Incidence of microdochium patch disease in golf putting greens and a relationship with arbuscular mycorrhizal fungi

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Abstract
A survey of pesticide records of golf courses was conducted to ascertain the incidence of microdochium patch disease, the most important turf grass pathogen in the UK. Disease incidence has remained steady over the last 10 years, but a wide variation was found in the incidence between courses. On average, golf courses suffered more than seven separate attacks of the disease per annum, with two distinct patterns being found. In one, disease was confined to spring and autumn, while in the other, outbreaks occurred with a similar frequency throughout the year. A negative correlation was found to exist between arbuscular mycorrhizal (AM) fungal abundance and disease incidence, while addition of AM fungi to a putting green produced some evidence that this resulted in a reduction in pathogen attack. It is concluded that AM fungi may have potential for use in a biocontrol programme against microdochium patch in fine turf.

Keywords: Microdochium nivale, Poa annua, arbuscular mycorrhiza, turfgrass, pathogen

Introduction
Microdochium patch, also known as fusarium patch or pink snow mould, was first recorded on UK golf greens in 1931 (Baldwin, 1990). The causative fungus is Microdochium nivale (Fries) Samuels and Hallett, formerly known as Fusarium nivale (Fr.) Sorauer (Smith et al., 1989). It is the most damaging and important disease of fine turf on putting greens or bowling greens in the UK, northern Europe and the Pacific North-west of America (Vargas, 1994). M. nivale has a wide host range, for example Couch (1973) lists 85 grass hosts, many of which are forage grasses. This fungus is also responsible for loss of sward productivity of Lolium perenne L. as well as being an important pathogen of cereal crops, where it causes seedling blight, foot rot and head blight (Simpson et al., 2000).

The biology and epidemiology of microdochium patch in fine turf has been reviewed comprehensively by Smith et al. (1989). M. nivale is considered to be a low-temperature pathogen of turf, with an optimum range for disease development of 0–7.8°C, and a maximum of 18–3°C (Fermanian et al., 1997). High humidity is also required and cool, wet conditions tend to promote disease outbreaks in spring and autumn (Smiley et al., 1992).

Biological control is being investigated in the turf industry for the control of the weed grass, Poa annua L. (annual meadow grass or annual bluegrass). Gange (1998) and Gange et al. (1999b) have found that arbuscular mycorrhizal (AM) fungi have the potential to reduce the abundance of this grass in fine turf. Although these fungi generally form mutualistic associations with many plants, there are occasions when the fungi can become parasitic (Gange and Ayres, 1999). In fine turf, it has been found that AM fungi are antagonistic to the growth of P. annua, while being beneficial to the growth of desirable grasses such as Agrostis spp. (Gemma et al., 1997; Gange, 1998). As P. annua is very susceptible to microdochium patch (Perris and Evans, 1996), we hypothesized that, if AM fungi can reduce the abundance of this weed, then there may be a concomitant reduction in the pathogen frequency. AM fungi may be useful as pathogen antagonists in a range of field crop plants, for example alfalfa (Volpin et al., 1995) and flax (Dugassa et al., 1996). However, no study has examined whether AM fungi can reduce the severity of disease caused by M. nivale.

The aims of this paper are to report the level of disease incidence in a range of golf courses over a 10-year period, and to show how this varies from (a)
course to course and (b) seasonally within courses. Furthermore, we describe a possible relationship between M. nivale occurrence and AM fungal abundance and report the results of an experiment in which mycorrhizal inoculum was added to a putting green and disease incidence recorded.

Materials and methods

Incidence of microdochium patch

Pesticide application records for the period 1991–2000 were obtained from 10 golf courses in southern England. These record the occurrence of microdochium patch and were used to document the incidence of this disease. Each course has asked to remain anonymous and is hereafter referred to as A, B, C, etc. Every course was aged between 90 and 100-year-old and had putting greens with soil-based root zones (Perris and Evans, 1996).

Data were summarized to provide information on the mean number of attacks per course over the 10-year period and the mean number of attacks per month for each course. Differences between years were examined with one-factor ANOVA, using courses as replicates. For each course, variation in time of attack (i.e. differences between months) was examined with one-factor ANOVA, using years as replicates. However, this analysis contained many zero data points and therefore did not satisfy the assumptions of normality. The analysis was therefore conducted using a Poisson error structure and log link function in GLIM (Crawley, 1993).

