 50 cm deep into the soil profile (York, 1998).

The wetting agent, Revolution, was then applied to the plots as appropriate using the rate advised for monthly application of 1.9 ml of product in 14.1 ml of water per 1 m^2, as instructed on the product label, using a 5-litre knapsack sprayer with a flat fan nozzle. The plots were then further treated with either fungicide (Heritage Maxx at 0.25 ml of product in 15.75 ml of water per 1 m^2), biological treatment (AquaCept at 0.5 mg of product in 15.5 ml of water per 1 m^2), or surfactant (Clearing at 4 ml of product in 12 ml of water per 1 m^2) if any, using the same sprayer in accordance with the manufacturers' instructions for spot treatments on the product labels. AquaCept, which was granular, was pre-dissolved in warm water first, as per the label instructions. In order to water the treatments in, two litres of water were applied to each plot immediately post-application using a watering can. For consistency, every plot was watered, including the untreated.

Following treatment, some of the plastic pegs marking the corners of the plots were left in place, flush with ground level, so that the plots could be marked out again in the same the place on subsequent visits. One month later, in September 2013, both fairy rings were plotted out and assessed again for turf quality and soil moisture content, and samples taken as before. A second application of treatments was then carried out. Six weeks later, in November 2013 a final assessment of the fairy rings was performed, again, using the methods above.

5.3.1.3 Results

To assess efficacy of the treatments, the data taken before, during and after the fairy rings were treated were compared to look for potential changes, such as increase in soil moisture content, which may represent a reduction in fungal biomass in the soil, or improvement in visual turf quality.

As shown in Table 13, when comparing pre-treatment (August) turf quality with post-treatment (November) turf quality, the majority of plots saw an improvement in turf quality score of 1-2. One plot on the Hoylake ring (Figure 55) and one plot on the Shropshire ring (Figure 56) stayed the same and two plots on the Hoylake ring declined in turf quality by a score of 1.

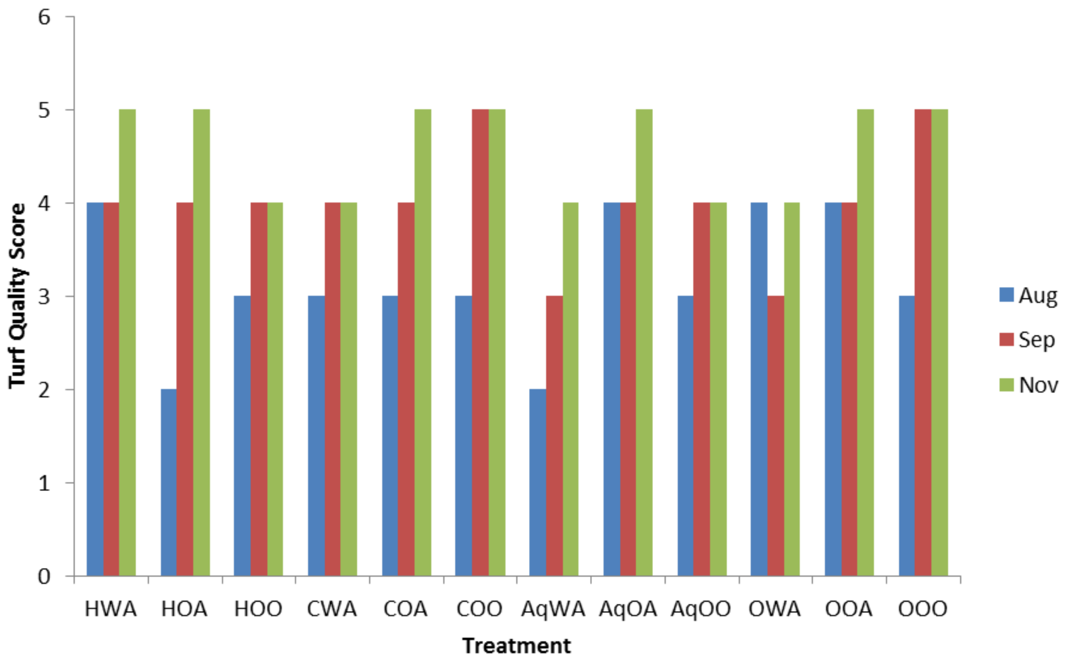


Figure 55: Turf quality of fairy ring plots at Hoylake before, during, and after treatment

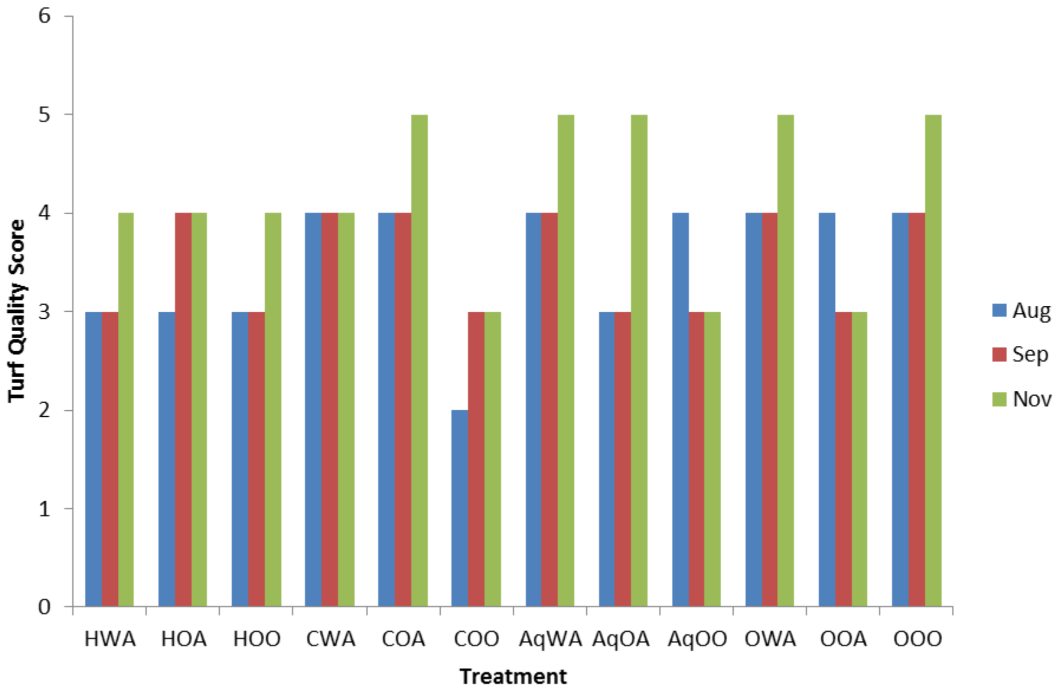


Figure 56: Turf quality of fairy ring plots at Shropshire before, during, and after treatment

Table 13: Change in turf quality of each fairy ring plot between pre-treatment (August) and post-treatment (November)

Abbreviation	Treatment	Change in Turf Quality		
		Shropshire	Hoylake	Average
HWA	Heritage Maxx, Revolution and aeration	+1	+1	+1
HOA	Heritage Maxx and aeration	+3	+1	+2
HOO	Heritage Maxx only	+1	+1	+1
CWA	Clearing, Revolution and aeration	+1	0	+0.5
COA	Clearing and aeration	+2	+1	+1.5
COO	Clearing only	+2	+1	+1.5
AqWA	AquaCept, Revolution and aeration	+2	+1	+1.5
AqOA	AquaCept and aeration	+1	+2	+1.5
AqOO	AquaCept only	+1	-1	0
OWA	Revolution and aeration	0	+1	+0.5
OOA	Aeration only	+1	-1	0
OOO	Untreated	+2	+1	+1.5

Whilst turf quality data for the two rings could be pooled for analysis, as the data sets were not significantly different (t-test, $p = 0.302$), the data were too few and the range of turf quality values too limited to draw any conclusions that could be rigorously supported by statistical analyses. The turf quality of the majority of test plots improved over time, as the turf became wetter into the autumn months. With only two fairy rings and two untreated plots to compare, there is no way to differentiate whether the improvements seen in treated plots are enhanced in comparison to those seen in the untreated plots and no evidence of any improvement in turf quality that can be attributed to treatment given the scores for control plots.

For soil moisture, the set of results taken mid-treatment (i.e. directly before the second application of treatments, in September) was not significantly different from the pre-treatment results taken in August and the mid-treatment data sets were, therefore, excluded from further analyses and only the August and November sets were used

The Shropshire and Hoylake rings were analysed independently, as they were found to be significantly different from each other statistically when their data sets were analysed as a whole (ANOVA $p < 0.001$). The Shropshire ring was found to be much wetter than the Hoylake ring, having approximately twice the soil moisture content. The differences are likely to be a result of varying soil type, location, and management regime.

In order to determine whether the change in soil moisture over time varied between treatments, a repeated measures two-way mixed design ANOVA was used on the data. As the repeated measures ANOVA assumes sphericity of the data, a Mauchly's Test of Sphericity was performed first in order to confirm that the data conformed to sphericity and the repeated measures ANOVA was, hence, a valid test.

For the Shropshire ring, the biggest change in soil moisture was seen in OWA (wetting agent and aeration only), shown in orange (Figure 57). The smallest change was in HWA (Heritage Maxx, wetting agent and aeration), shown in purple. The HWA plot, however, started with a markedly higher soil moisture level than the other plots before the first treatment was applied.

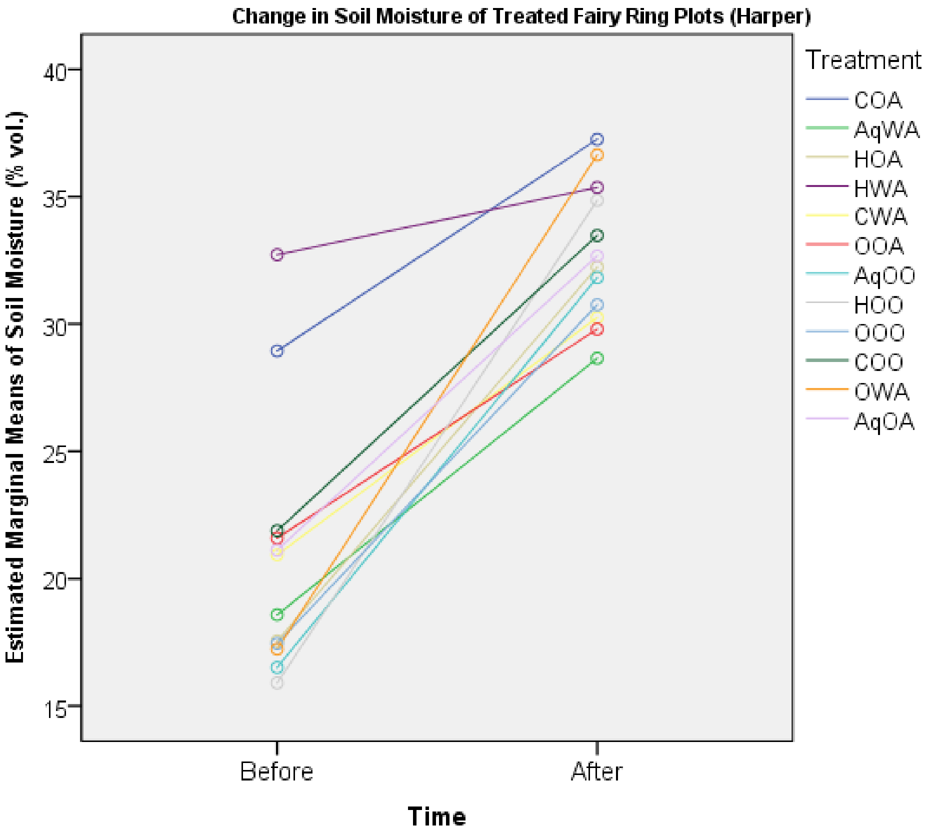


Figure 57: Change in soil moisture content of fairy ring plots at Shropshire before and after treatment

When comparing soil moisture by treatment over time at Shropshire, Maulchly's Test of Sphericity confirmed that the data did conform to sphericity (p = 0.196) and the tests associated with the repeated measures ANOVA showed that soil moisture did vary between treatments over time (Pillai's Trace p= 0.002, Wilks' Lambda p = 0.002, Hotelling's Trace p = 0.001, Roy's Largest Root $p < 0.001$). *Post-hoc* analyses were implemented to isolate where these differences occurred. Parametric tests LSD and Tukey's HSD identified several pairs of differing treatments, although Levene's and Box's tests of variance/covariance showed that variance in the data was not equal and parametric tests, therefore, were not suitable for the data.

The non-parametric test Dunnett's T3, which does not assume equal variances, was then performed *post-hoc* to the repeated measures ANOVA. The test showed six pairs of treatments that were significantly different from each other:

- AqWA and HWA
- HOA and HWA
- OOA and HWA
- AqOO and HWA
- HOO and HWA
- OOO and HWA

Treatment HWA (see Table 13 for abbreviations), Heritage Maxx, wetting agent and aeration, was one of the treatments in every case. As a fungicide well-established in the sports turf market and alongside two practices which help deliver product into the soil profile, HWA was expected to be the most effective of all the treatments.

As shown in Figure 57, however, the HWA plot, and to a lesser extent the VOA plot, were particularly wet, in comparison to the other plots, before the first treatment was applied and did not show a significant increase in soil moisture different to that of the other treatments. On referring to the raw data to investigate these anomalies, it can be seen that the inner and right-hand side of the HWA plot and the outer edge and inner left-hand side of the VOA plot were abnormally wet in comparison to the rest of the ring. The anomalies were represented by a series of higher values, rather than one or two rogue values, and the way they were localised within the plots suggests that moisture pockets had been detected. The moisture pocket measured in the HWA plot before treatment is expected to be the cause of differences picked up by the statistical analyses between HWA and other plots. It is not representative of any post-treatment increase in soil moisture content.

There was, therefore, no evidence to suggest that any of the treatments had an effect on soil moisture.

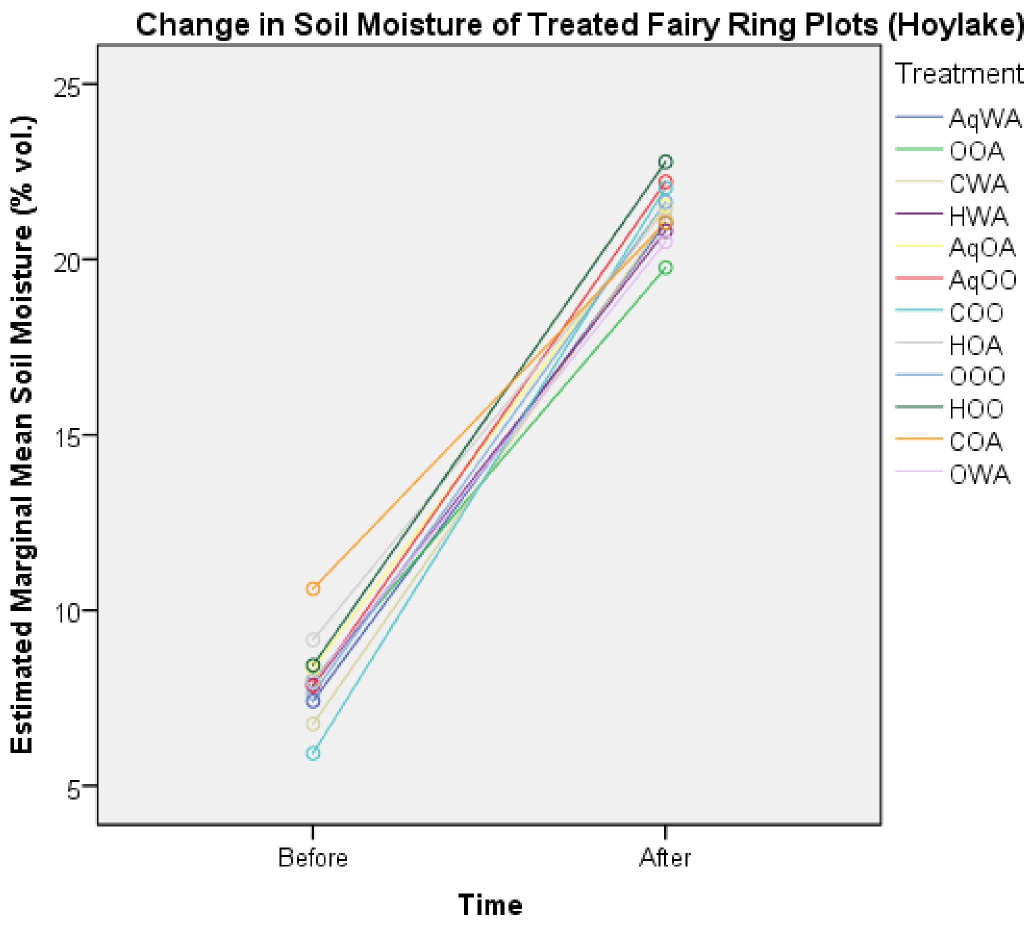


Figure 58: Change in soil moisture content of fairy ring plots at Hoylake before and after treatment

A repeated measures ANOVA performed on the Hoylake data to look for differences between treatments over time showed that there were no significant differences (*p* = 0.564; Figure 58).

Comparison by product, i.e. Heritage Maxx, Clearing or AquaCept, irrespective of whether wetting agent or aeration had also been used, showed that there was no significant difference (*p* = 0.240) in change in soil moisture between products for either ring (Figure 59).

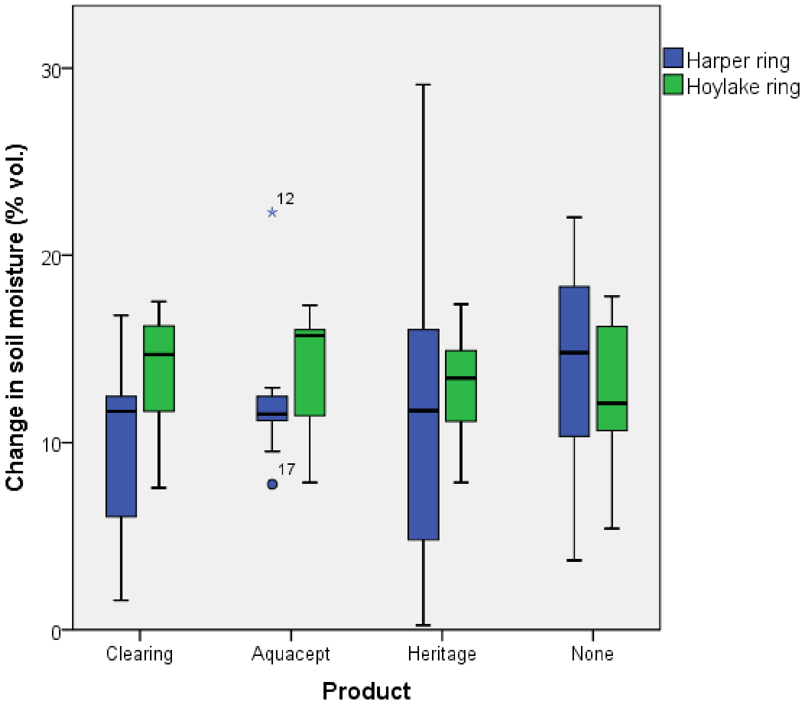


Figure 59: Change in soil moisture over the course of the experiment by product

Figure 60 shows results from both rings pooled, showing there was no significant difference in change of soil moisture between plots that had had the wetting agent Revolution applied pre-treatment and plots that had not (p = 0.359).

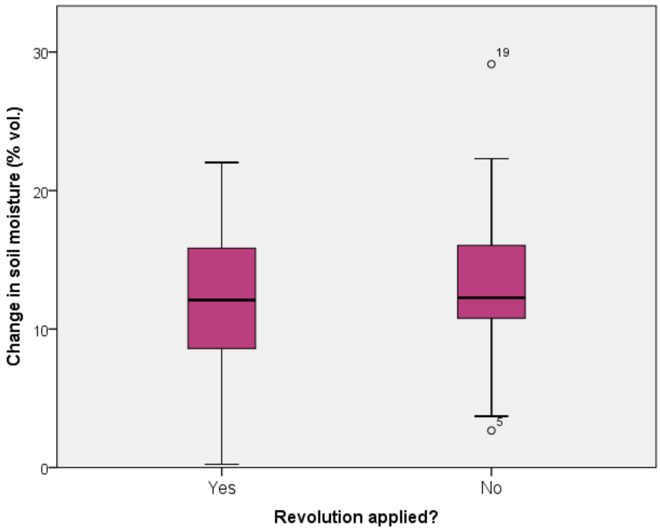


Figure 60: Change in soil moisture before and after treatment for plots that had the wetting agent Revolution applied pre-treatment and plots that did not

As shown in Figure 61, plots that had been aerated manually with a fork prior to treatment were significantly wetter post-treatment than those that had not (p = 0.011).

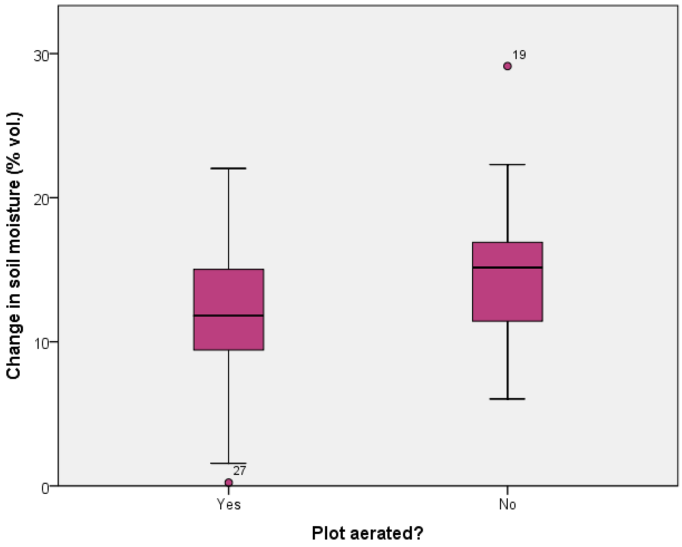


Figure 61: Change in soil moisture before and after treatment for plots that were manually aerated with a fork pre-treatment and plots that were not

5.3.2 Year two

5.3.2.1 Introduction

In late summer to autumn of 2014, an adapted field control experiment was carried out, following the aforementioned findings from the previous year.

It was predicted that the lack of significant results from the first field experiment may have been due to the nature of *M. oreades* as a subject. The density of mycelium in the soil, associated hydrophobicity, and depth of growth exhibited by this species may all contribute to a reduced likelihood of the control treatment penetrating into the soil profile effectively and making contact with the causal fungus. Hence, the control treatments used in year one may not have made sufficient contact with *M. oreades* to have any effect.

