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Exploration and exploitation strategies of powdery mildew on barley cultivars with different levels of nutrients

Adrian C. Newton and David C. Guy
Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK (Fax: +44 1382 562426)

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Abstract

The mass fractal dimension (MFD) of colonies of mildew (*Erysiphe graminis* f.sp. *hordei*) growing on barley was calculated as a measure of their spatial structure. Despite the elongated shape of the colonies imposed by the leaf cellular structure, the MFD remained constant with scale. The mildew MFD differed on different cultivars of barley, and was greater on leaves produced under higher nutrient level indicating a physiological component. Lower MFD values correspond with the thin spreading growth associated with exploration strategies and higher values correspond to the denser, more branched structure associated with exploitation of the substrate. Cultivars showing exploration strategies induced by resistance expression responded to increased nutrient levels more than those expressing little resistance such as Golden Promise.

Introduction

Inter-cultivar differences in not only the overall severity but also the appearance of powdery mildew on barley are clearly visible under field conditions. Still greater are the effects of nutrient levels on this appearance, high levels generally giving the appearance of much more mildew being present. This is often attributable to better colony growth rather than to more colonies being present (Newton and Hackett, 1994). Microscopic examination shows that nutrient levels affect the structure of colonies; low nutrient colonies giving a much more diffuse appearance (AC Newton, unpublished data). Formation of secondary hyphae and spore production is increased by nitrogen, but not all cultivars respond to the same degree (Jenson and Munk, 1997).

The morphological response to nutrient levels is likely to be a reflection of the ability of the substrate (the leaf) to supply sufficient nutrients to the pathogen for its development. Low nutrient levels in a saprophytic fungus result in it adopting an exploration strategy, branching little and instead apportioning resources to radial growth in search of nutrients (Dowson et al., 1989; Crawford et al., 1993). Conversely, high nutrients result in a much denser more branched colony structure, exploiting the adequate substrate it has encountered. In the case of a biotroph, such as powdery mildew, the resources of the substrate are compartmentalised into cells which, in the case of barley, have an elongate structure. This tends to impose a preferred axis for mildew colony growth on its surface producing elongated colonies with a width : length ratio of 0.6 : 1 (AC Newton, unpublished data). However, in addition to treating the leaf as a compartmentalised substrate, cellular resistance mechanisms will frequently be triggered even in compatible reactions, each of which may also have some effect on the cell itself and its immediately adjacent cells.

In order to analyse the effects of nutrients and differences in resistance expression, the effect on mildew colony structure needs to be quantified. Visual assessments of colony appearance are possible but highly subjective. An image analysis technique was adopted whereby the ability of the colony to fill the space and therefore exploit the resources within the space, was measured by calculating the mass fractal dimension

(MFD) of the image (Ritz and Crawford, 1990). This is based on the assumption that fungal colony structures tend to have the property of self-similarity where the mycelia are clustered in a heterogeneous manner such that large scale branching patterns resemble those at smaller scales. The following experiments were therefore carried out to test the methodology:

Barley leaves and inoculum

Seedlings of five different spring barley cultivars: Golden Promise, Proctor, Prisma, Camargue and Triumph, were sown in John Innes No 2 compost in propagators and grown for 14 days at 14°C in a Fisons G600 growth cabinet with 16 h light at 140 $mMol^{-1}$. Different nutrient levels achieved by applying either water (n1) or nutrient solutions or 5.0 g/L (n2), using 0.25 L/pot. The nutrient solution used in all experiments was 'Vitax Vitafeed III Soluble nutrient' (N, P and K = 19%, Mg = 0.15%, Bo = 0.013%, Cu = 0.025%, Fe = 0.05%, Mn = 0.025%, Mo = 0.0003%). The first leaves were removed and a 3.0 cm section cut 1.0 cm from the tip, and placed on 0.5% (w/v) agar containing 120 ppm bemzimidazole in sealed plastic boxes measuring 115 × 45 × 19 mm (Stewart Plastics, Croydon, UK).

Inoculation and leaf preparation

Leaves were inoculated with isolate IM82 (Newton, 1989) and incubated for five or eight days at 15°C under continuous light. A range of inoculation densities was used to enable colonies to be photographed without immediately adjacent colonies causing any interference. Leaves were then cleared in boiling lactophenol : ethanol (50 : 50 v/v), stained with aniline blue and mounted in lactophenol.