Arbuscular mycorrhizal colonization

Pesticide data from one course (course E) were summarized to provide information on the number of disease attacks per annum per green. To obtain AM colonization data, all 18 greens were sampled on four occasions (March, June, September and December) from 1993 to 2000. On each sampling occasion, three cores, measuring 2·5 cm diameter × 5 cm deep were removed from random positions on each green. Roots of the dominant grass, P. annua, were removed from each and washed free of soil. Slide preparations of roots were examined at ×200 magnification using a Zeiss Axiophott epifluorescence microscope fitted with a UV lamp and filters giving a transmission of 455–490 nm blue, to reveal arbuscules (Gange et al., 1999a). Colonization levels were assessed using the cross-hair eyepiece intersection method of McGonigle et al. (1990). Approximately 200 intersections per slide were recorded, to give a measure of per cent root length colonized (% RLC). Mean mycorrhizal levels per green were calculated as the average of all 32 values obtained (4 samples × 8 years). To examine whether there was any relationship between AM abundance and disease incidence, the Pearson product moment correlation was calculated, using greens as replicates.

Manipulative experiment

A practice putting green at course E was used for this experiment. The sward was composed of 85% P. annua, and 15% A. stolonifera as measured by the optical point quadrat method (Laycock and Canaway, 1980) and bicarbonate-extractable P concentration (measured by the molybdenum blue method given in Allen, 1989) was 34 ± 5·2 μg g⁻¹.

Forty plots, each 0·5 × 0·5 m and separated by 2 m, were marked out in a randomized block design. In each block, two plots were randomly allocated to one of two treatments: (1) addition of AM inoculum [Vaminoc, MicroBio Ltd, Hemel Hempstead, UK, containing a mixture of Glomus fasciculatum (Thaxt.) Gerd. & Trappe and G. mosseae (Nicol. & Gerd.) Gerd. & Trappe] at the rate of 5 g per plot (20 g m⁻²); and (2) addition of sterilized inoculum. These two AM species dominated the spore counts from this course in previous work (Gange et al., 1999b). Infectivity of the inoculum was 1274 (standard error = 119) propagules g⁻¹, giving an application rate of 25 480 propagules m⁻². The treatments were applied once, in late May 1999, and immediately after application the sprinkler system was turned on to irrigate the green for 30 min. The incidence of microdochium patch in each plot was recorded by counting the number of disease scars at monthly intervals from 1 September – 31 December. No fungicide was applied to the green during this time. The effect of treatment on disease incidence over the autumn was examined with a Repeated Measures Analysis Of Variance, after square root transformation of the data to satisfy the assumptions of normality and homogeneity of variances.

Results and discussion

Incidence of microdochium patch

The incidence of microdochium patch per course remained constant over the 10-year period ($F_{9,57} = 1·89$, $P > 0·05$), with a range from 6–6 attacks in 1998 to 8–5 attacks in 1992. Over the 10-year period, there was an average of 7·6 attacks of the disease per year.

There was a significant difference in the incidence of the disease between courses ($F_{9,57} = 5·06$, $P < 0·001$). The most severely affected course (course J) suffered an average of 9·3 separate attacks per annum, while the least-affected course (course A) suffered 3·5 attacks per
annum. Local climate, topography, drainage and amount of play are likely to account for these differences.

However, one surprising feature of this study was that two distinct patterns of disease incidence were found. In the first pattern (Figure 1a and b) there were two separate periods of attack, and in these cases there were significant differences between months (course C, $\chi^2 = 36.50$; course H, $\chi^2 = 39.24$; all d.f. = 11, all $P < 0.01$). However, in the second pattern (Figure 1c and d), attacks occurred with equal frequency throughout the year (course I, $\chi^2 = 1.54$; course J, $\chi^2 = 2.14$; all d.f. = 11, $P > 0.05$). Of the 10 sampled courses, three displayed pattern one with significance at the 5% level, while seven displayed the non-significant pattern two.