It was, therefore, decided that the subsequent year's field experiment should focus on the efficacy of control treatments on an alternative species of fairy ring fungus, rather than *M. oreades*, which may be easier to target within the soil profile.

5.3.2.2 Methodology

In July 2014, a series of complete and incomplete type-2 fairy rings appeared on one of the football pitches at Harper Adams University, Shropshire. Their late appearance in the fairy ring season suggested they were not caused by *M. oreades*. Three of the largest and most well-defined fairy rings were selected on which to apply a new set of control treatments. The rings were later identified as being caused by *A. campestris*, when fruiting bodies began to appear around the edges of all of the selected rings.

The products used in the second year field experiment were:

1. Heritage Maxx by Syngenta
   A systemic strobilurin fungicide containing 95 g/l of the active ingredient azoxystrobin. The label does state that the product is approved for control of type-2 fairy rings only (http://www.greencast.co.uk/uk/products-offers/fungicides/heritage-maxx.aspx)
2. Banner Maxx by Syngenta (which indicated control potential during the preliminary investigations leading up to Part 1 of this chapter)
   A broad spectrum foliar fungicide with systemic properties, containing 156 g/l of the active ingredient propiconazole, advertised for control of several turf diseases, including Fusarium, Dollar Spot, and Anthracnose, but *not* Fairy Ring (http://www.greencast.co.uk/uk/products-offers/fungicides/banner-maxx.aspx)
3. Potassium bicarbonate
   A simple salt that can change the pH of the soil and may make edaphic conditions unfavourable for certain fungi

The three rings selected were adjacent to each other within an area covering approximately $225m^2$. They were large enough to accommodate 15 sample plots around their ring circumference each, but plot size had to be reduced to $0.25m^2$. The grass species here was largely *Poa annua*, but a substantial component of the turf was also herbaceous species, such as clover.

Despite the lack of evidence to suggest any effect of wetting agent in the previous experiment (again, thought to be linked to the impenetrability of *M. oreades* rings), both aeration and wetting agent were this time applied to all sample plots as a matter of good cultural practice, with the exception of the untreated plots.

Using the same application methods as in the previous experiment, the 15 plots on each ring received three replicates of five different randomly-assigned treatments:

1. Heritage Maxx with aeration and Revolution
2. Banner Maxx with aeration and Revolution
3. Potassium bicarbonate with aeration and Revolution
4. Aeration and Revolution only
5. Untreated

First, all plots, with the exception of three untreated plots on each ring, were passed over six times using a manually-operated, rolling aerator with 6 cm-deep tines at approximately 5 cm spacing. Revolution was then applied to these same plots using the rate for monthly application of 1.9 ml of product in 14.1 ml of water per 1 m^2, as instructed on the product label, using a 5-litre flat nozzle knapsack sprayer, before applying Heritage Maxx (at 0.25 ml of product in 15.75 ml of water per 1 m^2), Banner Maxx (at 0.3 ml of product in 15.7 ml of water per 1 m^2) or potassium bicarbonate (at 0.1 mg of product in 15.9 ml of water per 1 m^2, added slowly to cold water to avoid a volatile reaction) using the knapsack sprayer at the rates instructed for spot treatments on the product labels for Heritage Maxx and Banner Maxx and at a rate slightly higher than advised in an article from the magazine Sports Turf Manager (http://archive.lib.msu.edu/tic.stnew/article/1996jun11.pdf) for potassium bicarbonate. All plots were then irrigated immediately post-treatment using a watering can to deliver one litre of water per plot.

The first set of treatments was applied on 17 August 2014 and a second application of treatments was carried out approximately five weeks later, on 23 September 2014. Assessment of the rings was carried out directly prior to the first treatment application and four weeks after the second application, on 23 October 2014.

As with the previous year's experiment, assessment involved a subjective visual assessment of turf quality (in line with STRI's Standard Operating Procedure No. 1B0712 at Appendix II), whereby the turf quality of each plot was scored qualitatively on a scale of 1 to 10, with 1 representing '*turf in very poor condition, largely dead grass or bare ground*' and 10 indicating '*turf perfect*'. Turf quality scores were assigned as an average for each plot as a whole rather than for each plot at its worst point. A subjective estimation of percentage symptom cover was also recorded for each plot, to the nearest 10%.

Soil moisture content (%) was measured at a depth of 7.5 cm using the Fieldscout TDR 100 soil moisture meter (Spectrum Technologies, Inc.). For each plot, three measurements were taken at equal intervals in the outer zone, centre of the active zone, and inner zone of each fairy ring, equating to nine soil moisture measurements per plot.

After the soil moisture measurements had been taken, a 20mm-diameter open-sided soil auger with a foot pedal was used to extract a soil core from the point between which the two Fieldscout probes had entered the ground for the measurement taken in the centre of the active zone. Each core extracted was tested for soil hydrophobicity immediately, *in situ* using the water drop penetration time (WDPT) test (Dekker *et al*., 2009) in the uppermost layer of the soil profile, directly below the thatch (see Chapter 6 for photograph of WDPT test). For the WDPT test, a Gilson pipetman P100 pipette was used to deliver a 50 µl droplet of sterile distilled water (as recommended by Dekker and Ritsema, 2009) by holding the pipette approximately 3 cm above the sample, at a right angle to it.

A small, supplementary experiment was carried out alongside the second year field control experiment in order to test whether soil moisture content was representative of the incidence of fairy ring symptoms. A type-1 *M.oreades* ring and the normal turf both within it and directly surrounding it at Harper Adams University (which was not used in any of the other experiments) was divided into a square grid of 121 484 cm^2 plots. On 9th August 2014, each plot was measured with the soil moisture meter at a depth of 3.8 cm and presence or absence of symptoms and turf quality score recorded.

Data were analysed statistically by ANOVA, Mann-Whitney U-Test, and linear regression using IBM SPSS Statistics (version 23).

5.3.2.3 Results

In the weeks between the second application of treatments and the final assessment, the configuration of the football pitches was changed and the area containing the rings, which was once off the north-west edge of the pitch, where foot traffic appeared limited, became the area of play directly in front of the football goal (Figures 62 and 63). The turf in this area was heavily damaged by game play and marked with imprints from studded football boots and skid marks. The rings were very difficult to visually distinguish and, whilst soil moisture measurements could still be taken, estimations of turf quality and percentage symptom cover could not be made. Hence, no results were acquired in relation to any potential change in turf quality or symptom cover as a result of any of the treatments.

Figure 62: Looking east to *A. campestris* fairy rings on Harper Adams University football pitch at time of first treatment

Figure 63: Looking south-west to *A. campestris* fairy rings on Harper Adams University football pitch at time of final assessment

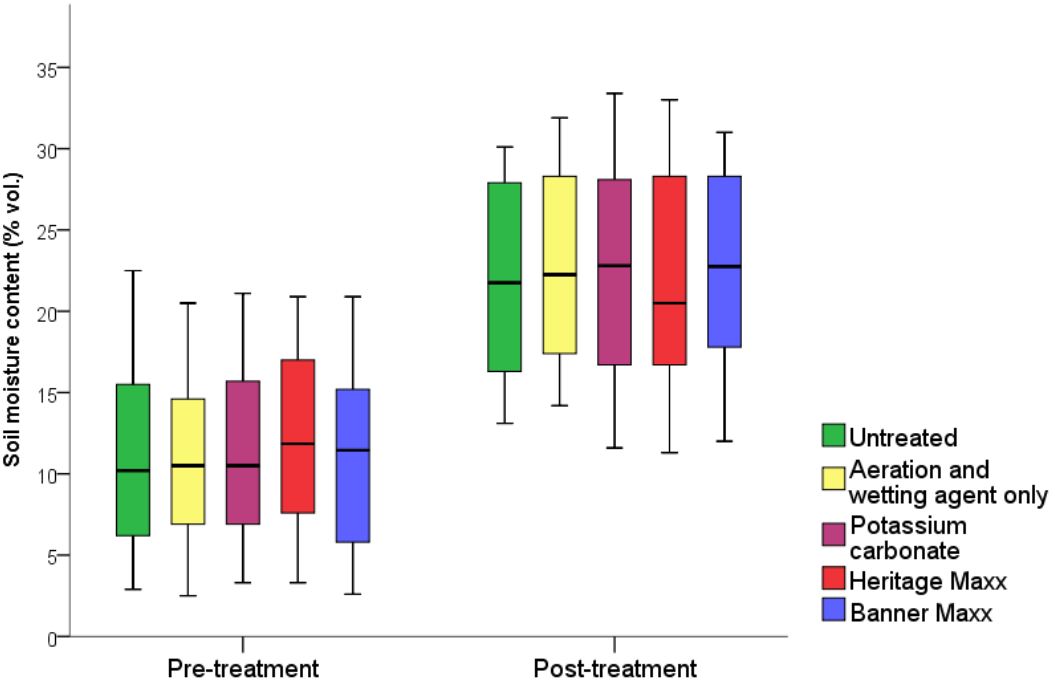


Figure 64: Soil moisture content of fairy ring plots before (17 August 2014) and after (23 October 2014) treatment with either aeration and the wetting agent Revolution alone, aeration, wetting agent and potassium bicarbonate, Heritage Maxx or Banner Maxx, or no treatment

As with the previous year's experiment, all plots were wetter at the point of final assessment in late October than they were pre-treatment in August, but there was no significant difference in change in soil moisture between treatments (ANOVA, p = 0.945) (Figure 64).

There was no incidence of hydrophobicity recorded for any of the soil cores from any of the sample plots throughout the experiment, with water drops soaking into the cores instantly on application in every case.

Data from the supplementary experiment on the *M. oreades* ring showed that soil moisture in plots with fairy ring symptoms present was significantly lower than plots with no symptoms (Mann Whitney U-Test, p <0.001; Figure 65) and turf quality was found to be positively associated with soil moisture content (R^2 = 0.256, p <0.001).

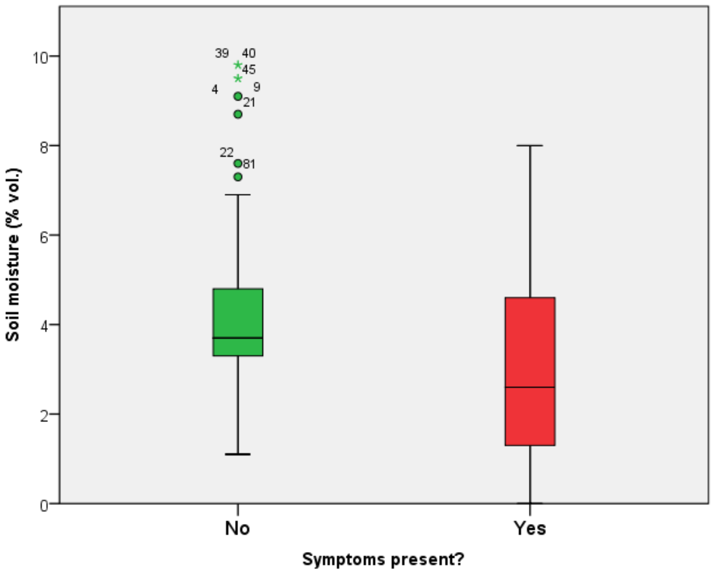


Figure 65: Soil moisture content of fairy ring plots with and without visible symptoms

5.4 Discussion

No evidence was found that any of the products tested in the two years of field experiments provided any control of fairy ring symptoms. The only significant finding in these two experiments was that, unsurprisingly, aeration of plots in the first year resulted in a significant increase in soil moisture content. The use of wetting agent was expected to have a similar effect, but this was not the case, presumably because the *M. oreades* rings tested in the first year were already so hydrophobic that even the wetting agent was unlikely to be properly absorbed. In hindsight, the first year’s experiment should not have been conducted on rings that were so mature and hydrophobic that the products were unlikely to be adequately delivered. It was, however, difficult to find rings that were large enough to accommodate replicate plots and visible enough to be subjectively measured.

The hydrophobic condition of the soil does not, however, explain why none of the treatments, including aeration and wetting agent, had any effect of soil moisture in year two. None of the treatments significantly differed from the untreated control. Hydrophobicity testing in the second year using the WDPT test showed that there was no hydrophobicity present in the rings.

The problem with the second year experiment was the reconfiguration of the football playing area, so that the rings were in front of the goal opening. It is not known how long after treatment this occurred, but it is expected that the soil compaction and disturbance as a result of the foot traffic would have sealed up the holes made during aeration, may have led to the soil holding more water than usual, and could have affected the action of the products.

As soil moisture content was the only measure by which to quantify fairy ring severity in the second year (since the ruined turf could not be assessed for turf quality or percentage symptom cover), it must also be questioned whether soil moisture is indeed representative of fairy ring severity.

As with many other diseases, it is difficult to find quantitative ways, rather than qualitative, of measuring the severity of the disease and, hence, efficacy of control treatments.

In other studies, the visual assessment of turf quality on a scale of 1-9 is a commonly used method (Fidanza, 2002b; Settle *et al.*, 2006; Miller, 2010), although it is highly subjective and unstandardised. Other highly subjective measures used include ‘ring intensity’ on a 0-4 scale (Settle *et al.*, 2006).

Percentage symptom cover or percentage turf loss of treatment plots may provide a more accurate measure of severity, particularly if a quadrat or similar can be used to provide a fairly accurate percentage. Type-2 symptoms, however, can be difficult to distinguish from healthy turf, so there is still an amount of subjectivity involved.

Some of the more quantitative methods used include: number of basidiocarps per plot (Fidanza, 2002b, Miller, 2010) and number of fairy rings per plot (Fidanza, 2002b), but even these methods can be interpreted differently. Is a partial fairy ring still counted as a ring? What if two rings have merged together? Does a basidiocarp have to be fully formed to be counted? Is presence of basidiocarps even a good measure of severity of the other fairy ring symptoms?

Being able to quantify the presence of the fairy ring fungus itself in the soil through DNA analysis would provide an excellent way to measure the severity of fairy ring and the efficacy of treatment (if quantity of fungal DNA in the soil does indeed correlate with severity of symptoms in the turf). Attempts were made during this investigation to quantify basidiomycete DNA within soil samples taken from the fairy rings using qPCR, but the DNA failed to successfully amplify.

The supplementary experiment in the second year provided evidence that soil moisture content was correlated with fairy ring symptoms. However, this investigation was only carried out on one *M. oreades* ring. It is not known whether soil moisture content is representative of symptoms in other species, which may offer an explanation as to why no significant results were seen in the second year experiment on *A. campestris* rings.

Both Settle *et al*. (2006) and Miller (2010) provide data in support of the efficacy of azoxystrobin (Heritage) and propiconazole (Banner Maxx) in the field in controlling fairy ring. Miller shows that azoxystrobin is significantly more effective when applied with the wetting agent, Revolution, against *Vascellum curtisii*. Settle *et al.* (2006) do not specify the species that they worked on, but found that azoxystrobin was significantly more effective than propiconazole. Both measured fairy ring severity as percentage symptom cover, but also used ‘visual quality’ as an alternative measure. No authors appear to use soil moisture as an indicator of fairy ring severity.

5.5 Limitations

Notable limitations were experienced during the course of the field experiments. As already mentioned, it was difficult finding suitable rings on which to work. Ideally, more fairy rings would have been included in experiment one and more sites would have been included in experiment two.

The fleeting nature of some fairy rings became apparent during this study. A third site of small type-2 fairy rings at Gaudet Luce Golf Club in Droitwich was originally included in the experiment, but the rings disappeared not long into the fairy ring season for unknown reasons.

Within the limited timeframe of this study, only two species were tested *in situ*: *M. oreades* and *A. campestris*. There are a number of other species, particularly the puffballs that seem to cause the watermarked effect on greens (Miller, 2010) that need investigation.

As it seems to be impossible to culture fairy rings in the field (i.e. inoculate areas of turf) for experiments (personal communications Lee Miller and Roy Watling), this type of research is completely reliant on finding areas that are already infected. This is perhaps the most restrictive aspect of fairy ring research.

5.6 Conclusions

As the species usually implicated in causing the damaging type-1 fairy ring symptoms, *M. oreades* has a reputation as being particularly aggressive and difficult to control (Dernoeden, 2000). Interestingly, the *in vitro* test showed that *M. oreades* was actually quite susceptible to fungicidal action, whilst the *A. campestris* isolates appeared to be more tolerant to the chemicals tested. The *A. campestris* isolates all came from localities where they were not expected to have come into contact with fungicides before, so it is unlikely to be a resistance issue.

From observations made during this study, *A. campestris* tends to cause what can be fairly large and fairly thick type-2 fairy rings, but does not persist for long, as rings only appear around July and start receding around September. This would suggest that chemical control of *A. campestris* may be generally unnecessary. Fidanza (2007), who has been studying fairy ring for decades, however, has encountered *A. campestris* causing very severe type-1 symptoms. Whilst this may be a result of environment and/or climate, with Fidanza working in the United States and this study being UK-based, further investigations into the control of *A. campestris* would be advisable.

The responsiveness of *M. oreades* isolates to chemicals *in vitro* provides evidence that the incidence of hydrophobicity, reducing contact with the chemical, may be the major factor in controlling them *in situ*. It shows that the products are not ineffective, meaning that focus should be on ways to maximise product delivery and/or minimise soil hydrophobicity. Very little research has ever been done on the hydrophobicity associated with fairy ring, so the next chapter intends to explore how fairy ring fungi impact soil moisture levels.

5.7 Acknowledgements

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6.0 Fairy Rings and Soil Moisture

6.1 Abstract

Fairy rings formed by soil-dwelling fungi are a common phenomenon found in woodland, grassland and arable ecosystems worldwide. A reduction of moisture content in soil colonised by fairy ring fungi can lead to development of soil hydrophobicity, inducing drought stress in the vegetation above that can lead to loss of agricultural crops or degradation of amenity turf.

Soil moisture content of three *Marasmius oreades* fairy rings and an adjacent asymptomatic area on a links golf course were monitored during 2013 using a soil moisture meter. Rainfall, temperature, and irrigation data were gathered for comparison. Soil moisture was significantly lower in the fairy rings than in the asymptomatic area throughout the experimental period of April to November. Soil moisture deficit in fairy rings was more pronounced after periods of low water input and was at its worst in April, which is earlier in the year than expected, as fairy ring symptoms are not always obvious around this time.

During 2014, soil moisture of the *M. oreades* rings and asymptomatic area on the links golf course was measured again, this time alongside three *M. oreades* rings from a parkland golf course some 60 miles away. In this experiment, soil moisture was measured at three different depths and the water drop penetration time test was used to detect hydrophobicity in soil cores taken from the sample plots in order to look for a relationship between soil moisture content and the development of hydrophobicity.

Fairy ring moisture deficit was similar to that seen in year one and was most prominent at the beginning of the season, in March. Two rings were already hydrophobic in March and the rest were all hydrophobic by June. Hydrophobicity had disappeared in all rings by October. Hydrophobicity predominantly occurred in the upper layer of the soil, at 3.8 cm, but moisture deficit between fairy rings and asymptomatic areas was detectable to a depth of 12 cm.

Some high water content samples were found to be hydrophobic and some low water content samples were not, showing that there is no clear soil moisture threshold at which hydrophobicity develops and there are likely to be factors other than soil moisture also involved in the development of hydrophobicity.

6.2 Introduction

Mechanisms leading to the development of soil hydrophobicity, a phenomenon with major repercussions for the viability of land for agricultural production or amenity use, are still poorly understood. It is generally thought that, if soil moisture content drops below a critical level, particularly when there is an accumulation of waxy organic compounds in the soil, the soil can abruptly fail to absorb water (Dekker *et al*., 2009). The resulting drought stress that this inflicts on any plants growing in this soil can lead to loss of vegetation.

Whilst there are numerous causes of hydrophobicity development, such as root exudates (Dekker and Ritsema, 1996), wildfires (DeBano, 2000), and microbial activity (Hallett and Young, 1999), the cause under investigation in this work was the presence of basidiomycete (mushroom-producing) fungi in the soil (Shantz and Piemeisel, 1917). All filamentous fungi, including basidiomycetes, produce amphiphilic, surface active proteins called hydrophobins, which allow them to manipulate hydrophilic and hydrophobic interfaces with their environment (Woston and de Vocht, 2000, Rillig, 2005). These hydrophobins are thought to coat soil particles, leading to the development of fungus-related soil hydrophobicity (Rillig, 2005), but there is, however, little evidence to support this theory (Spohn and Rillig, 2012).

The basidiomycetes often implemented in causing hydrophobicity are those that grow in a circular pattern known as a fairy ring. Fairy rings occur commonly in woodland, grassland and arable ecosystems all over the world (Ainsworth and Bisby, 1950), and are particularly notorious for affecting turf on sports pitches, golf courses and domestic lawns. Of the many species that grow in ring formation, only some have the potential to cause the loss of vegetation described earlier and the fungus *Maramius oreades* appears to be most frequently associated with these symptoms. These are the most destructive type of fairy ring and are known as 'type-1' (Shantz and Piemeisel, 1917) (Figure 66).

A fairy ring begins with the germination of a single spore or mycelial fragment, which produces hyphal tips that grow radially outward. The ring grows larger as the active edge of the fungus forages outward into the soil, leaving nutrient-depleted soil behind it in the centre of the ring, which eventually recovers. A type-1 fairy ring is characterised by an active zone comprising of a ring of dead vegetation flanked on the inside and outside by rings of stimulated plant growth, which have benefitted from the nutrients released as the fungus feeds on organic matter in the soil beneath (Shantz and Piemeisel, 1917).


Figure 66: Loss of vegetation caused by a type-1 fairy ring of unknown species growing on amenity turf (photograph courtesy of STRI)

Fairy ring-related hydrophobicity is highly localised and can often go unnoticed unless occurring on intensively managed areas, such as amenity turf (Dekker and Ritsema, 1996). It is a particular problem on golf courses, where symptoms such as those shown in Figure 66 can impact both playability and aesthetics (Keighley *et al.*, 2013). This has led to 'fairy ring' being classified as a disease of turfgrasses, which requires specific management techniques to be controlled effectively. The epidemiology of fairy ring as a disease, however, and indeed the number of different species that cause these symptoms, is still unclear.

A previous study has provided a detailed description of the spatial distribution of soil moisture and severity of hydrophobicity within fairy rings (York and Canaway, 2000), but this only described one snapshot in time (date not specified) and no published work has followed the development of fairy rings as dynamic systems over any period of time. Spohn & Rillig (2012) showed that severity of fungus-related hydrophobicity caused by the cultivated mushroom, the basidiomycete *Agaricus bisporus*, was positively correlated with temperature and negatively correlated with moisture *in vitro*. This would suggest that noticeable differences in fungus-related hydrophobicity may well be seen over seasonal changes in the field.

This investigation sought to test the hypothesis that fairy ring soil moisture would change in comparison to asymptomatic soil over a typical growing season. Only severe fairy rings are visible all year round. Many only become apparent when they become active in spring. The associated loss of vegetation will typically be at its worst in August and September, but most rings will be returning to dormancy by December (Keighley *et al*., 2013). Thus, the typical fairy ring growing season in the UK is considered to be April to November.

By measuring soil moisture content over two fairy ring seasons, the epidemiology of type-1 fairy ring symptoms, including associations between climatic and environmental factors, could be explored. In particular, this phase of the project aimed to determine how soil moisture content impacts the development of the hydrophobicity that is thought to be the primary driver in the onset of type-1 symptoms.

This chapter is divided into two parts, for each of the fairy ring seasons studied. Year one focused on three *Marasmius oreades* fairy rings on one site – Royal Liverpool Golf Club, Hoylake, Merseyside, and year two also incorporated three *M. oreades* rings at a second site – The Shropshire Golf Centre, Telford, Shropshire. Locations of the fairy rings studied at each site are shown in Figures 67 and 68.


Royal Liverpool Golf Club

Soil moisture experiments – years 1 & 2
3no. *Marasmius oreades* rings

Figure 67: Locations of the three *M. oreades* fairy rings at Royal Liverpool Golf Club, Hoylake used for the soil moisture experiments in years 1 and 2, shown in yellow


The Shropshire Golf Centre

Soil moisture experiment – year 2
3no. *Marasmius oreades* rings

Figure 68: Locations of the three *M. oreades* fairy rings at The Shropshire Golf Centre, Telford used for the soil moisture experiment in year 2, shown in yellow

6.3 Experiments

6.3.1 Seasonal changes in soil moisture – year one

6.3.1.1 Introduction

In the first of two years of soil moisture experiments, the primary aim was to see how fairy ring soil moisture changed throughout the year in comparison to asymptomatic turf in response to the changing climate. Both fairy ring and asymptomatic soil moisture were expected to be correlated with air temperature and water input (a combination of rainfall and irrigation), but to be distinctly different from each other.

Following on from the findings in Chapter 3.0, soil moisture was expected to be lowest in the hotter, drier summer months of July and August, which is also when greenkeepers reported symptoms to be at their worst, but to have a moisture content comparable with the asymptomatic turf during the dormant season of November to April.

6.3.1.2 Methodology

Three *Marasmius oreades* (Bolton) Fr. fairy rings growing in a sandy soil on the practice area of Royal Liverpool Golf Club, a coastal (also known as links) golf course in Hoylake, north-west England were monitored from April 2013 to November 2013. At the start of the investigation, ring 1, ring 2 and ring 3 covered areas of 10.69m^2, 11.59m^2 and 25.68m^2 respectively. These rings were chosen because they were clearly visible, complete, of sufficient size to provide a viable data set, and accessible to work on without obstructing golfers. Ring 1 was originally just in front of golf tee-off point (the 'tee'), but became integrated into it as the tee area was gradually moved forward throughout the season to allow the divots made by golf clubs when teeing-off to repair. Rings 2 and 3 were located next to each other, several metres south of ring 1, located just outside of the in-play area. Turf in the in-play area where ring 1 was located was mowed to a height of 10mm, whereas rings 2 and 3 were mowed to 40mm. Ring 1 was therefore subjected to more foot traffic (compaction), golf club damage (disturbance), and management (mowing) than rings 2 and 3. Positive identification of the causal species was determined by examining the fruiting bodies that appeared on the outer edge of the rings between June and October, using an identification guide by Phillips (2006).

Soil moisture measurements were taken on five occasions at approximately bimonthly intervals: 15th April, 28th June, 21st August, 1st October and 29th November 2013. Soil moisture content (% volume) was measured in the centre of the active zone at 15 cm intervals around the circumference of each fairy ring (clockwise, north-north) using an ML2x ThetaProbe (Delta T Devices), which measured to a depth of 5 cm, in April and June, and a Fieldscout TDR 100 (Spectrum Technologies, Inc.), which measured to a depth of 3.8 cm, in August, October and November. Both devices work on the principle of time-domain reflectometry, whereby percentage volumetric water content is measured between the full depth of the probes that are inserted into the ground. The ThetaProbe sustained damage during the experiment from being inserted into very dry ground and was, hence, replaced with the more robust Fieldscout. For comparison, the same number of measurements as collected from the biggest of the three fairy rings was then taken from an adjacent asymptomatic area of turf of equivalent dimension to act as a control.

Weather data from the weather station on Hilbre Island, a tidal island lying 2.5 km west of the sampling site, were obtained by registering online (http://cobs.noc.ac.uk/cobs/met/hilbre/) to download data files, courtesy of the National Oceanography Centre at Liverpool. Data obtained were various weather readings taken every ten minutes, every day from 00:00-23:50. Air temperature and rainfall data were used to give an indication of conditions during the period leading up to each sampling date. Ten-minutely readings for 30 complete days directly proceeding each sampling date were averaged to obtain mean air temperature (°C) and summed to give total accumulated rainfall (mm).

Soil moisture was not purely a result of rainfall, as the golf course practice area was also irrigated for part of the year as part of the management regime. The Golf Course Manager provided details of the irrigation schedule, which consisted of 3 mm applications of water, 3 times per month at approximately regular intervals during June, July and August. This equates to an extra 9 mm of water received by the turf leading up to the sampling dates in June and August, which is taken into consideration alongside rainfall data.

Data were analysed statistically using SPSS v.21 (IBM). Fairy ring data sets were pooled and averaged to give a mean fairy ring soil moisture value for each month. Control data sets were also averaged to obtain one value per month, before plotting both against the weather and irrigation data. Mann-Whitney U-tests were used to compare fairy ring and asymptomatic soil moisture by month. Linear regression and Spearman's rank correlation co-efficient were used to examine relationships between the four variables, and the Shapiro-Wilk test was used to measure normality of distribution within the data sets.

6.3.1.3 Results

Mean fairy ring soil moisture was 8.5-42.4% lower than the asymptomatic control throughout the season, which was a significant reduction for every sampling month (Mann-Whitney U Tests, $p < 0.001$) apart from June (Mann-Whitney U Test, $p = 0.334$) (Figure 69). Irrigation in June and August raised water input to relatively high levels of 28.2 mm and 36.1 mm respectively, comparable with the rainfall-only figure of 35.6 mm in November. In the two months when rainfall was at its lowest, at <20 mm in April and October, there was no supplementary irrigation. These were also the two months that showed the most pronounced difference in soil moisture between the fairy rings and the asymptomatic control (April: fairy ring 42.4% drier than asymptomatic area; October: fairy ring 42.3% drier than asymptomatic area). Mean monthly temperature peaked to 18.44°C in August, which saw the asymptomatic control soil moisture drop to its driest point of 11.45% vol., with the fairy ring soil even lower at 8.36% vol. Whilst asymptomatic control soil moisture had recovered slightly by October, the fairy ring soil had become even drier, reaching its seasonal low of 7.96% vol.

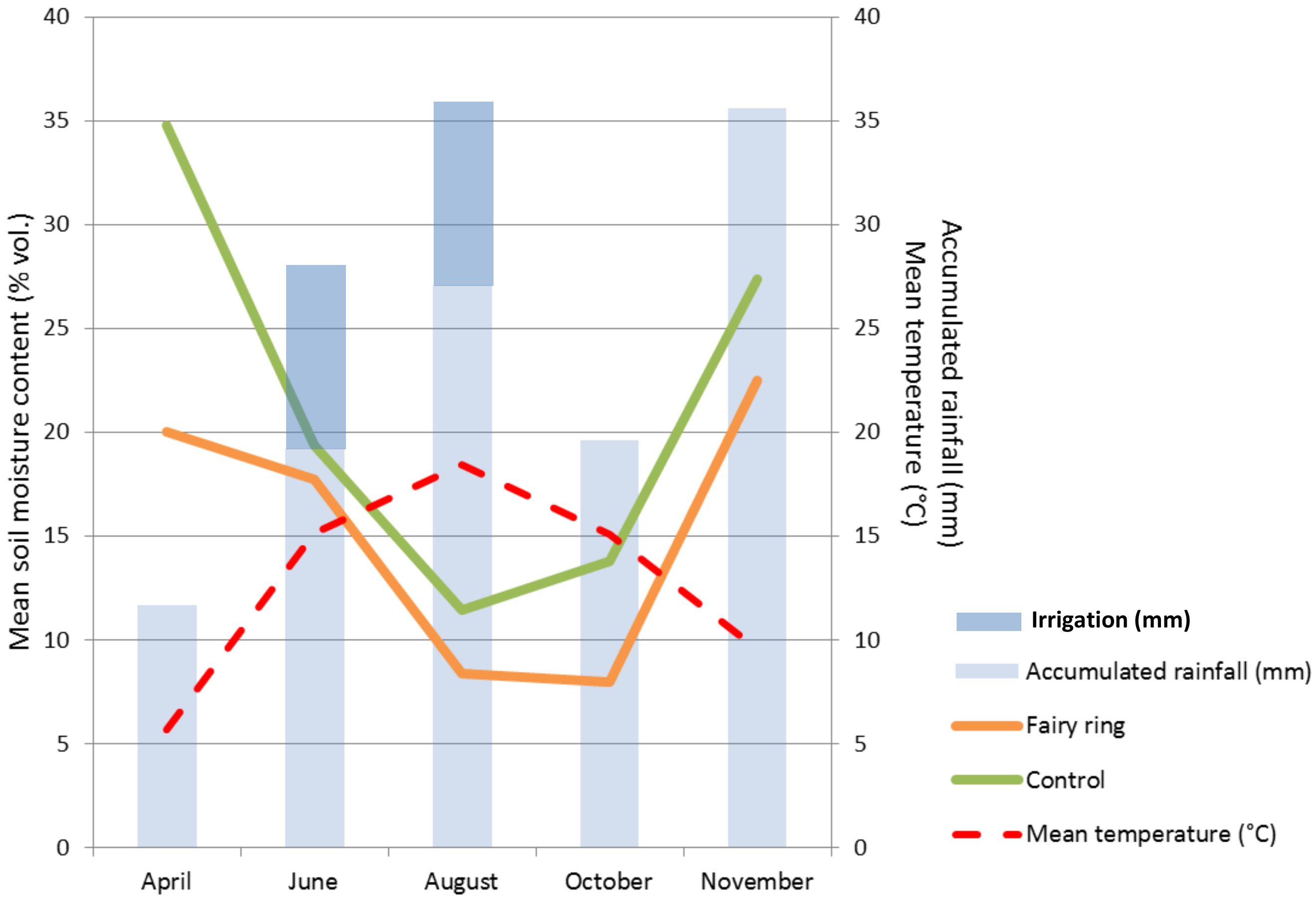

Figure 69: Mean soil moisture content of fairy rings and an asymptomatic control area on a coastal golf course in northwest England during the 2013 growing season. Weather data, from the weather station on a tidal island 2.5km west, and the irrigation schedule, supplied by the golf course manager, show average air temperature (°C), total accumulated rainfall (mm) and accumulated irrigation water (mm) during the 30 days prior to each of the sampling dates of 15th April, 28th June, 21st August, 1st October and 29th November.

Table 14: Linear regression (R^2) and Spearman's rank co-efficient (p) results showing significance of association and correlation respectively. Significant relationships shown in bold.

	Fairy ring soil moisture	Control soil moisture	Rainfall plus irrigation	Mean monthly temperature
Fairy ring soil moisture		**R^2 = 0.527** p = 0.094	R^2 = 0.010 p = 0.500	**R^2 = 0.607** p = 0.142
Control soil moisture			R^2 = 0.075 p = 0.252	**R^2 = 0.696** **p = 0.019**
Rainfall plus irrigation				R^2 = 0.062 p = 0.094
Mean monthly temperature				

Linear regression showed weak associations between fairy ring soil moisture and control soil moisture (R^2 = 0.527); fairy ring soil moisture and temperature (R^2 = 0.607); and control soil moisture and temperature (R^2 = 0.696) (Table 14). Spearman's rank correlation coefficient showed the only statistically significant of these relationships was between control soil moisture and temperature (p = 0.019). Water input, as the sum of both rainfall and irrigation, was not statistically linked to any of the other variables.

Soil moisture data from the fairy ring active zones were not normally distributed, except in November, whereas data from the asymptomatic control were normally distributed throughout the season (Table 15). Soil moisture was also highly variable in fairy ring active zones, particularly at the beginning of the season in April (mean 20.02 (SD 7.21) % vol.), but became less so in the driest months of August (mean 8.36 (SD 2.85) % vol.) and October (mean 7.96 (SD 2.44) % vol.) (Figure 70).

Table 15: Normality of soil moisture data distribution

	Shapiro-Wilk normality test (*p*)	
	Fairy ring soil	Asymptomatic soil
April	0.002*	0.298
June	0.002*	0.400
August	0.001*	0.836
October	0.024*	0.079
November	0.106	0.717

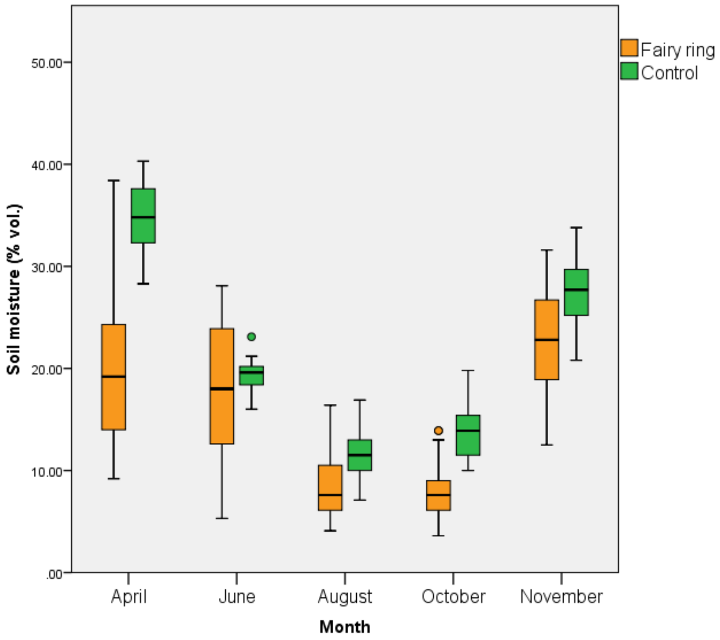

Figure 70: Soil moisture of fairy rings compared to asymptomatic control by month

6.3.1.4 Discussion

Fairy rings' soils had consistently lower moisture content than asymptomatic soil (although this was not statistically significant in June), rendering them more likely to drop below the critical level that is thought to prompt the development of hydrophobicity, particularly in late summer through to autumn, when they are at their driest. As expected, higher temperatures were associated with lower soil moisture.

Changes in fairy ring soil moisture followed the same seasonal pattern as asymptomatic soil until a period of lower water input (rainfall plus irrigation) appeared to result in a pronounced difference between the two. This was particularly noticeable in April, which also had the lowest mean temperature, showing that this is a phenomenon that is not limited to the hotter summer months. A pronounced difference was also seen in October, where fairy ring soils were at their driest, but irrigation in the 30 days leading up to sampling had stopped. This may be a scenario in which hydrophobicity is likely to develop, suggesting that irrigation may need to persist into the autumn months. The severity of fairy ring dryness in the autumn months may be masked by the improving moisture status of healthy, asymptomatic turf. Whilst there was no good statistical evidence of a relationship between soil moisture and water input, there was some evidence that low water input promotes high differences in soil moisture between fairy rings and asymptomatic soil.

Data sets from fairy ring active zones highlighted how variable soil moisture can be. In this study, for example, in one fairy ring active zone a soil moisture reading of 8.1% vol. was neighboured by a reading of 28.1% vol. just 15 cm away. Interestingly, the initial high ranges of soil moisture seen in fairy rings at the beginning of the year became more constant in the driest months of August and October. It was originally considered that this could be a discrepancy associated with changing to a different model of soil moisture meter, but this was disregarded when results from November, also taken with the new equipment, were found to resemble patterns seen at the beginning of the year with the original equipment.

The change of soil moisture meter was due to failure of the ThetaProbe and discontinuation of the ML2x model. A decision was made to swap to the more robust Fieldscout which measured at a slightly shallower depth of 3.8cm rather than the

5cm depth previously measured by the ThetaProbe. As a previous study had found hydrophobicity of *M. oreades* fairy rings to be at its highest at a depth of 3-5 cm (York and Canaway, 2000), the risk of the 1.2 cm measuring depth difference affecting the results was considered to be minimal.

High spatial variability of moisture in hydrophobic soils has been noted for some time (Dekker and Ritsema, 1996). Dekker and Ritsema (1996) explain that under grass cover, such as amenity turf, hydrophobicity can cause water to flow vertically down through the soil profile in distinct channels known as fingered flow rather than distributing evenly throughout the soil. This means, when measured from the surface, that dry areas can be directly adjacent to wet areas. Fingered flow would explain why uneven moisture patterns were seen in the fairy ring active zones. The channels involved in fingered flow are notoriously unstable (Dekker and Ritsema, 1996), so it is possible that the collapse of channels under decreasing soil moisture would explain the reduction in soil moisture variability seen in August and October.

The high variability of soil moisture in fairy rings discovered during this study shows that anyone managing fairy ring soils should take numerous measurements from the full circumference of the active zone in order to get a true representation of how dry each ring is. This may be of lesser importance during drier, summer months when variability is less pronounced. An insufficient number of soil moisture readings has the potential to mislead a turf manager into thinking that dryness in the fairy ring is less severe than it actually is. If control methods are not implemented soon enough, hydrophobicity could develop, making the soil even more difficult to treat.

Further research aims to identify the critical soil moisture level at which fairy ring-related hydrophobicity is induced. Once a soil has become water repellent, it can be very difficult to ameliorate, but when the soil is still wettable, fungicides, wetting agents, and other chemicals can be delivered into the soil profile more effectively (Smith, 1980a; Cisar *et al*., 2000). The prevention of hydrophobicity should, therefore, be a priority for anyone managing fairy ring soils. It is recommended that this is achieved by maintaining high levels of soil moisture through frequent irrigation, alongside the use of wetting agents and aeration techniques (Cisar *et al.*, 2000; Hallett, 2008). As the critical soil moisture level for fairy ring-related hydrophobicity development is not yet known, water use efficiency may not be being optimised. Finding the exact level at which soil moisture must be maintained to avoid development of hydrophobicity would aid not only management of fairy ring as a disease, but also contribute to water conservation through reduced irrigation.

As with Spohn and Rillig's (2012) experiments with the related basidiomycete *A. bisporus in vitro*, evidence has shown that soil moisture content of *M. oreades* fairy rings in the field is influenced by both temperature and water input, resulting in seasonal changes in moisture status. It must also be considered that there are other species of fungus reported to cause hydrophobic type-1 fairy rings (Fidanza, 2009), although none have been identified during this project, that may differ to *M. oreades* in their dynamics within the edaphic environment.

This first year's study has shown that turf managers should be aware that soil moisture of fairy rings can continue to fall in the autumn months, even when asymptomatic turf is appearing to regain moisture. In severe cases, this lag-effect may warrant the continuation of irrigation into September, if rainfall is low, to avoid development of hydrophobicity. Those managing fairy ring soils should also be aware that moisture in the active zone can be highly variable and monitoring the entire circumference at frequent intervals is the best way to get an accurate measure of the ring's dryness.

6.3.2 Seasonal changes in soil moisture – year two

6.3.2.1 Introduction

To build upon the findings of the first year's soil moisture field experiment, a similar experiment was carried out in year two, this time including a second site to look for differences between golf course types, a test to detect the presence of soil hydrophobicity, and soil moisture measured at three different depths, to see how moisture changed through the soil profile.

Links golf courses are always coastal and, hence, sit atop sandy soils. Fungi are thought to move more easily through sandy soils (York, 1998), so it is possible that fairy rings may proliferate more readily and, potentially, be more severe on links golf courses. Parkland golf courses are, generally, on loamier soils, inland, more sheltered, and would usually have a far greater presence of trees than links courses. By testing both a links course and a parkland course, comparisons by course type could be made. With more space between the sand particles for the waxy fungal mycelium to accumulate, the links golf course was expected to have a higher incidence of hydrophobicity than that of the loamy, parkland course.

Measuring soil moisture at different depths aimed to determine where moisture was lowest and, hence, at what level the fairy ring fungus might be most concentrated within the soil profile. The water drop penetration time (WDPT) test was used alongside this to see both where and when hydrophobicity occurred.

6.3.2.2 Methodology

In 2014, the same three *M. oreades* rings and asymptomatic area used in the previous year at Royal Liverpool Golf Club in Hoylake were monitored on a monthly basis from March to November. In addition, three rings from Shropshire Golf Centre in Telford, England were similarly monitored. Shropshire Golf Centre is classed as a ‘parkland’ golf course, which are generally characterised as being inland, tree-covered, and sat on loamy soils. The aim of using a parkland golf course was to look for how differences in soil type and climate may affect fairy ring soil moisture in comparison to the links golf course, which are in coastal ecosystems, generally devoid of trees, on sandy soils.

At the Shropshire site, the three rings chosen were located in an area of turf by the side of the car park, rather than on the golf course itself. As with the Hoylake site, they were identified as being caused by *M. oreades*, due to the presence of basidiocarps around the edge of the rings, and were chosen as they were clearly definable, complete rings, and all of a similar size. An asymptomatic area between rings one and two was selected for sampling in order to act as a control. There were some small trees in the area accommodating the Shropshire rings, meaning they were largely shaded. There was no formal maintenance regime, other than occasional mowing as and when required, according to weather conditions.

The methodology was adapted from that of the first year, in order to account for the different ring sizes and to generate equal sample sizes. A tape measure was used to measure each ring diametrically in order to ascertain and mark out its central point. A peg was inserted into the ground at the central point and a length of twine attached that was long enough to reach the active zone of the fairy ring. The Apple iPhone 4 compass application was then used at the centre of the ring in order to determine the direction of north. The twine was stretched out along the indicated line of north from the centre point, to the active zone of the fairy ring, where north was marked with a peg. In order to mark 20 points around the active zone of each ring at which to sample, the twine was moved around the circumference of the ring, from north, in a clockwise direction, marking with pegs at 18° intervals, determined using the compass located at the centre point of the ring.