One possible explanation is that M. nivale is known to occur in two varieties, var. nivale and var. majus (Diamond and Cooke, 1999). In the only study to date, Mahuku et al. (1998) found that all of 100 isolates of M. nivale from four turfgrass sites in Canada were var. nivale. However, only one commercial golf course was included in that study and a comprehensive survey of variety occurrence in UK putting greens has yet to be performed. It is possible that var. majus, which is most prevalent on wheat (Parry et al., 1995) and which can occur throughout the summer (Pettit et al., 1996), was present on some or all of the seven courses. It is interesting that Smith (1983) found that isolates from turfgrass were pathogenic towards cereals, and this may be tentative evidence that var. majus does occur on turf grass, but has yet to be recorded. Meanwhile, the three courses that displayed the ‘expected’ spring and autumn pattern may have been attacked by var. nivale.

It is possible to distinguish between the two varieties using RAPD markers (Nicholson et al., 1996) and this technique needs to be applied to the populations of fungi found on golf putting greens in the UK.

Relations between disease incidence and mycorrhizal occurrence

A significant negative correlation was found between the extent of AM colonization of P. annua roots and the number of attacks of microdochium patch per green per year ($r = -0.546$, d.f. = 16, $P < 0.01$; Figure 2). Therefore, when AM fungi were abundant, disease incidence was rarer and vice versa.

One must acknowledge that such a result does not imply cause and effect, which can only be established by a manipulative experiment. However, there are three possible explanations for the negative correlation. The first is an indirect one, mediated through abundance of P. annua. On some occasions, it has been found that the abundance of P. annua is negatively related to

![Figure 1](image_url)

Figure 1 Seasonal incidence of microdochium patch at four golf courses. At course C (a) and course H (b) there was a distinct seasonal pattern, while no such pattern was seen at course I (c) or course J (d). Values plotted are monthly means over a 10-year period; vertical bars represent one s.e. of mean.
that of AM fungi (Gange et al., 1999b) and thus greens with more AM fungi and less P. annua in the sward may suffer fewer attacks of the disease. However, in this course the greens were dominated by P. annua (often forming over 90% of the sward) and there was no evidence that the abundance of this grass varied between greens (Kruskall-Wallis ANOVA, $\chi^2 = 15.3$, $P > 0.05$ d.f. = 17).

A second explanation for the negative correlation is that all microbial components of the soil, including AM fungi, are likely to be higher in areas where less pesticide is applied. Indeed, overuse of fungicides can weaken the turf and actually lead to an increased level of attack by M. nivale (Reicher and Throssell, 1997). We compared the number of fungicide applications per green, using years as replicates, and found that there were 8 pairwise comparisons that were significantly different. Given that 153 multiple comparisons were made, one would expect about 5% (8) to be significant by chance alone, so we do not believe that variation in fungicide application is a reason for the observed correlation. Furthermore, we have recently completed an experiment in which three of the most-commonly used fungicides (chlorothalonil, fenarimol and iprodione) were applied twice to a putting green and AM levels measured over a six-month period. During this time, there was no measurable effect of the chemicals on colonization levels (A.C. Gange, unpublished data).

A third explanation is that there is a direct effect of AM fungal colonization on M. nivale, as AM fungi can reduce the disease severity of a number of pathogens (Whipps, 2001). The exact mechanisms are largely unknown, but include competition for root space, antibiosis, induced resistance and direct growth promotion of the plant. We believe that an investigation of the direct interactions between mycorrhizas and M. nivale would be worthwhile.

Inoculum addition successfully increased colonization levels ($z = 2.04$, $P < 0.05$) and decreased the abundance of P. annua ($z = 4.32$, $P < 0.001$), while control plots showed no change. Over the course of the study, no significant effect of AM addition on disease incidence could be found ($F_{1,38} = 3.31$, $P > 0.05$), but there was a significant interaction between the inoculum treatment and time ($F_{1,38} = 7.65$, $P < 0.01$). This was because there appeared to be a lower incidence of disease in treated plots in November, but not in any other month (Figure 3). We conclude that these data give sufficient evidence to suggest that an investigation of AM control of microdochium patch would be worth pursuing.

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References