Soil moisture content (% vol.) was measured at depths of 3.8 cm, 7.5 cm, and 12 cm in the centre of the active zone of the ring at each of the 20 marked sampling points around the circumference using the Fieldscout TDR 100 soil moisture meter (Spectrum Technologies, Inc.; Figure 71), which has interchangeable rods of the said lengths .

After the soil moisture measurements had been taken, a 20 mm-diameter open-sided soil corer with a foot pedal was used to extract a soil core from the point between which the two Fieldscout rods had entered the ground. Each core extracted was tested immediately for soil hydrophobicity using the water drop penetration time (WDPT) test (Dekker *et al*., 2009), as described in the previous chapter, at the three depths measured by the soil moisture probe – approximately 3.8 cm, 7.5 cm, and 12 cm, whilst it was still in the open-sided corer (Figure 72). It was then manually removed from the corer and placed back into the hole from which it came as fully as possible.

Figure 71: Fieldscout TDR 100 soil moisture meter on the active zone of a M. oreades fairy ring with the 7.5 cm length rods fitted

Figure 72: Water drop penetration time test carried out at three depths on a soil core *in situ*. The beaded drop at the ≈3.8 cm depth indicates severe hydrophobicity in this area

This time the experiment was carried out approximately monthly from March to November 2014. The Hoylake site was sampled on 24th March, 29th April, 27th May, 17th June, 18th August, 19th September, 30th October, and 30th November. Hoylake could not be sampled in July as The Open Championship golf tournament was being held there and the fairy rings were inaccessible. The Shropshire site was sampled on 27th March, 30th April, 27th May, 19th June, 22nd July, 28th August, 23rd September, 23rd October, and 20th November.

In the time since the first year's experiment, the website used to access weather data from the Hillbre Island weather station near Hoylake (http://cobs.noc.ac.uk/cobs/met/hilbre/) became unmaintained and the data no longer available. Instead, historic weather data was accessed via the MetOffice website (http://www.metoffice.gov.uk/public/weather/climate-historic/#?tab=climateHistoric). The nearest available weather station to the Shropshire site with accessible data was RAF Shawbury, which lies 12 miles west-north-west of Shropshire Golf Centre. The Hoylake site did not have a data-accessible weather station in as close proximity as the Shropshire site. The nearest data source was also RAF Shawbury, some 50 miles south-east, but as this site is inland and Hoylake is coastal, it was thought to be more suitable to use the second nearest weather station, which was RAF Valley, on the west coast of the isle of Anglesey, North Wales. Whilst located 55 miles west of Hoylake, RAF Valley was at a similar latitude and considered to have a similar maritime climate.

Unlike in the first year's experiment, the weather data from the MetOffice was provided as monthly totals, meaning that totals could not be calculated using data from the 30 days directly proceeding the sampling date. As sampling was generally carried out within the last week of each month, however, it was considered suitable that the monthly totals would be sufficiently representative of the conditions leading up to each sampling date.

As with the previous year's experiment, the practice area at Hoylake where the fairy rings were located was also irrigated during the summer months to supplement rainfall, as part of the maintenance regime. The Golf Course Manager provided details of the irrigation schedule for 2014, which was between 6 and 18 mm of water applied per month from April to September, inclusive. The Shropshire site did not receive any supplementary irrigation during 2014.

Data were analysed statistically using SPSS v.23 (IBM). Fairy ring data sets were pooled and averaged to give a mean fairy ring soil moisture value for each month. Control data sets were also averaged to obtain one value per month, before plotting both against the weather and irrigation data. Mann-Whitney U-tests were used to compare fairy ring and asymptomatic control soil moisture by month. Linear regression and Pearson's Coefficient were used to examine relationships between the four variables. Mann Whitney U-tests were used to compare hydrophobic against non-hydrophobic soil moisture contents.

6.3.2.3 Results

At the Shropshire site, fairy ring soil moisture content was significantly lower than that of the asymptomatic control area throughout the sampling season (Mar, Apr, May, Jun, Jul, Aug, Sep, Oct, Nov – Mann Whitney U-Tests all *p* <0.001) (Figure 73).

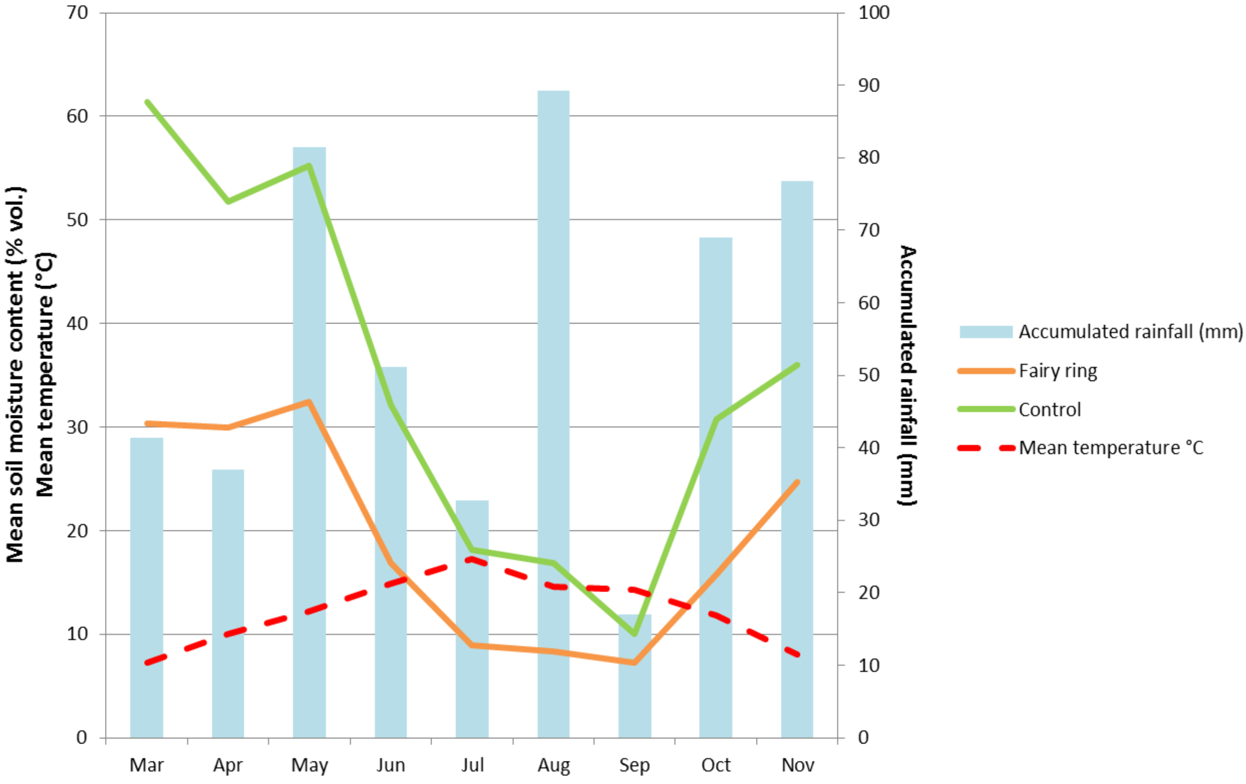

Figure 73: Mean soil moisture content of fairy rings and an asymptomatic control area at Shropshire Golf Centre during 2014. Weather data, from the weather station at nearby RAF Shawbury, show monthly totals for average air temperature (°C) and total accumulated rainfall (mm)

At Hoylake, fairy ring soil moisture was significantly lower in March (*p* = 0.001), May (*p* <0.001), and June (*p* <0.001), but not in April (*p* = 0.070), August (*p* = 0.092), September (*p* = 0.785), October (*p* = 0.069), or November (*p* = 0.245). The results for Hoylake, however, are likely to have been largely affected by the occurrence of The Open Championship golf tournament, which was held there in July (Figures 74 and 75).

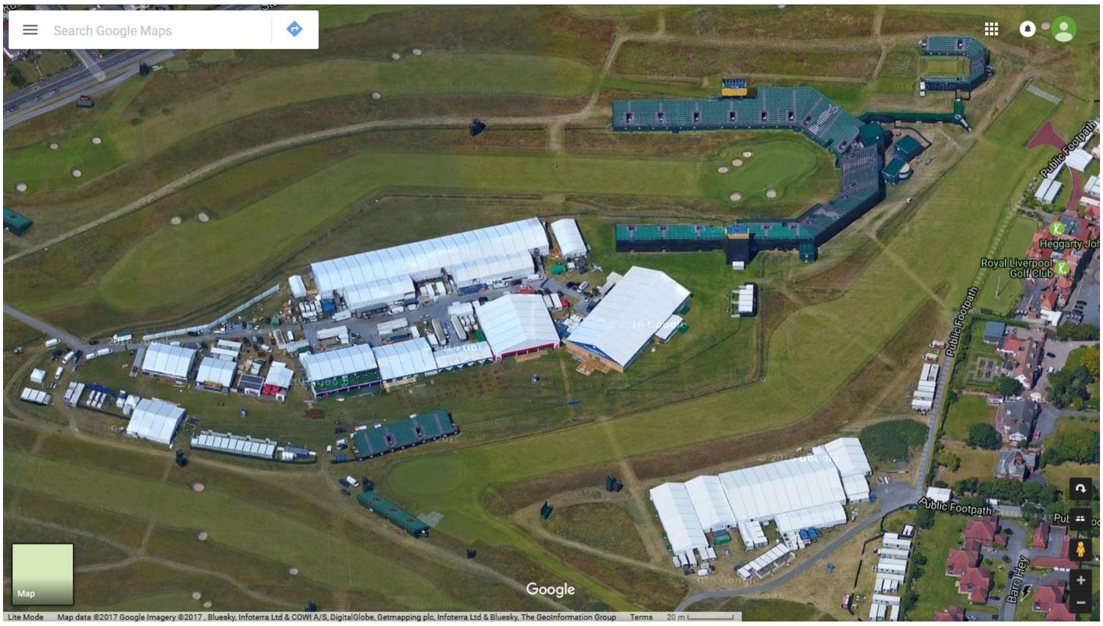

Figure 74: The Open Championship village, which was set up on Royal Liverpool Golf Club's practice area in July in order to host the competition

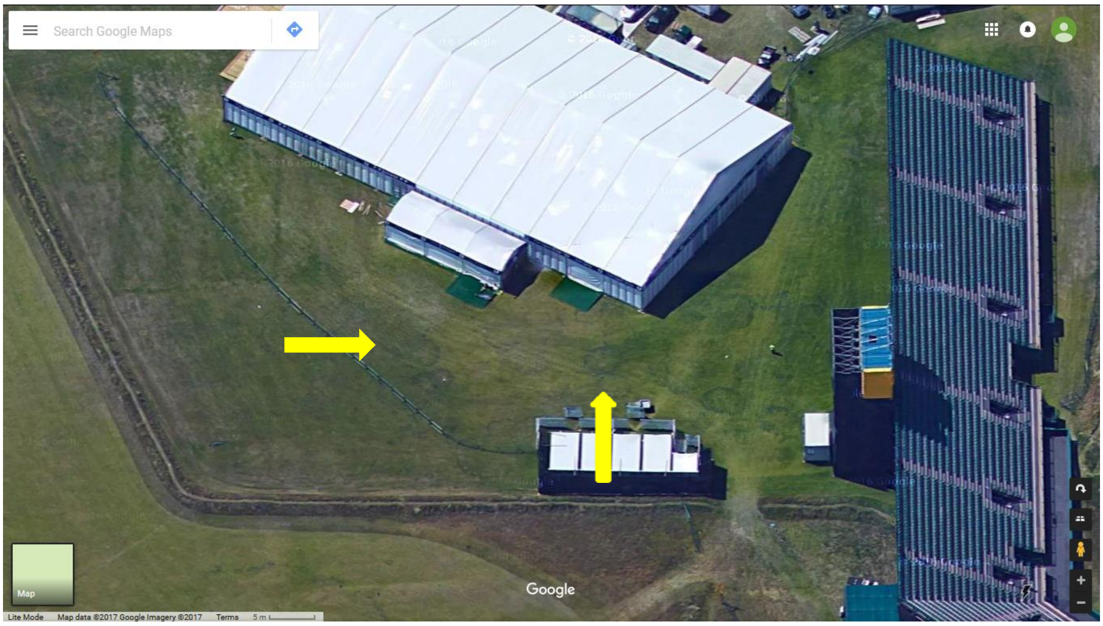

Figure 75: Some of the experimental fairy rings can be seen to the rear of this marquee on Google Earth satellite images

During The Open Championship, the practice area where the fairy rings were located accommodated various marquees and was subjected to heavy foot traffic (the rings themselves largely escaped this, as they were behind one of the marquees – Figure 75), which caused damage to the turf and soil compaction. To remediate this, the Links Manager said that the area was vertidrained (a method of mechanically aerating the soil) with half inch tines to a depth of ten inches three times, over-seeded, and treated with 110 kg of nitrogen per hectare. This occurred at some point between the September and October sampling dates.

The effect of this event and the subsequent turf management is evident in Figure 76, where, as of August, the fairy ring and control soil moisture readings become very similar.

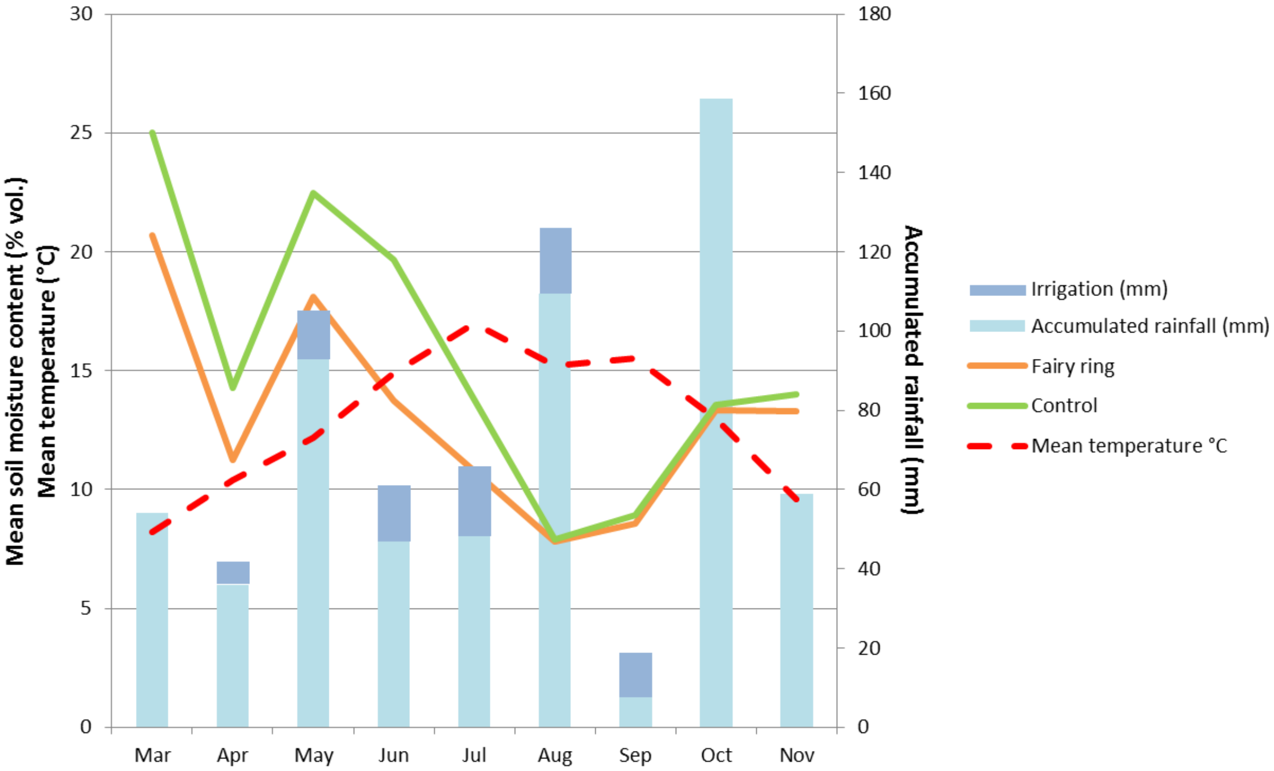

Figure 76: Mean soil moisture content of fairy rings and an asymptomatic control area at Royal Liverpool Golf Club during 2014. Weather data, from the weather station at RAF Valley, and irrigation data provided by the Golf Course Manager show monthly totals for average air temperature (°C), total accumulated rainfall (mm), and total accumulated irrigation water applied (mm)

At Shropshire, both the fairy rings and the control were at their driest in September, when rainfall was particularly low. Moisture deficit, the difference in moisture between fairy rings and asymptomatic turf, was at its greatest at the very beginning of the season, in March, which mirrors what was seen in last year's experiment at Hoylake.

Fairy ring and control soil moistures were at their lowest in August at Hoylake, but the inability to sample the rings in July and the management implemented around the impact of The Open Championship limits what can be drawn from the results from this site.

Linear regression and Pearson's Coefficient were carried out on the average soil moisture and weather data to look for evidence of potential correlation and/or association between the variables.

Table 16: Linear regression (R^2) and Pearson's Coefficient (*p*) results showing significance of association and correlation, respectively, between soil moisture and weather data at Hoylake. Significant relationships shown in bold.

Hoylake	Fairy ring soil moisture	Control soil moisture	Rainfall plus irrigation	Mean monthly temperature
Fairy ring soil moisture		**$R^2 = 0.918$** **$p < 0.001$**	$R^2 = 0.001$ $p = 0.477$	$R^2 = 0.448$ **$p = 0.035$**
Control soil moisture			$R^2 = 0.007$ $p = 0.421$	$R^2 = 0.318$ $p = 0.073$
Rainfall plus irrigation				$R^2 = 0.015$ $p = 0.378$
Mean monthly temperature				

Table 17: Linear regression (R^2) and Pearson's Coefficient (*p*) results showing significance of association and correlation, respectively, between soil moisture and weather data at Shropshire. Significant relationships shown in bold.

Shropshire	Fairy ring soil moisture	Control soil moisture	Rainfall	Mean monthly temperature
Fairy ring soil moisture		**$R^2 = 0.942$** **$p < 0.001$**	$R^2 = 0.038$ $p = 0.308$	**$R^2 = 0.591$** **$p = 0.008$**
Control soil moisture			$R^2 = 0.023$ $p = 0.349$	**$R^2 = 0.540$** **$p = 0.012$**
Rainfall plus irrigation				$R^2 = 0.027$ $p = 0.336$
Mean monthly temperature				

As Tables 16 and 17 show, statistically significant relationships exist between fairy ring soil moisture and control soil moisture at both sites. There was a correlation between fairy ring soil moisture and air temperature at Hoylake and Shropshire and also a relationship between control soil moisture and air temperature at Shropshire.

There was no relationship between water input (as rainfall and irrigation or rainfall alone) and any of the other factors.

To see if moisture deficit differed according to depth into the soil profile, average fairy ring soil moisture and average control soil moisture for each month were compared by depth. The three depths at which measurements were taken (3.8 cm, 7.5 cm, and 12 cm) are referred to as shallow, middle, and deep, respectively. Multiple depths were not measured until April, so only the value for the 7.5 cm depth is shown for March at each site.

As shown by the following sets of Figures 77 to 82, the two sites varied in the differences they had between fairy ring and control soil moisture content at the different depths measured. At Hoylake, there was little difference between fairy ring and control in the shallow layer. The graphs show that the variation lay in the middle and deep layers, showing that moisture deficit of *M. oreades* fairy rings is detectable up to a depth of at least 12 cm.

At Shropshire, the three depths bear far more resemblance to each other than at Hoylake and there is a clear distinction between fairy ring and control soil moisture at every depth. Moisture deficit is noticeably lower in the shallow layer during July and August than in the middle and deep layers.

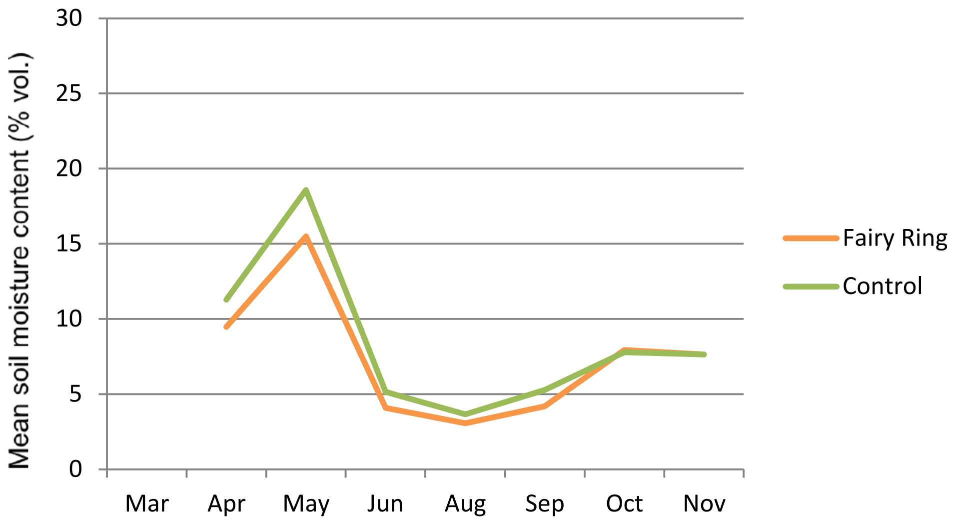

Figure 77: Mean soil moisture at shallow depth (3.8 cm) at Hoylake during 2014

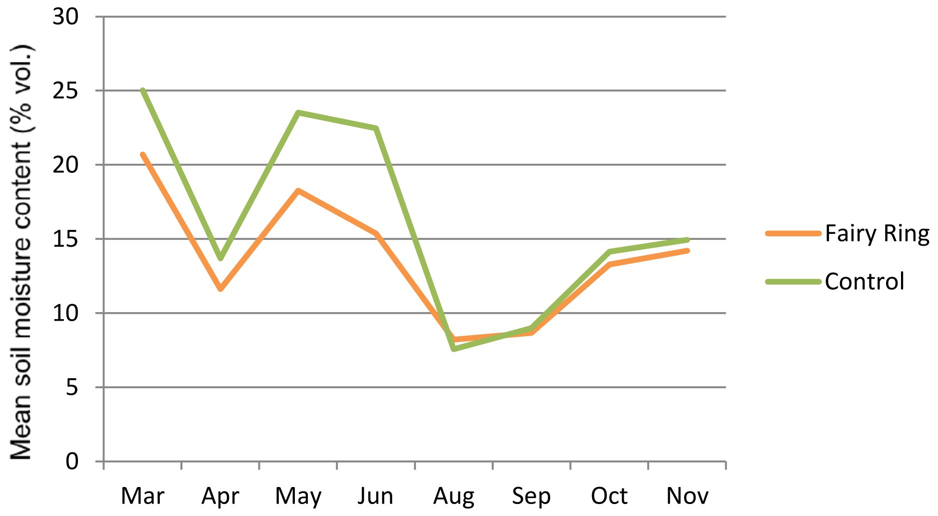

Figure 78: Mean soil moisture at middle depth (7.5 cm) at Hoylake during 2014

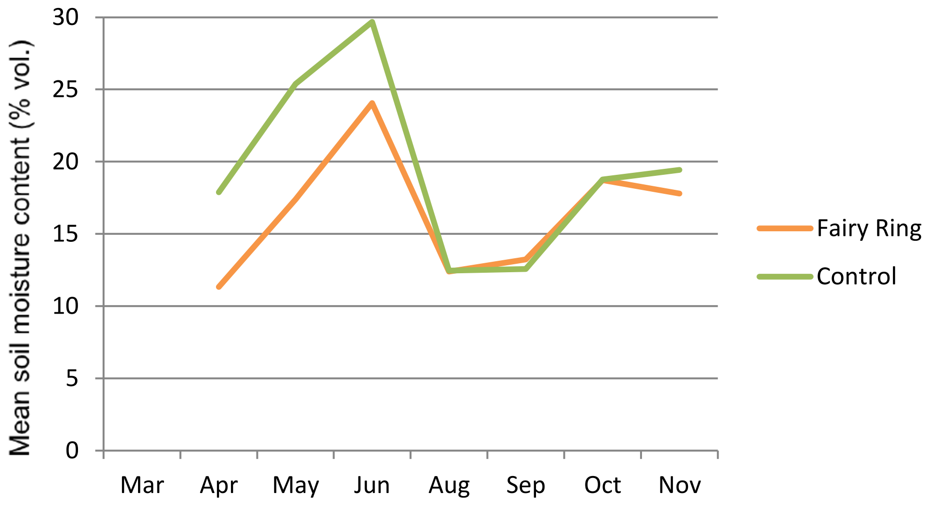

Figure 79: Mean soil moisture at deep depth (12 cm) at Hoylake during 2014

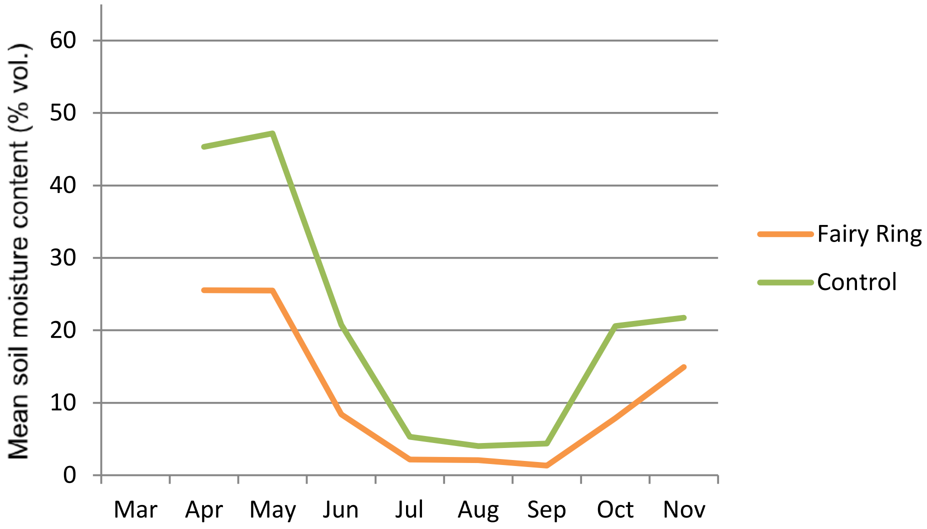

Figure 80: Mean soil moisture at shallow depth (3.8 cm) at Shropshire during 2014

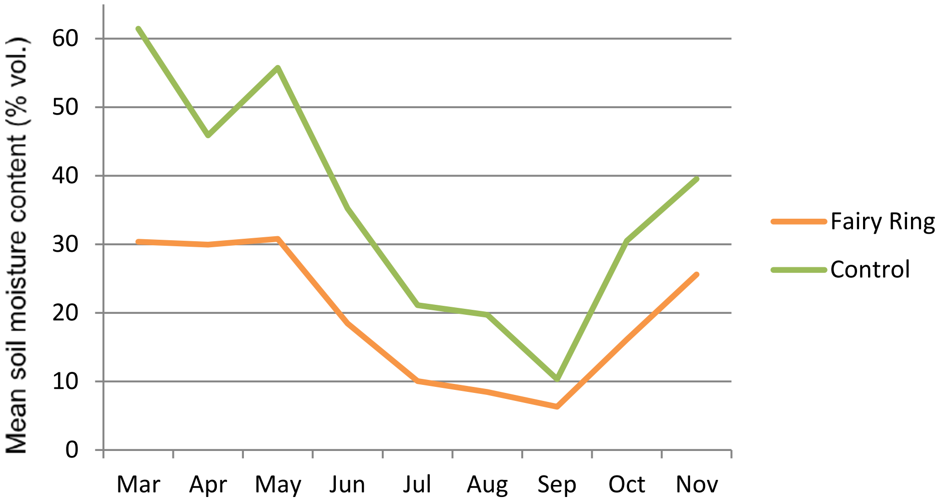

Figure 81: Mean soil moisture at middle depth (7.5 cm) at Shropshire during 2014

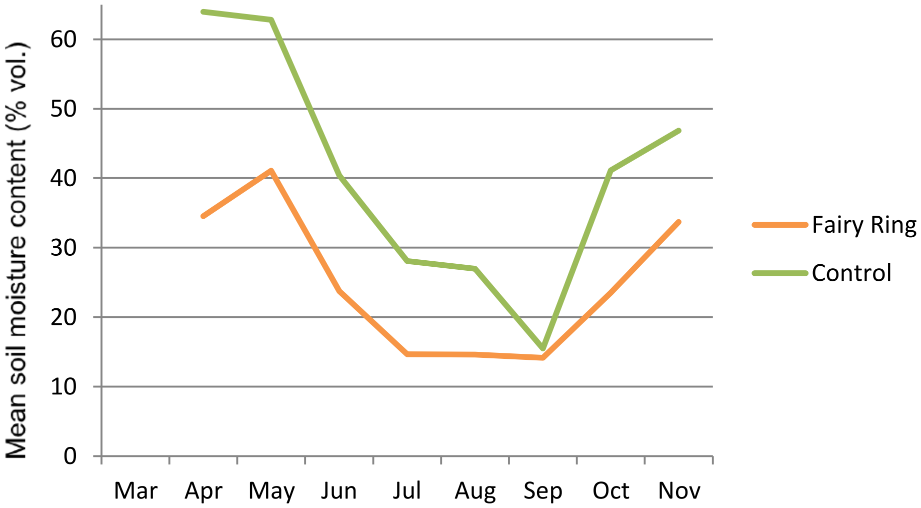

Figure 82: Mean soil moisture at deep depth (12 cm) at Shropshire during 2014

Whilst carrying out the WDPT test, it became evident that there was little variation in the time taken for a water droplet to soak into a soil core. The reaction was either instantaneous (which it was the majority of the time), had a slight delay of 2-5 seconds before fully absorbed, or, in rarer circumstances, sat beaded on top of the soil core for in excess of 60 seconds, indicating that the area was severely hydrophobic and barely penetrable to water.

Rather than looking for correlations between the amount of seconds for a droplet to absorb and potential severity of hydrophobicity, hydrophobicity for each sample plot was analysed as being either present or absent, with an instantaneous result representing absence of hydrophobicity and any time longer than that, where a delay was experienced, representing incidence of hydrophobicity.

One of the three rings tested at each site already had incidence of hydrophobicity when the experiment started in March. The other two rings had developed hydrophobicity by May at Hoylake (Figure 83) and by June at Shropshire (Figure 84).

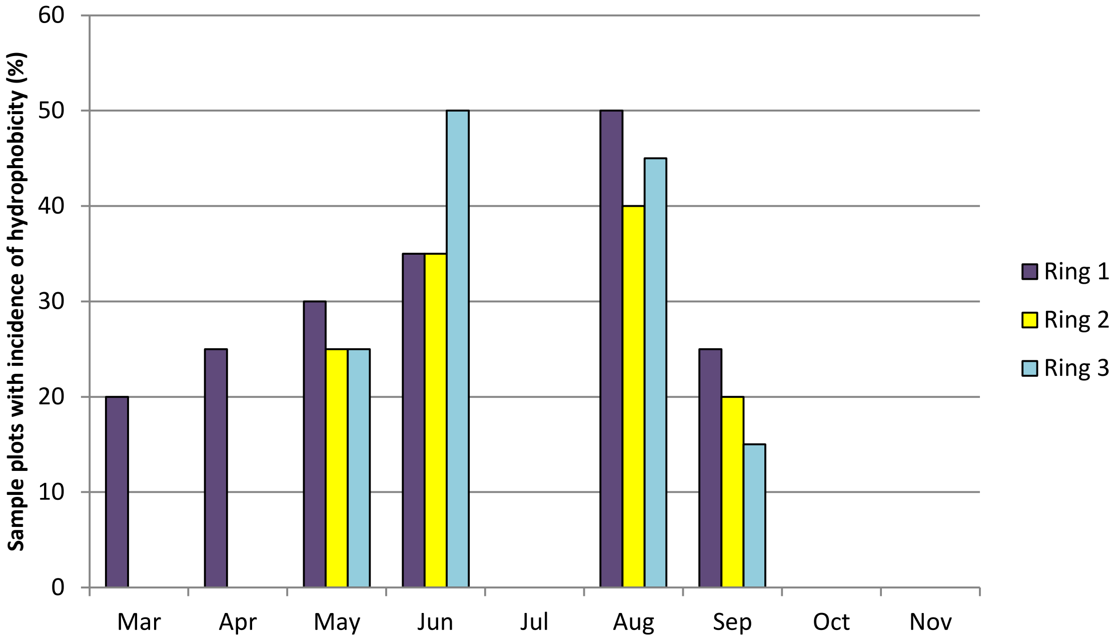

Figure 83: Percentage of *M. oreades* fairy ring sample plots with incidence of hydrophobicity, detected using a water drop penetration time test, at a soil depth of approximately 3.8 cm during 2014 at Royal Liverpool Golf Club, Hoylake. Rings were not accessible during July, due to The Open Championship golf tournament, and data therefore missing

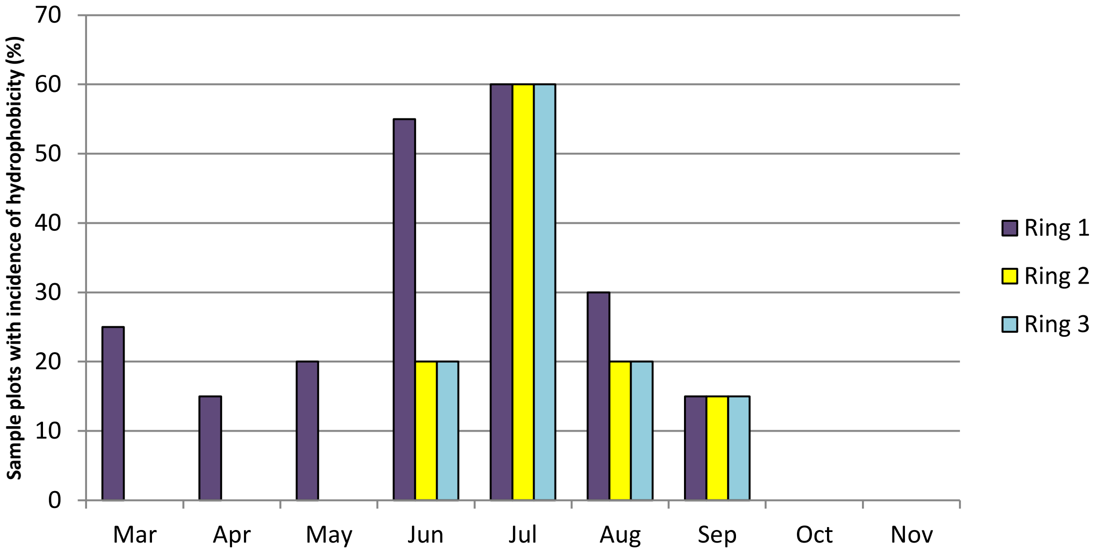

Figure 84: Percentage of *M. oreades* fairy ring sample plots with incidence of hydrophobicity, detected using a water drop penetration time test, at a soil depth of approximately 3.8 cm during 2014 at Shropshire Golf Centre, Telford

At Shropshire, hydrophobicity reached its peak incidence in July, where 60% of sample plots in all three rings were hydrophobic in the uppermost (3.8 cm deep) measuring level. This dropped off steeply in August and September and, by October, there was no incidence of hydrophobicity in any of the Shropshire rings.

Due to the rings at Hoylake being inaccessible whilst The Open Championship golf tournament was on, there is no data set for July, so what happened around this point is unclear. From the data available, hydrophobicity of the rings can be seen to peak, on average, in August. Again, a steep reduction in percentage of affected plots was seen by September and no incidence of hydrophobicity was recorded in October and November. Unlike Shropshire, however, the Hoylake site received the aforementioned maintenance between the September and October sampling dates, which is highly likely to have broken up any areas of hydrophobic soil and, hence, impacted the results.

Over the duration of the project, no hydrophobicity was recorded in either of the asymptomatic control areas. In the fairy rings, no incidence of hydrophobicity was encountered at the deepest measuring depth of approximately 12 cm. Incidence was recorded occasionally at the middle depth of approximately 7.5 cm at both sites, but was largely limited to the shallowest measuring depth of approximately 3.8 cm. Hydrophobicity was more than five times more likely to be found at 3.8 cm than it was at 7.5 cm (chi-square test p <0.001).

At Hoylake, non-hydrophobic samples had soil moisture contents ranging from 0 to 28.9% vol. (Figure 85). Hydrophobic samples ranged from 0 to 22.2% vol. soil moisture content. As hydrophobicity was thought to only occur at very low soil moisture contents, it was unexpected to experience it with a 22.2% moisture content sample. These data also show that fairy ring soil can be at 0% moisture without necessarily being hydrophobic. Whilst hydrophobic and non-hydrophobic samples from Hoylake appeared to fall within a similar range of soil moisture contents, statistical analysis showed that soil moisture did vary significantly according to the presence or absence of hydrophobicity (Mann Whitney U-Test, *p* <0.001).

Results from the Shropshire site followed a similar pattern. Hydrophobic soil moisture ranged from 0 to 38.6%, whereas non-hydrophobic soil moisture ranged from 0 to 58.6%. Statistical analysis showed that soil moisture did vary significantly according to the presence or absence of hydrophobicity (Mann Whitney U-Test, *p* <0.001).

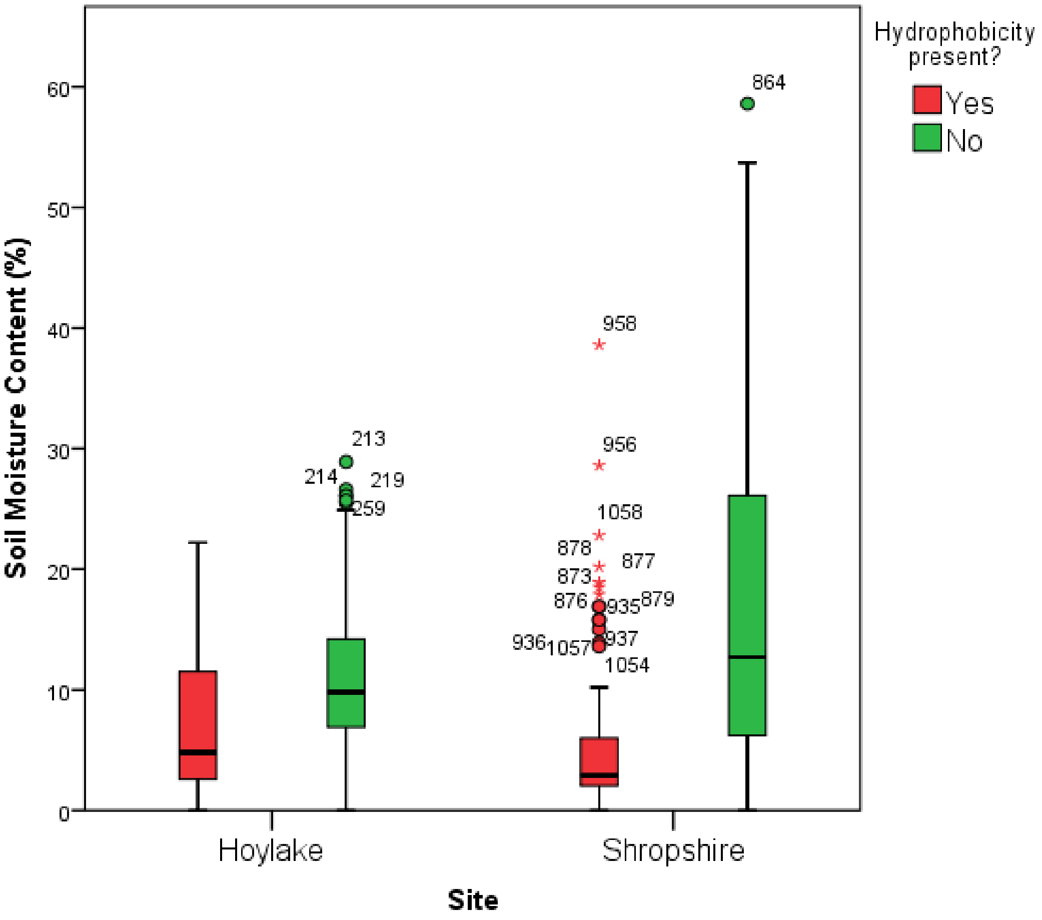

Figure 85: Range of percentage volumetric soil moisture content of hydrophobic and non-hydrophobic soil samples from *M. oreades* fairy rings at Royal Liverpool Golf Club, Hoylake and Shropshire Golf Centre, Telford

6.3.2.4 Discussion

From York and Canaway's (2000) claim that fungi find it easier to proliferate through sandy soils, it was expected that the links golf course, Hoylake, might be more badly affected by fairy ring, either through soil moisture deficit or incidence of hydrophobicity, than the parkland golf course, Shropshire, on loamy soil. Whilst the courses could not reasonably be compared statistically, due to the missing data for Hoylake from July, the raw data suggest that one course was not notably worse than the other.

Analysis by depth showed that, on the links golf course, variability between fairy ring and control soil moisture content was only pronounced at depths of 7.5 cm and 12 cm. At the parkland golf course, Shropshire, variability was also pronounced at a depth of 3.8 cm, meaning moisture deficit was greater in the upper layer than it was at Hoylake. This could be a result of soil structure and/or management regime. For example, a maintained golf course area that has received aeration, wetting agent, and perhaps top dressing with sand, applied to the surface, is going to have a more uniform upper soil layer than a natural, unmaintained turf, like that at Shropshire.

The incidence of hydrophobicity was at its worst in July for the Shropshire site and in August for Hoylake. This correlates with some of the data seen in Chapter 3.0, where respondents to the questionnaire reported that fairy ring symptoms were at their worst in July and August.

It was interesting to find that hydrophobicity was absent from both sites by the point of the October sampling dates. This was expected at Hoylake, as a result of the management that had been implemented following The Open Championship, but the same effect was also seen with the Shropshire rings, which had received no management.

Some of the rings experimented on at both sites contained patches of severe type-1 symptoms. As type-1 symptoms can often be visible year-round, it would not have been unexpected to find incidence of soil hydrophobicity persisting into the winter months, rather than vanishing by October.

Although hydrophobic soils were statistically drier, contrary to expectations, there was no clear cut moisture level below which incidence of hydrophobicity increased dramatically, showing that hydrophobicity is not a direct result of low soil moisture. Incidence of hydrophobicity was recorded in soil cores with up to 38.6% volumetric moisture content. There were also records of soils with 0% vol. moisture content not being hydrophobic. So, it would seem that there are more factors at work than soil moisture when it comes to the development of hydrophobicity.

Amongst the observations made whilst carrying out this experiment was that type-1 symptoms frequently occurred above soil cores that were not hydrophobic. So, what causes turf loss if it is not hydrophobicity?

Blenis (2004) produced data to support the hypothesis that *M. oreades* produces cyanide that it emits into the local rootzone, inhibiting the growth of both grass roots and beneficial rhizofungi. Blenis also discusses how this is exacerbated by the reduced soil moisture of fairy rings, as seen, which means that the cyanide is more concentrated and, hence, more damaging to the turf and rootzone than if water was present to dilute it. This is one possible explanation as to why turf could be lost without hydrophobicity being detected.

Attempts were made to include analysis of soil samples for cyanide content as part of the second year experiment, but an institution that had the necessary equipment to test for cyanide could not be sourced within the timeframe.

Another explanation for non-hydrophobic type-1 symptoms comes from Fidanza (2007). He found that concentrations of ammonium, potassium, sulphur, and soluble salts were significantly higher in fairy ring necrotic zones of *Agaricus campestris* than in asymptomatic samples. He puts this largely down to a reduction in microbial activity in the rootzone. This can occur as soil moisture becomes reduced or as conditions in the soil become hydrophobic. Toxic levels of ammonium can build up if the actions of microbes involved in nitrification or in the conversion of ammonium to nitrate become impaired. An accumulation of sulphur can also result from inhibited microbial activity and lead to production of hydrogen sulphide, which can be toxic to plant roots. In this circumstance, aerating the turf allows oxygen to penetrate into the rootzone, stimulating productivity, and releases some of the built up toxins.

In both of these cases, reduced soil moisture appears to be the pre-requisite for what may potentially be a series of feedbacks leading to the eventual necrosis of turf. If tackled early enough and regularly enough, it seems feasible that symptoms could be remediated, through aeration and wetting techniques.

6.3.2.5 Limitations

Like some of the work on fairy ring control, this is another experiment that was impacted by unforeseen events that affected results (when the Hoylake rings were selected at the beginning of the project, it was not known that The Open Championship was due to take place there two years later). This further highlights some of the difficulties associated with experimenting on fairy rings *in situ*, especially over any prolonged period of time.

6.4 Conclusions

In contrast to points raised in year one of the soil moisture study, this second year study would suggest that there is no particular soil moisture threshold at which hydrophobicity develops.

The fact that type-1 symptoms can persist without the presence of hydrophobicity, suggests that there are more factors involved in the necrosis of turf in fairy rings than simply soil moisture. The likelihood is that it is due to a combination of effects, which may include accumulation of toxins in the rootzone.

Perhaps the most useful finding across experiments one and two is that both significant moisture deficit and hydrophobicity can be present in rings as early in the season as March, and maybe even before. For species such as *M. oreades*, where development of type-1 symptoms appears to be a common occurrence, early treatment could go a long way in ameliorating symptoms. By treating fairy rings early in the year, even if they are not looking like too much of a problem from the surface, it could tackle the unfavourable edaphic conditions that could lead to severe symptom expression in the summer months.

6.5 Acknowledgements

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7.0 General Discussion

Despite fairy ring being a common turf disease, and by no means a new phenomenon, this is the first time that it has been studied in such detail in the UK. Previous studies have focused mainly on type-1 fairy rings caused by *Marasmius oreades* (Smith and Rupps, 1978; Smith 1978; Smith 1980a) or puffball species growing on golf putting greens (Terashima *et al*, 2002; Miller *et al*, 2007; Miller and Tredway, 2009; Miller, 2010) and been limited to studies at individual sites (Fidanza, 2002b) or individual seasons (York and Canaway, 2000) or have not specified the causal species (Settle *et al*, 2006). The most complete individual study on fairy ring to date is that of Miller (2010), who worked in the USA and, whilst his study generated some notable findings, they are not directly relevant to the UK because many of the chemicals tested are not licensed for use here in the UK and because the geographic and climatic differences between continents mean there are likely to be considerable differences in the fairy ring causal agents, if not in species composition, then certainly in genetic variation. Miller's (2010) study also focused exclusively on golf putting greens, whereas this study covered the golf course as a whole and potentially a greater range of fairy ring niches.

Firstly, an online questionnaire emailed to every golf course in the UK and Ireland aimed to gather information on the incidence, distribution, and severity of the disease for the first time. Results showed that type-2 fairy ring, where turf growth had been stimulated, occurred most frequently and the impact was predominantly aesthetic. Symptoms peaked in July and August and geographic analysis suggested that courses in the south-east of the UK may suffer from fairy ring more severely, possibly resulting from drier weather and higher temperatures.

In support of York's (1998) claim that fungi proliferate more readily through sandy soils, which could increase severity of fairy ring symptoms, the questionnaire results generated evidence that the links golf courses, characterised by sandy rootzones, were particularly badly affected. It is, however, difficult to ascertain whether symptoms were visually worse on links courses or whether the expectations of links golf courses to provide a higher quality turf surface exacerbate the perceived severity of flaws.

Some evidence was generated to support the theory that fairy ring symptoms are exacerbated by hot, dry weather, which could be expected to worsen hydrophobicity. Albeit a rather crude north-west/south-east divide of Great Britain showed that incidence of golf courses with problematic fairy ring was higher in what weather data showed to be the hotter and drier half of the country.

In *in vitro* tests, a product which does not currently include fairy ring on the product label, Banner Maxx (with the active ingredient propiconazole), proved to be significantly better at inhibiting fungal growth of every isolate tested, of species *Marasmius oreades*, *Agaricus campestris*, *Bovista plumbea*, and *Handkea utriformis*, than any of the other chemicals, including those labelled for fairy ring control. Miller (2010) carried out a similar experiment, but did not include *A. campestris* or *H. utriformis*, which were both tested here. Until this experiment, there were no existing data available on the control of *A. campestris* and *H. utriformis in vitro*. Miller (2010) also found propiconazole to be one of the most effective chemicals tested, but less so than triticonazole and tebuconazole, neither of which are available for amenity use in the UK.

Despite being the product often recommended for fairy ring control in the field in American literature (Fidanza, 2002a; Nelson, 2008), this study found that flutolanil showed poor performance *in vitro* compared to the other chemicals. Miller (2010) reported inconsistent results from flutolanil in his *in vitro* study.

An *in vitro* isolate pairing experiment aimed to identify whether mycelial growth provided any evidence of interspecific, intraspecific, or self-antagonism in *M. oreades* and *A. campestris.* Post-experimental research suggested that interpretation of the results may have been misconstrued and what, on the surface, appeared to be self-antagonism in isolates paired with themselves may not have been indicative of hyphal interactions submerged in the agar. The results did, however, show how vigour of growth can vary between, and also within, species, as certain isolates were dominant over others.

Propiconazole (Banner Maxx) provided very effective control of fairy ring isolates in the lab, but this was not replicated in the field. The field experiments in both years, however, experienced problems, with the fairy rings used in the first year potentially being too hydrophobic to absorb any chemical and the rings used in the second year being subjected to a disturbance event prior to final evaluation, by being incorporated into the new football pitch goal area.

Agaricus campestris appeared to be the most tolerant to the chemicals tested *in vitro*, having the greatest growth of all species tested, but since the rings generally only persist from July to September in the field, control is less of an issue than with the more persistent and/or damaging species, such as *Marasmius oreades*.

Marasmius oreades responded well to control treatments *in vitro*, although one isolate showed evidence of resistance to azoxystrobin, but was difficult to treat in the field. This is thought to largely be due to the incidence of soil hydrophobicity, which makes it difficult for water and, hence, control treatments to penetrate into the soil profile and make contact with the fungus. This is a problem that has also been reported by Smith (1980a) and Cisar *et al* (2000).

Until this project, little was known about the soil moisture of fairy rings and there was only one existing piece of literature on fairy ring related soil hydrophobicity (York and Canaway, 2000). York and Canaway's (2000) study, however, was only carried out on one date and no one had ever studied how soil moisture and hydrophobicity of fairy rings changes throughout the year.

In agreement with York and Canaway's (2000) conclusion that hydrophobicity occurs mostly in the top 3-5 cm of the soil profile, hydrophobicity was encountered at the 3.8 cm sampling depth used in this experiment five times more often than at the 7.5 cm sampling depth.

The experiments on soil moisture showed that *M. oreades* fairy rings can have significant moisture deficit and incidence of hydrophobicity as early in the year as March. However, hydrophobicity was found to have disappeared by October. This would suggest that timing of treatment may be the issue in treating fairy ring, as the standard procedure is often to treat in spring, when the symptoms first start to appear. As symptoms generally appear around April at the earliest, results show that this may be too late, as hydrophobicity may have already developed. These results provide evidence that treating fairy ring chemically may be more effective at the end of the year, perhaps in November, when hydrophobicity may have subsided or very early on in the year, before March, before hydrophobicity develops.

8.0 Conclusion

The aim of this study was to generate and disperse information on the causes, epidemiology, and control of fairy ring in order that turf managers could learn how to better manage the disease on their golf courses.

Dissemination of the findings into the greenkeeping community in order that the knowledge could be practically applied in golf course management was a vital part of the project. This was, and will continue to be achieved through publications and presentations. To date, an overview of the questionnaire findings has been published in the International Turfgrass Society Research Journal (Keighley *et al*, 2013; Appendix III) and a poster presented at their conference in Beijing, China (Appendix IV), the first year of soil moisture work was published in the European Journal of Turfgrass Science (Keighley *et al*, 2014; Appendix V) and a poster and short talk presented at their conference in Osnabrück, Germany (Appendix VI), and some of the early findings were written in an article for STRI's Bulletin for Sports Surface Management, which is distributed to golf clubs and other sports organisations. Towards the end of the project, summaries of the main findings were presented to many greenkeepers and other turf managers at the STRI Research Days in September 2014 and at the BIGGA Turf Management Exhibition (BTME) in January 2015. A final presentation was also delivered to the sponsors, The R&A, alongside guests from The Open Championship Venues, STRI, and other related organisations, at their annual meeting in St Andrews in March 2015.

The findings herein have shown how, when, and where fairy ring is a problem in the UK, alongside recommendations for managing disease symptoms, such as the importance of water management in preventing hydrophobicity and the potential of propiconazole as a fungicidal treatment.

There must be a greater emphasis on determining which species is causing the symptoms before attempting control. Referring to ‘fairy ring’ as a turf disease promotes a misconception that this is one disease with one potential cure, whereas fairy ring symptoms can be caused by a number of distinctly different species that may respond dissimilarly to treatments. By focussing on each causal species as a separate disease, control methods could be researched and tailored more effectively, but to do this, we need to improve the way we identify individual fairy rings to genus or species level.

9.0 Recommendations

Whilst this study has greatly furthered understanding of fairy rings on UK golf courses, there is still much to learn.

Development of a DNA catalogue of basidiomycete species implicated in causing fairy ring symptoms was one of the primary aims of this project, but, with time as the limiting factor, and various other aspects of the project to deliver, the continued attempts to extract, amplify, and sequence fairy ring DNA eventually had to be abandoned.

With more time, the development of a DNA catalogue for fairy rings in the UK, similar to that compiled by Miller (2010) for the USA, should be achievable. Perhaps the Commentary of Laboratory Work, shown at Appendix I, could give another researcher a head-start on this. With today’s rapidly advancing molecular techniques, it should become increasingly easy in the future to identify fairy ring fungus species from samples.

There is still much to find out about the mechanisms leading to the development of hydrophobicity. Results from this study suggest that there are other factors at work than purely soil moisture content. A better understanding of what hydrophobicity is and why it develops will be integral in further advising on how to prevent it. Investigations into the chemical make-up of hydrophobic soils would be useful, including substances such as cyanide, which could not be tested for during this study.

Fungicidal control of fairy rings is an unsustainable solution and future research should aim to develop ways to manage symptoms through cultural practices, such as water and nutrient management, wherever possible.

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Appendix I

Fairy Rings on Golf Courses: Commentary on Laboratory Work

The following notes give a brief overview of some of the laboratory procedures undertaken during the project with the aim of sequencing fungal DNA from fairy ring samples.

Isolating pure fungal cultures from fairy ring soil cores

Autumn 2012

- Visible, white mycelium growing around some of soil cores peeled off with tweezers, washed with distilled water and placed on half strength potato dextrose agar (19.5g/L amended with 10ml streptomycin) establishes well in culture
- Other cores do not develop visible mycelia, even when incubated
- Attempts to bait the fungi by placing wooden dowels (birch and beech; moistened and dry) in with the soil cores are unsuccessful
- Attempts to culture anything other than what is later found to be *M. oreades* become contaminated

Spring 2013

- Now more soil cores with visibly growing mycelia. Eleven further unidentified cultures established on PDA by removing mycelial growth from soil cores. Varying mycelial characteristics seen in this batch, rather than the familiar thick, brown/yellow-tinted growth of *M. oreades*

Isolating fungal cultures from fairy ring basidiocarps

Autumn 2012

- Sections of *M. oreades* basidiocarps generally establish well in culture on PDA
- Establishment of an identified culture allows existing cultures from soil cores to be identified as *M. oreades* due to shared characteristics (see Figure 1)

Figure 1 (right): Young *M. oreades* culture starting to exhibit characteristic dense growth with brown/yellow colouration

- It is noted that, throughout the project, *M. oreades* rarely succumbs to contamination in comparison to other isolates

Summer 2013

- Contamination of many of the cultures with ‘the black fuzz’ means some isolates have to be re-established

Autumn 2013

- Developed a number of new cultures from basiocarps, including *Agaricus campestris*, using spore drop method (section of gill suspended from top of Petri dish with petroleum jelly and left for 2-4 hours for spores to drop onto PDA)
- Fresh puffballs are pierced and squeezed to blow spores onto agar surface, but fail to establish on PDA
- Tried a medium recommended by Leonian (1924) for culturing of puffball species. Containing /L:
    - 1.25g KH_2PO_4 (monopotassium phosphate)
    - 0.625g Mg_2SO_4 $7H_2O$ (magnesium sulphate)
    - 0.625g peptone
    - 6.25g maltose
    - 6.25g malt extract
    - 20g agar
    - 10ml streptomycin
- *Bovista plumbea* and *Handkea utriformis* establish for the first time and grow, albeit slowly, on this medium (Figures 2 and 3)

Figure 2: Relatively slow growing *Bovista plumbea* culture, which was eventually established on Leonian agar

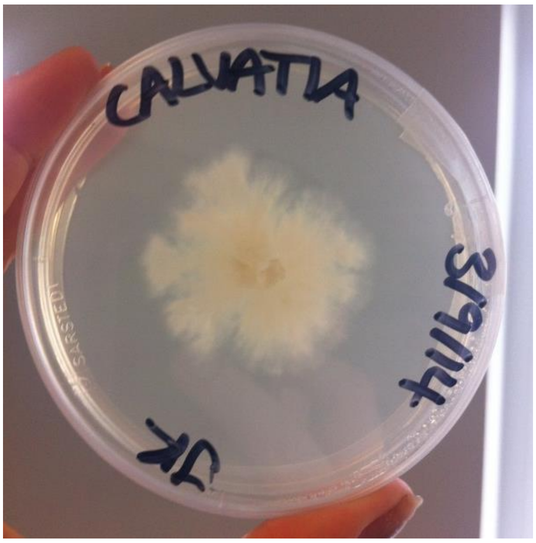

Figure 3: Distinctive hyphal branching of *Handkea utriformis* culture (formerly *Calvatia utriformis*), which was eventually established on Leonian agar

- Approximately half of culture collection gets wiped out by contamination expected to be a *Penicillium* sp. that has been circulating in LEV cabinet

Summer 2014

- Mite infestation in the lab wipes out large proportion of culture collection
- Sets of new cultures developed during this year's fruiting season from fresh basidiocarps

DNA extraction and PCR amplification

Spring 2013

- Comparison between freeze thaw method (CTAB) and rapid method with Chelex carbon buffer (10% Chelex, 5% carbon) shows them to be similarly effective
- 1/10-1/1000 Serial dilutions established with sterile distilled water
- Started PCR protocols with fungal primers ITS4 and ITS5 on ANN50 programme (annealing temperature 50˚C, full thermocycle details not recorded) on *M. oreades* cultures from basidiocarps and soil cores. Nanodrop results show DNA successfully extracted by both aforementioned methods, but gel run after PCR indicates high primer dimer
- Soil extraction method scaled down from a paper by Woodhall (2012) using ball bearings in Nalgene bottles to homogenise soil yielded some results which amplified poorly and were not strong enough to proceed with
- Series of qPCR runs on all cultures plus basidiocarps with ITS4 and ITS5, new Evagreen buffer, and programme changed to ANN58. Only samples from *M. oreades* basidiocarp tissue were positive (MO 10^{-2} being the strongest result)
    - ANN58 thermocycle:
    - 94°C for 1:15 mins
    - 94°C for 15 secs; 58°C for 15 secs; 72°C for 45 secs X34
    - 72°C for 4:15 mins
    - ∞ 4°C
- DNA extracted from basidiocarps of five other fairy ring fungus species to try and build a reference of positives with which to compare other samples
- Moved onto basidiomycete-specific primers of ITS4b and ITS1F with Evagreen buffer, using qPCR. Good results for three basidiocarp extractions 10^{-2} and 10^{-3}

Summer 2013

- Tried FastDNA Spin Kit for Soil – results all negative (apart from positive control)
- Tried 'BASIDIO' PCR programme from Miller (2010)
    - BASIDIO thermocycle:
    - 94°C for 1 min
    - 94°C for 30 secs; 55°C for 1 min; 72°C for 1 min X32
    - 72°C for 2 mins

- Ruled out 10^{-3} dilutions – not strong enough to yield results
- Started using fresh basidiocarp tissue of *M. oreades* and *Agaricus campestris* in soil kits (i.e. no soil) to try and develop positive controls, before slowly incorporating soil with basidiocarp
- Started freezing extractions immediately, as nanodrop results show that DNA samples deteriorating quickly when refrigerated

Autumn 2013

- FastDNA Spin Kit eventually yields some genomic DNA from fairy ring infested soil (ITS4B/1F on BASIDIO thermocycle), but not clean enough to proceed with
- Compared an alternative kit: Powersoil by MoBio – conclude Powersoil more effective with primers ITS4/5 and FastDNA more effective with primers ITS4B/1F
- Ran some new soil samples of unidentified infection causing thatch collapse at Royal Liverpool Golf Club – samples test positive for basidiomycete DNA (i.e. with primers ITS4B/1F)
- Tried BAS-2R+ and BAS001 primers, as used by Miller (2010). Run with all three primer sets for quite some time, but find results generally better with ITS4B/1F
- Developed some reasonably good quality DNA samples from fresh *A. campestris* and *M. oreades* basidiocarps, with the aim of sequencing these and using them as a reference for further samples

Winter 2013-2014

- Tried soil extractions from start again using method used by Miller (2010), where samples washed in sodium pyrophosphate (tetrabasic ≥ 95%) and sieved through 850, 300, and 63 μm sieves with tap water prior to running through Powersoil kit
- Reasonably clear gel bands for several of samples. Undiluted samples were clearest, showing that washing and sieving removes need to dilute

Spring 2014

- BAS-2R+ and BAS001 primers show some evidence of working better with thatch and puffball species, but not so good with *A. campestris* and *M. oreades*
- Five best washed/sieved soil extraction samples taken through to cloning stage.

DNA purification and sequencing

Winter 2013-2014

- Erroneously used Wizard Genomic DNA Purification Kit (not suitable) before switching to Wizard SV Gel and PCR Clean Up System

- Tried cloning of samples from fresh *A. campestris* and *M. oreades* basidiocarps using Promega pGEM-T Kit and *E. coli* (JM109 high efficiency), which is successful
- PCR'd clones show positive for target DNA, showing correct inserts were accepted
- Ran Promega Pureyield Plasmid Miniprep Kit
- Four samples with strongest gel bands sent to Eurofins for sequencing (2no. *M. oreades* and 2no. *A. campestris* samples, both originating from fresh basidiocarps)
- Only one of the four samples yielded a result.
- *A. campestris* (extracted from a basidiocarp) returns a fractional sequence of 20 base pairs, which a BLAST search shows a 100% match with a *Helicobacter* sp. (bacterium) and 94% match with the cultivated mushroom *Agaricus bisporus*
- Sequence is too short to draw any firm conclusions
- Subbing-off clones is repeatedly unsuccessful and samples with correct insert appear to get lost

Spring 2014

- Five best washed/sieved soil extraction samples taken through cloning process, show no DNA in PCR'd clones, indicating that insert has not been taken up

Summer 2014

- Nanodrop results show that purified soil DNA extractions have deteriorated considerably, even though stored in freezer
- Another cloning run yields negative results post-PCR

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Appendix II
STANDARD OPERATING PROCEDURE NO. 1B0712

VISUAL ASSESSMENT OF TURF QUALITY

1. Scope

This standard operating procedure specifies methods for assessing the visual quality of sports and amenity turf.

2. Principle

Turf quality is determined by subjective visual assessment using a 1 to 10 scale. Factors taken into account are sward density, uniformity, turf colour, grass cover, weed content and disease and pest invasion.

3. Procedure

One of two assessment methods shall be used: (a) if there is a need to define the overall quality and acceptability of turf in relation to usage e.g. golf green, winter sports pitch or (b) if it is desirable to score visual differences which are apparent among treatments but are not necessarily linked to one particular use.

3.1 (a) Assessment to define the quality and acceptability of turf

The turf is assessed on a 1 to 10 visual scale where a score of 1 represents very poor turf quality and a score of 10 signifies very good turf quality. A value of 5 represents turf that is just acceptable and values below 5 shall be used if turf quality is not considered acceptable. To help visualise this scale, subjective descriptions for rating turf quality are provided in Table 1.

TABLE 1
Scale for scoring turfgrass quality

1 = Turf in very poor condition, largely dead grass or bare ground.

2 = Turf in poor condition, majority of sward showing signs of discoloration, stress or damage. Some obvious dead or bare patches.

3 = Turf appearance uneven and showing signs of discolouration or stress. May be some evidence of turf thinning and a few dead or bare patches.

4 = Grass cover largely complete. Obvious but not severe discoloration or evidence of disease activity or other factors that affect sward uniformity.

5 = Acceptable turf (fit for intended purpose). Grass cover complete. Some evidence of stress factors that limit visual quality (low fertility, drought, wear, disease, etc.). Variation in sward uniformity apparent.

6 = Turf appearance generally good. A few stress factors (superficial disease activity, uneven growth or colour) or small variations in uniformity apparent on inspection.

7 = Turf appearance good. May be able to find first signs of disease or evidence of other stress factors on close inspection. Few but some differences in sward uniformity.

8 = Turf appearance good, no evidence of disease or other stress factors on close inspection. Sward very uniform in appearance. Little but some scope for improvement left.

9 = Turf appearance and uniformity very good. No evidence of disease or other stress factors present.

10 = Turf perfect

3.2. (b) Subjective assessment of observed variations among grass cultivars, species and mixtures

Individual plots will be assessed on a 1 to 10 scale (1 = very poor, 10 = very good). For each assessment a score of 5 will be used to describe plots which could be placed in the middle of the ranking order for that particular assessment. Scores below 5 should be given to plots which fall below this average and above 5 for those which are observed to have greater visual appeal. For each assessment the maximum range between 1 and 10 which can be reasonably scored should be used.

Where two or more individuals are carrying out the assessment, each should obtain a unique score by acting independently.

4. Expression of the results

Where two or more individuals make assessments, the mean turf quality value should be calculated.

NOTE:

This is a copy based on an electronic format for inclusion in reports.

The definitive and signed copy can be viewed at STRI.

FAIRY RING DISTRIBUTION, INCIDENCE AND SEVERITY ON UK GOLF COURSES

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ABSTRACT

Fairy ring, a turf disease caused by many basidiomycete species, affects golf courses worldwide. In the UK, however, fairy ring has not been extensively studied. An invitation to complete an online questionnaire, which sought to determine the distribution, incidence and severity of fairy ring, was emailed to 3,475 golf clubs in the UK. A total of 169 questionnaires were completed, which confirmed that fairy ring occurred throughout the UK, with 68% of respondents having recently been affected. 62% of affected respondents considered fairy ring to be a problem on their course. Type 2 fairy ring was the most common, with 90% of fairy ring-affected courses reporting it present, and the negative impact was predominantly aesthetic. Type 1 fairy ring had the greatest effect on playability. Both fairy ring types were considered most severe when occurring on golf putting greens. The links courses had the highest incidence of problematic fairy ring when compared to parkland and heathland courses. The higher proportion of courses suffering from problematic fairy ring were located in the generally hotter and drier south and east of the UK compared to the colder and wetter north and west UK suggesting a potential association between climate and fairy ring incidence.

INTRODUCTION

The activity of over 60 different species of basidiomycete fungi can cause the turf disease known as ‘fairy ring’ (Fidanza, 2009). These species vary in their individual response to a range of control methods, making it a difficult disease to manage.

The symptoms generated by the different species are categorised into four ‘types’ which cause various problems on a golf course. Type 1 fairy ring manifests as a circle or arc of necrotic turf, resulting from soil hydrophobicity (Fidanza *et al*., 2007). Type 2 fairy ring is characterised by darker and/or taller grass, where growth has been stimulated; and type 3 fairy ring is the occurrence of mushrooms or puffballs, which is not necessarily detrimental to the turf (Couch, 1995). The fourth type, known as superficial fairy ring, occurs as patches of discolouration with mycelial growth at the base of the sward (Dernoeden, 2013).

The incidence and severity of these different types of fairy ring on UK golf courses is poorly understood due to a lack of previous research. More information is needed on the occurrence of fairy ring in order to understand the disease’s epidemiology and to develop effective control strategies. A questionnaire was designed to investigate associations between fairy ring incidence and severity and geographic distribution which may indicate a relationship with environmental or climatic factors.

MATERIALS AND METHODS

The data for the study were collected via a survey of UK golf course managers and greenkeepers. Containing details of 3,475 UK golf courses, the database of the Sports Turf Research Institute (STRI) was determined to be the largest available contact list and so was used as the sampling frame for this study. An email and a series of reminders were sent between April and June 2012 requesting that recipients take part in the survey and providing a link to an online questionnaire supported by Survey Monkey. The questionnaire had been developed from a review of the fairy ring literature, consultation with experts, and piloting with a small number of agronomists from STRI. The final version of the questionnaire comprised ten questions (see Appendix), supported by pictorial aids which sought to ensure that respondents properly appreciated the different types of fairy ring.

Recipients were asked about the presence of the four types of fairy ring on their course and if and why they caused a problem. Respondents recently affected by fairy ring that they considered problematic were asked to score the severity of each different fairy ring type occurring on each different part of their course (i.e. tee, carry, fairway, rough, putting green or other) on a Likert scale of 1 (not serious) to 5 (very serious). These severity scores were then pooled and added to an assigned value indicating the proportion of the course affected by fairy ring, using golf holes as units (i.e. 1 or 2 holes, several holes, lots of holes, or all holes), to calculate a severity index (SI) for each course.

Postcodes provided by the respondents were used to geographically map fairy ring incidence using GIS software Esri ArcMap (v. 9.2) and Ordnance Survey map data downloaded from EDINA Digimap. This map was divided into two climatic zones – the hotter and drier south and east UK and the colder and wetter north and west – by using MetOffice rainfall and temperature maps to determine the best fit of a straight line, which then intersected the map diagonally from Sidmouth, Devon to Newcastleupon-Tyne. Golf courses were assigned to one of six incidence categories: never had fairy ring; previously had fairy ring; have fairy ring, but it is not a problem; have problematic fairy ring with low- (SI <20); medium- (SI 20-39); or high (SI 40+) severity level. Using SPSS (v. 19), incidence categories and SI of golf courses were compared statistically by climatic zone and golf course type (i.e. links, parkland, or heathland).

RESULTS

A total of 169 useable responses to the survey were received, which while only a 5% response rate, still means that the results are significant at the 95% confidence level with 7.5% accuracy (West, 1999). The majority of courses (68%) had been affected by fairy ring within the past 12 months. Respondents reported that disease symptoms were at their worst in July and August. Of the respondents recently affected by fairy ring, 62% said it caused a problem by negatively impacting their course. Type 2 fairy ring occurred most frequently, with 90% of respondents reporting its presence on

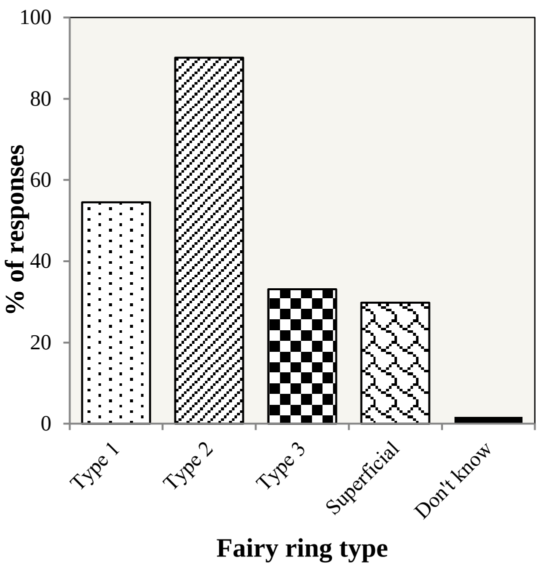

Figure 1: Incidence of the four fairy ring types on UK golf courses, as reported by questionnaire respondents, n = 253 (respondents could select multiple types ∴ n = no. of selections).

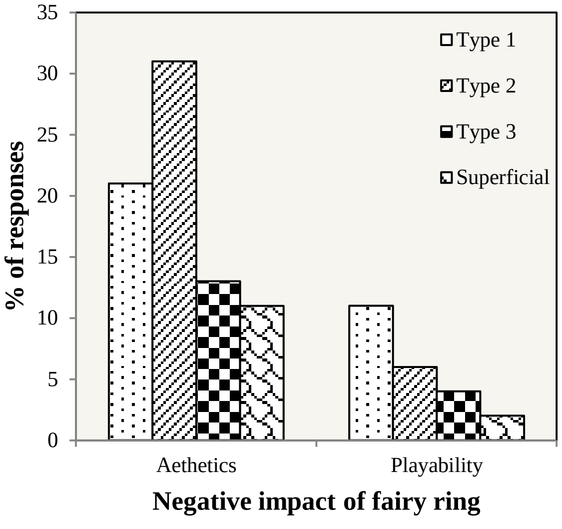

Figure 2: Reason the four fairy ring types were considered problematic on UK golf courses, as reported by questionnaire respondents, n = 435 (respondents could select reasons for multiple fairy ring types on multiple parts of the course ∴ n = no. of selections).

their course (Figure 1). Of those affected solely by type 2, only 44% considered it problematic. Most courses (65%) were affected by more than one type of fairy ring. For fairy ring that was considered problematic, the negative impact was predominantly visual, particularly type 2 (Figure 2). Playability was affected the most by type 1 fairy ring, which occurred most frequently on fairways. Problematic type 2 and superficial fairy ring occurred most frequently on greens, whereas the highest incidence of problematic type 3 was in the rough.

Mapping fairy ring incidence showed that the disease is widely distributed across England, Wales, Scotland, and Northern Ireland. On analysis of incidence categories by climate zone, a greater proportion of courses in the hotter and drier south and east of the UK were found to be affected by fairy ring, particularly by problematic fairy ring of medium- and high-level severity, when compared with the colder and wetter north and west ($\chi^2 = 144.45$ $p = <0.001$; Table 1). Incidence of problematic fairy ring was higher for links courses, especially medium- and high-level severity categories ($\chi^2 = 14.49$ $p = 0.025$; Table 2). Heathland courses were the most likely to have never had fairy ring and the disease was perceived to be less of a problem on parkland courses.

Severity scores showed that all four types of fairy ring were considered most serious when occurring on greens. Numerically, type 1 fairy ring was considered the most severe, although severity scores between fairy ring types were not quite significantly different at the 5% level ($p = 0.052$). Severity indices (SI) could be calculated for 63 courses and ranged from SI 5 to SI 68. Severity indices did not differ significantly by climate zone (Mann-Whitney U test $p = 0.355$) or golf course type (Kruskal-Wallis test *p* = 0.507) and courses with high level severity (SI 40+) were found throughout the UK; from the far north of Scotland to the Channel Islands in the far south near the French coast. A large majority (91%) of respondents with affected courses wanted to know more about dealing with fairy ring on their course.

Table 1: Fairy ring incidence of UK golf courses by climate zone

Region of UK	No. of responses	Fairy ring incidence (%)*					
		Never had fairy ring	Previously had fairy ring	Have fairy ring, but it is not a problem	Have problematic fairy ring and SI =		
					<20	20-39	40+
NW	77	18	22	35	12	10	3
SE	80	14	10	28	13	29	8

* Chi-square (χ^2) analysis for a 2 x 6 contingency table, where $\chi^2 = 144.45$ at $p \leq 0.05$.
SI = severity index.

Table 2: Fairy ring incidence by UK golf course type

Course type	No. of responses	Fairy ring incidence (%)*					
		Never had fairy ring	Previously had fairy ring	Have fairy ring, but it is not a problem	Have problematic fairy ring and SI =		
					<20	20-39	40+
Links	38	8	11	18	16	37	11
Parkland	117	15	15	38	9	21	3
Heathland	23	30	22	17	13	13	4

* Chi-square (χ^2) analysis for a 3 x 6 contingency table, where $\chi^2 = 14.49$ at $p \leq 0.05$.
SI = severity index.

CONCLUSION

Type 2 fairy ring was the most common fairy ring overall, but it was only considered to be a problem on less than half of the affected courses. It was considered to be most problematic on greens where its impact was mainly aesthetic. Type 1 fairy ring had the greatest effect on playability and had a higher incidence of being problematic on fairways than on greens. Type 1 symptoms may be suppressed on greens compared to fairways due to more intensive management practices, such as aeration and wetting. Severity was highest on greens for every fairy ring type and there was some evidence to suggest that type 1 may be the most severe. Whilst severity was not linked to either golf course type or climate zone, incidence of problematic fairy ring was found to be highest on links courses and on golf courses in the south and east of the UK. This suggests that fairy ring symptoms may be exacerbated by sandy rootzones and hotter, drier climatic conditions, supporting previous observations by York (1998) and Fidanza (2009). Incidence of courses that had previously had fairy ring was double in the north and west compared to the south and east, suggesting that the disease was less persistent there or easier to treat successfully.

This investigation provides a foundation on which to develop further research on fairy ring in the UK. Control methods should concentrate on possibly disguising type 2 fairy ring symptoms, and dealing with hydrophobicity associated with type 1; with management on links courses and preventative and curative treatment of greens needing particular attention. Results show that different types of fairy ring can present different issues on different types of golf course; meaning that there is no one control strategy for managing fairy ring. Thus, the multifarious nature of the disease may be best addressed with an integrated approach to turf management.

ACKNOWLEDGEMENTS

The authors would like to thank: the golf course managers for completing the online questionnaire; The R&A, Fife, Scotland and Harper Adams University, Shropshire, UK for funding the project; Carolyn Beadsmore and Helen Waite from the STRI marketing department for help with questionnaire design and delivery; and STRI soil scientist Dr Christian Spring and the STRI agronomists, particularly Henry Bechelet, for reviewing the questionnaire.

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APPENDIX

Fairy Ring Questionnaire

Question	Answers
1. Please enter the post code of your golf course	…………………….
2. Has your course had any fairy ring symptoms in the past, at any time prior to the last 12 months i.e. before April 2011?	Yes/No/Don’t know
3. Has your course had any fairy ring symptoms in the past 12 months?	Yes/No/Don’t know
4. At what time of year do you find that fairy ring symptoms are at their worst? Please tick all that apply.	Jan/Feb/Mar/Apr/May/ Jun/Jul/Aug/Sep/Oct/ Nov/Dec/Don’t know
5. Please select which type(s) of fairy ring occur on your course. Please tick all that apply.	Type 1/Type 2/Type3/ Superficial/ Don’t know
6. Do any of these types cause a problem on your course? A fairy ring may be considered a problem if it affects game play or visual appearance of the course in a negative way.	Yes/No/Don’t know
6*. Does fairy ring cause a problem on your course? A fairy ring may be considered a problem if it affects game play or visual appearance of the course in a negative way.	Yes/No/Don’t know
7. Considering each type of fairy ring on your course, please select the relevant table below and tell us where it is on the	Tables with drop-down boxes: part of hole, reason

hole, why it is a problem and how severe it is. Please select all that apply and give as much information as possible.	for problem, severity score (1-5)
8. Please indicate where fairy ring (all types) is a problem on your course.	1 or 2 holes/Several holes/Most holes/All holes/Don’t know
9. Where fairy ring (all types) is a problem, please indicate how many rings or partial rings there are.	1 or 2 rings/Several rings/Lots of rings/It varies/Don’t know
10. Would you like to know more about dealing with fairy ring on your course?	Yes/No

FAIRY RING DISTRIBUTION, INCIDENCE AND SEVERITY ON UK GOLF COURSES

J Keighley, M Hare, S Edwards, R Mann and K Walley
Harper Adams University and STRI

Corresponding author: Jennie Keighley

Introduction

Management of the fungal turf disease ‘fairy ring’ is poorly understood in the UK due to a lack of previous research, and is complicated by the large number of different basidiomycete species capable of causing symptoms.

A questionnaire, delivered to every known golf course in the UK, aimed to establish background information on the disease, including: the prevalence of the different types of fairy ring symptoms (Figure 1); where they occur on the golf course; and the severity of the symptoms.

Type 1: Ring of necrotic turf

Type 2: Ring of stimulated turf

Type 3: Ring of mushrooms or puffballs

Superficial: Patch of discolouration in the thatch

Figure 1: Classification of fairy ring symptoms

Methodology

In an online questionnaire, emailed to 3,475 UK golf courses, golf course managers were asked about the presence of different types of fairy ring on their course. If they had fairy ring incidence which they considered to be a problem, they were asked to score severity for each case.

Responses were used to map UK fairy ring incidence (Figure 5) and severity indices (SI) were calculated for golf courses that considered their fairy ring incidence to be problematic.

Results

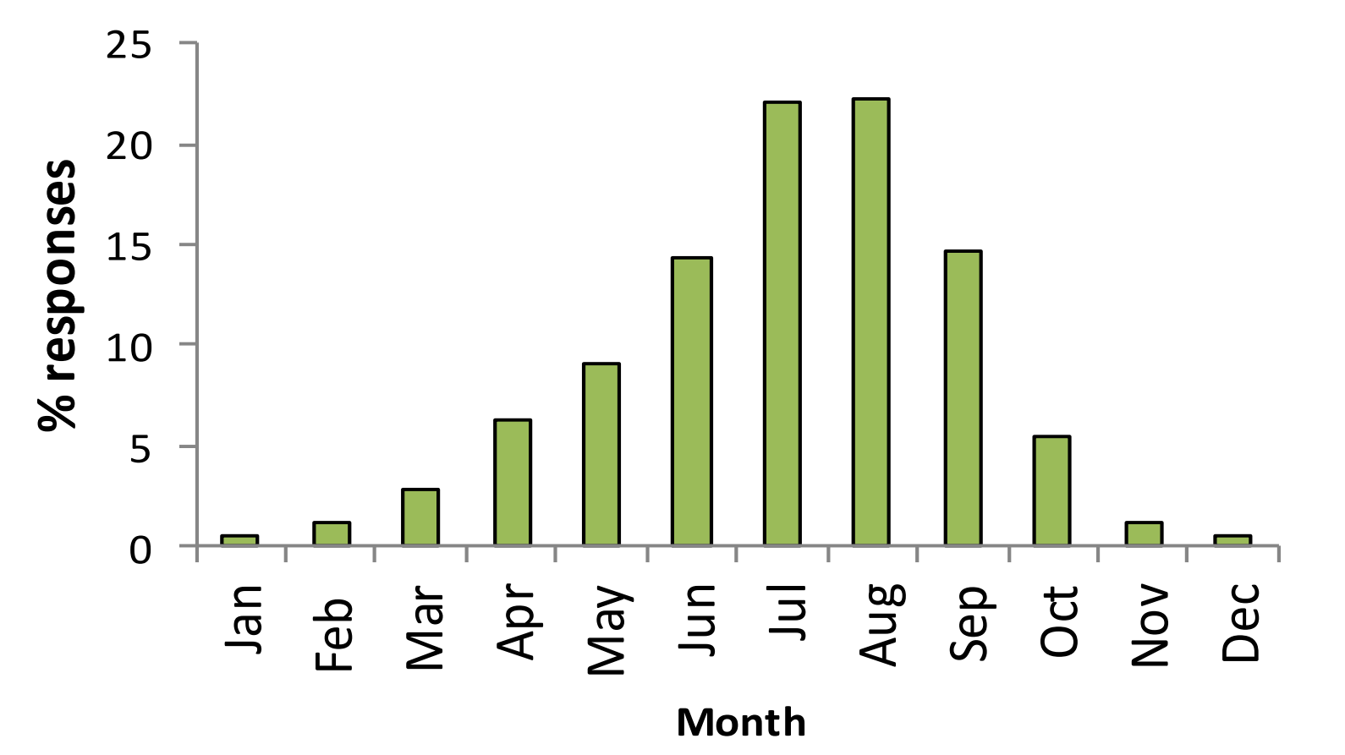


Figure 2: Time of year questionnaire respondents considered fairy ring symptoms on UK golf courses to be at their worst

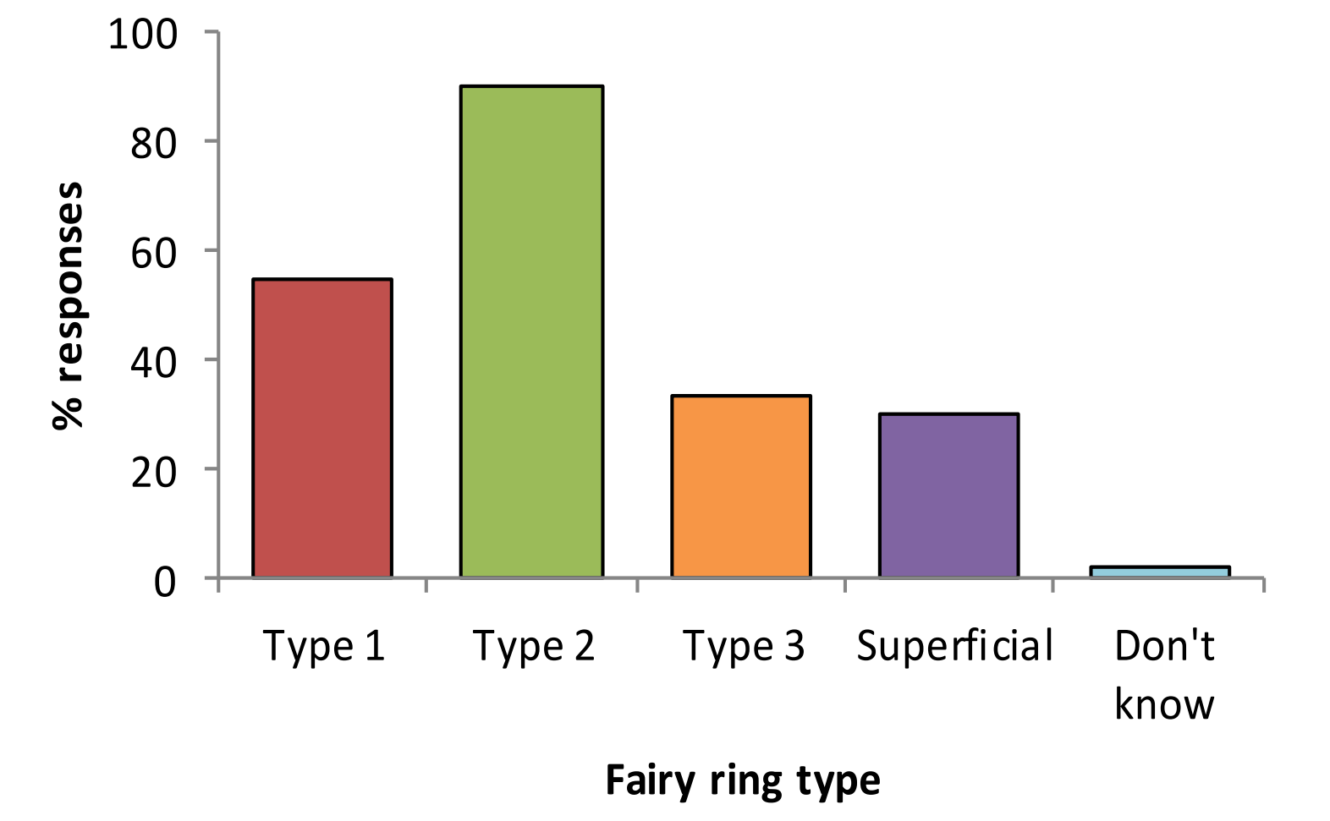


Figure 3: Incidence of the four fairy ring symptom types on UK golf courses as reported by questionnaire

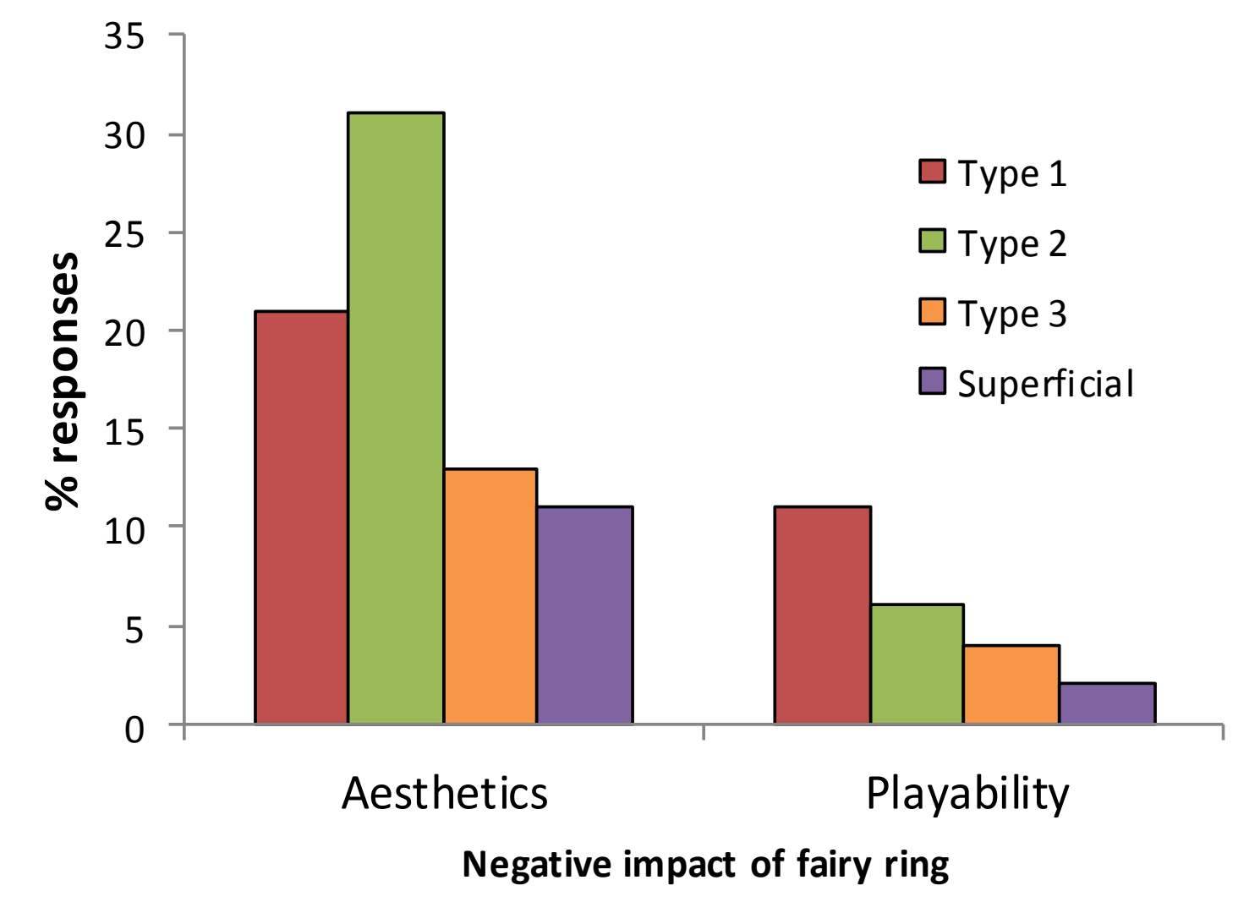


Figure 4: Reason the four fairy ring symptom types cause negative impact on UK golf courses as reported by questionnaire respondents

- Symptoms at their worst in July and August (Figure 2)
- Type 2 fairy ring most frequent (Figure 3)
- Negative impact of type 2 predominantly aesthetic, whereas type 1 most frequently affects playability (Figure 4)
- Type 1 fairy ring most frequent on fairways; type 2 and superficial on greens; and type 3 in the rough
- All four types most severe when occurring on golf putting greens
- Type 1 is most severe fairy ring symptom, but difference not quite statistically significant at 5% level (*p* = 0.052)

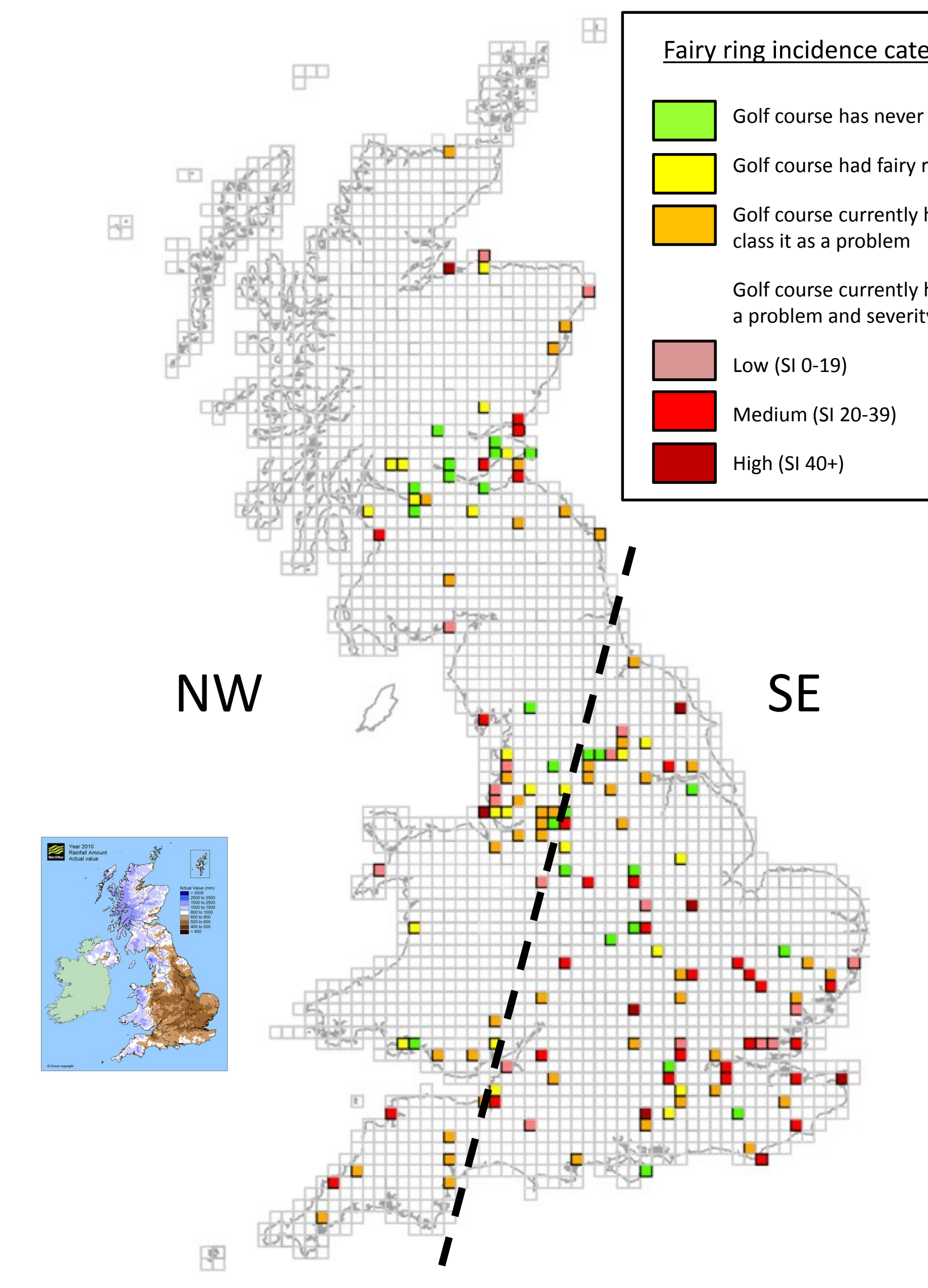
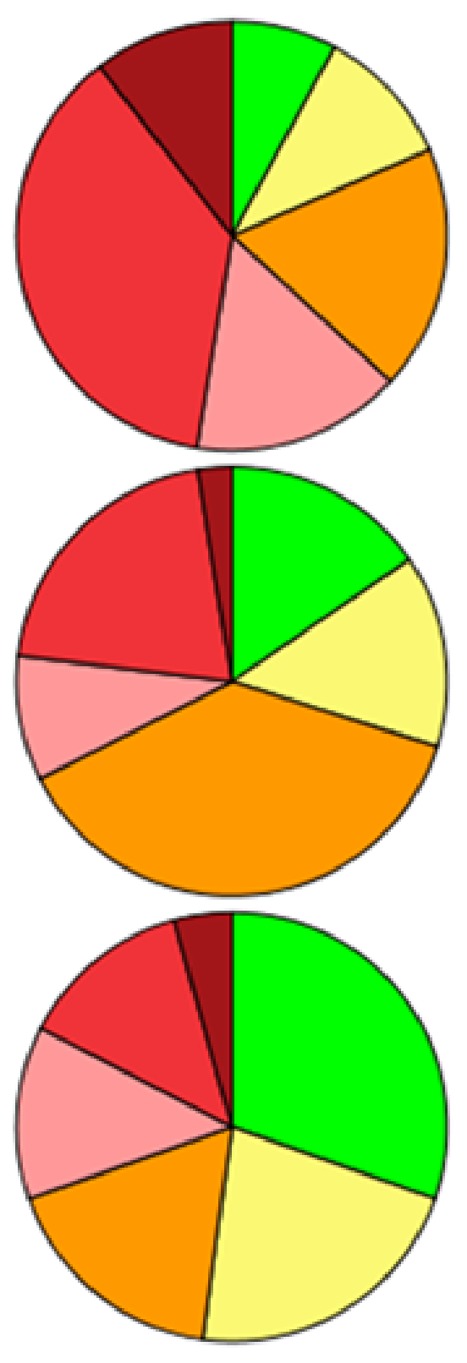


Figure 5: Map of fairy ring incidence on UK golf courses. Inset: UK MetOffice map of annual rainfall demonstrates the climatic divide seen between the north and west (NW) and south and east (SE) of the country. A line intersected across the incidence map to represent this divide allows for comparison by climatic region.

Golf course type

Incidence of problematic fairy ring on links courses is double that of parkland and heathland courses.



Links—coastal, sandy rootzones

Parkland—inland, loamy soils, often wooded

Heathland—upland moors, heavy clay soils

Figure 6: Fairy ring incidence by golf course type

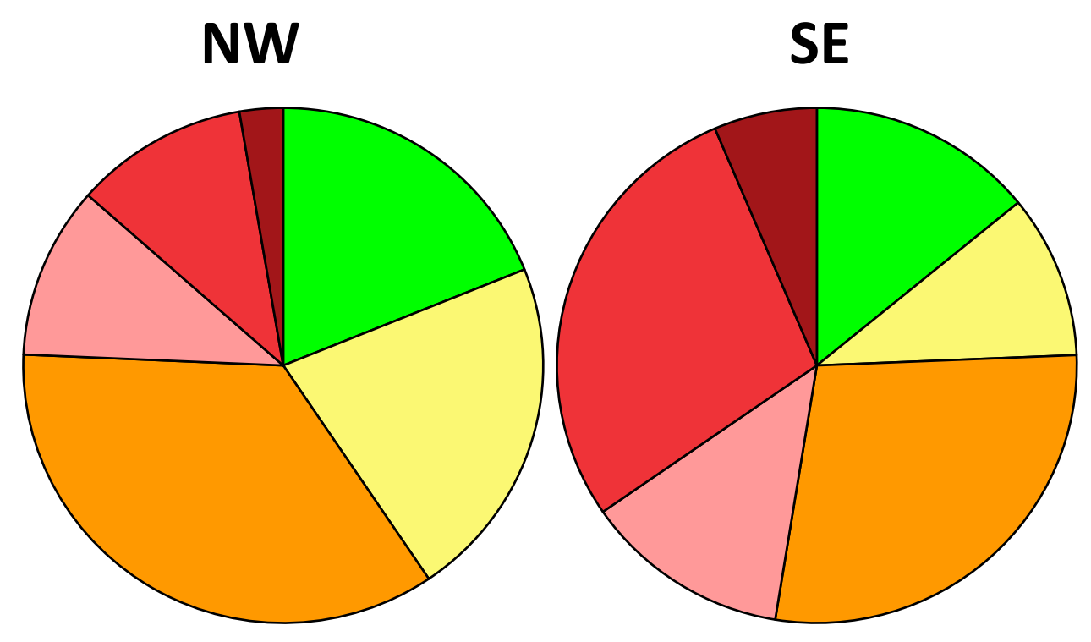


Figure 7: Fairy ring incidence by UK climatic region

Climatic region

Incidence of problematic fairy ring is significantly higher in the hotter and drier SE region of the UK when compared to the colder and wetter NW (Figure 7).

Conclusion

- Rings of stimulated turf growth (type 2) occur more frequently than other fairy ring types, predominantly affecting aesthetics, and are mostly an issue on golf putting greens.
- Loss of turf associated with type 1 fairy ring has the greatest effect on playability, but is found more commonly on fairways where effects on ball roll are less integral to game play.
- Links courses have a higher incidence of problematic fairy ring than other course types, suggesting that sandy soils may exacerbate symptoms.
- Golf courses in the south and east of the UK have a higher incidence of problematic fairy ring, showing that the hotter and drier climate may exacerbate symptoms.

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Seasonal changes in soil moisture of fairy rings

Keighley J. M., R. L. Mann, S. G. Edwards, and M. C. Hare

Summary

The onset of type-1 fairy ring symptoms is understood to largely be a result of decreasing soil moisture, yet guidance on mitigating symptoms through moisture management during the fairy ring season is currently anecdotal. By measuring the active zones of *Marasmius oreades* fairy rings with a soil moisture meter, it was found that fairy rings were considerably drier than asymptomatic soil, even as early in the season as April, when symptoms are only just appearing. As the asymptomatic soil regained moisture in October, the fairy ring soil continued to dry out, showing a potential lag phase in fairy ring recovery.

Introduction

The turf disease fairy ring is a worldwide phenomenon, occurring frequently on sports pitches, golf courses and domestic lawns. Fairy ring symptoms, which can be caused by a number of different fungus species, can vary from relatively benign circles of mushrooms or puffballs to circles of stimulated turf growth. Some of the more aggressive fairy ring fungi can colonise the soil with their waxy mycelium to the point where it repels water. The failure of water to sufficiently penetrate into the soil profile under these circumstances can induce drought stress and consequential necrosis of the turf. Notoriously difficult to control and widely considered the most damaging form of fairy ring (KEIGHLEY et al., 2013), these circles of bare ground (Figure 1), often in otherwise healthy-looking turf, are termed ‘type-1’ (SHANTZ and PIEMEISEL, 1917).

Figure 1: Necrotic circle of turf characteristic of a type-1 fairy ring (Image: STRI)

The key to managing type-1 fairy rings is to prevent soil moisture from dropping beneath the threshold at which water repellency develops through frequent irrigation, aeration, and use of surfactants (CISAR et al., 2000). In the UK, threat of fairy ring damage is thought to be at its greatest in the hotter, drier summer months, when soil moisture is at its lowest. In a recent questionnaire, UK greenkeepers reported that fairy ring symptoms on golf courses are at their worst in August and September (KEIGHLEY et al., 2013). It is, therefore, understandable that focus on type-1 fairy ring management occurs mainly in late summer in response to worsening visual symptoms. Fairy ring symptoms often first materialise in April/May when the fungus becomes active, but what we do not know is how they then develop during their active period to a point where soil moisture levels become detrimental to the turf. This study aimed to investigate the way in which soil moisture in fairy rings changes throughout their active season in comparison with healthy turf.

Materials and methods

Three fairy rings caused by the common type-1 forming fungus *Marasmius oreades* (Bolton) Fr. (also known as the fairy ring champignon), growing in the sandy soil of a links golf course in northwest England were monitored. The rings, covering areas of 11 m^2, 12 m^2 and 26 m^2, were measured on five occasions on an approximately bimonthly basis between April and November 2013. A soil moisture meter (originally an ML2x ThetaProbe, which was later replaced with the more robust Fieldscout TDR 100, after testing for consistency) was used to measure moisture content (% volume) of the topsoil at 15cm intervals around the circumference of the fairy ring active (necrotic) zones. For comparison, measurements were similarly taken from an adjacent asymptomatic area of turf. Fairy ring data sets for each month were pooled and statistically compared with the control data using Mann-Whitney U-tests.

Results

Fairy ring active zones were significantly drier than the asymptomatic control area in every month (April, August, October and November $p < 0.001$) apart from June (p = 0.334). This difference was particularly pronounced in April and October, when fairy ring soil moisture was 42.4% and 42.3% drier than the control, respectively (Figure 2).

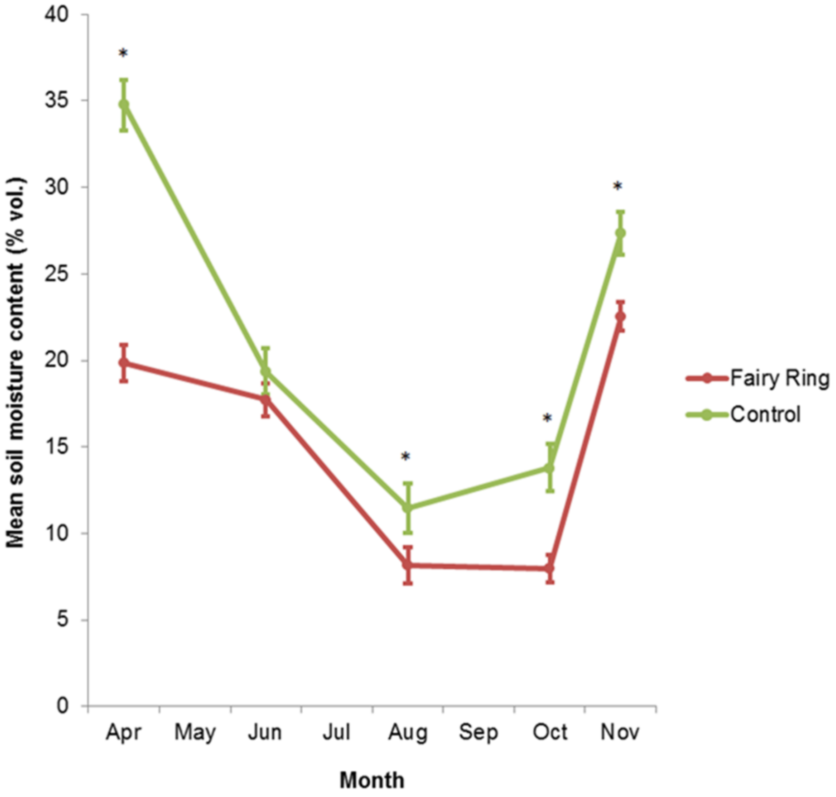

Figure 2: Mean soil moisture content of fairy rings compared with an asymptomatic

control area on a links golf course in northwest England during the 2013 fairy ring active season of April to November. Error bars represent 95% CI and * indicates $p < 0.05$.

Soil moisture in the control area was at its lowest in August at 11.45% vol. Whilst soil moisture in the control area had increased by October, the fairy ring soil had become even drier, reaching its seasonal low of 7.96% vol. mean soil moisture.

Conclusion

As soon as fairy rings become active in the spring, soil moisture may already be considerably lower than that of the surrounding healthy turf. Turf managers should, therefore, be aware that moisture deficit may start earlier in the year than originally anticipated. Findings also suggest that soil moisture in fairy ring active zones may continue to decrease even when surrounding healthy turf is appearing to recover. This research reinforces the need to be vigilant of fairy ring soil moisture in order to mitigate symptoms, even at the beginning and end of the season.

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Seasonal changes in soil moisture of fairy rings

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Introduction

The fungal mycelium of a fairy ring can cause a reduction in soil moisture that can lead to soil hydrophobicity and necrosis of turf. Understanding the advancement of this process in response to seasonal changes could improve the way we irrigate to mitigate symptoms.

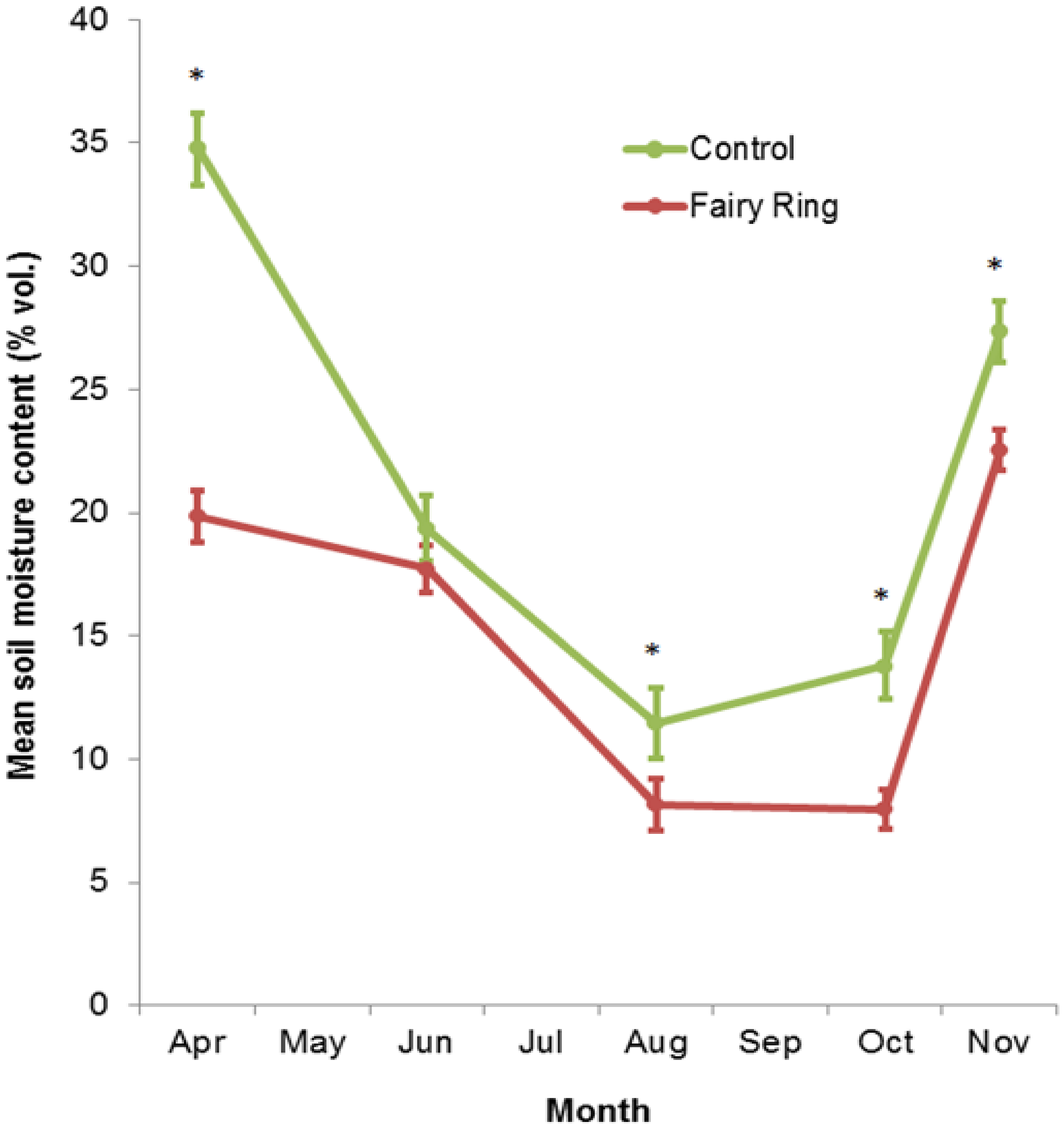
Methodology

Active zones of *Marasmius oreades* fairy rings on a links golf course in northwest England were measured from April to November 2013 using a soil moisture meter.

Results

Fairy rings were significantly drier than asymptomatic turf in every sampling month, apart from June.

Asymptomatic turf was at its driest in August, with a mean soil moisture content of 11.45% vol., whereas mean soil moisture of fairy rings did not reach its seasonal low of 7.96% vol. until October.



Mean soil moisture content of fairy rings compared with an asymptomatic control area on a links golf course in northwest England during the 2013 fairy ring active season of April to November. Error bars represent 95% CI and * indicates $p < 0.05$.

A fairy ring caused by the fungus *Marasmius oreades*

Inset: close-up of *M. oreades* mushrooms

Conclusion

Moisture deficit in fairy rings may already be considerable very early on in the growing season.

Later on in the season, when asymptomatic turf may appear to be regaining moisture, fairy rings can continue to dry out.

Further work

Field work in 2014 aims to identify the soil moisture threshold at which hydrophobicity develops in fairy rings.

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