Image preparation and analysis

Mildew colonies were selected for photography which did not overlap and had no immediately adjacent colonies. These were photographed at ×6.3 or ×10 objective magnification on a Zeiss RA microscope using Tmax ASA 100 black and white film. Digitisation of negatives or photographs proved unsuccessful due to background noise, so images had to be traced. Negatives were projected onto a 30 × 30 cm screen and colonies were traced onto clear acetate sheets using a black ink marker. Acetate images were then scanned and saved as '.tif' files for processing. The image was processed using Unix mounted 'Khoros' software in the following manner.

The MFD was calculated as follows: If $N(r)$ is the number of pixels in a circular region of radius r centred on a pixel belonging to the colony, in a larger region of radius R the number of pixels is simply $N(r)$ multiplied by a scaling factor n, eg. $N(R) = n(R/r)N(r)$. If the colony is fractal, then the scaling factor $n(R/r) \propto (R/r)^D$, where D is the fractal dimension. The relation between $N(r)$ and r, then has a solution of the form $N(r) \approx r^D$. The software determines $N(r)$ for a range in r and the fractal dimension (the slope) is calculated from a plot of $\log N(r)$ vs. $\log r$. This was repeated from 600 randomly chosen origins to calculate the average slope or mass fractal dimension (MFD). In this way a single number, D (the MFD), was derived to describe the space-filling property of a colony. These values were then subject to analysis of variance using Genstat release 5.3.1. The calculation and application of this method to fungal colonies on agar is explored fully in Ritz and Crawford (1990), but here we test whether it can be applied to substrates with an underlying structure which causes an elongate form on the colonies. The leaf substrate also introduces an additional factor which affects the availability of the nutrients: resistance to successful establishment of the powdery mildew feeding structures, the haustoria.

Experiments

In experiment 1, 21 leaves of Golden Promise and of Proctor were analysed at both low (n1) and high (n2) nutrient levels when colonies were 5 days old. In experiment 2 Golden Promise, Prisma, Camargue and Triumph were used. With 5 day old colonies errors were large and differences tended to be small so colonies were measured at 8 days old instead to improve discrimination. Colonies from 10 leaves of each cultivar were analysed from each nutrient level.

Results and discussion

Mildew colonies were successfully infected, cleared, stained and photographed on all cultivars of barley at all nutrient levels. In experiment 1, the mean MFD for each nutrient/cultivar treatment was between 1.52 and 1.59, and in experiment 2 between 1.30 and 1.60. Thus the MFD did not shift substantially with different colony

Table 1. Effect of nitrogen and barley cultivar on the mass fractal dimension of powdery mildew colonies

	n1	n2	mean
5 day old colonies			
Golden Promise	1.5387[a]	1.5579[a]	1.5483
Proctor	1.5204[a]	**1.5870**[b]	1.5537
mean	1.5296	1.5724	
nutrient lsd=0.0351, cvar lsd=0.0351, cvar× nutrient lsd=0.0497			
8 day old colonies			
Golden Promise	1.5072[a]	1.5081[a]	1.5076
Prisma	1.5252[a]	**1.5955**[b]	1.5649
Camargue	1.3050[a]	**1.4857**[b]	1.3954
Triumph	1.4144[a]	**1.4941**[b]	1.4542
mean	1.4402	1.5208	
nutrient lsd=0.0314 cvar lsd=0.0444, cvar×nutrient lsd=0.0627			

Superscript comparisons of significance within cultivars.
(Bold = highlights main within cultivar significantly differences).

sizes within a treatment despite the elongate shape of the colonies imposed by the leaf cellular structure.

In experiment 1 there was a significant nutrient effect ($p < 0.005$), but no significant cultivar effect (Table 1). High nutrient increased the MFD overall and on cultivar Proctor but not significantly from Golden Promise. In experiment 2 there was a highly significant nutrient and cultivar effect ($P < 0.001$) and interaction ($P < 0.005$) overall. The increase in MFD at high nutrient was highly significant for cultivars Prisma, Camargue and Triumph ($P < 0.001$) but not significant for Golden Promise (Table 1). Thus there are clear effects on colony morphology of both cultivars and nutrient levels as measured by the MFD indicating that powdery mildew has the ability to adapt its growth morphology in response to substrate availability.

The higher MFD under the higher nutrient conditions indicates greater space filling or an exploitation-type strategy, whereas the lower MFD under lower nutrient levels indicate more exploration. Thus there appears to be both genetic and physiological components affecting mildew growth on leaves. However, the effects could be further divided into resistance expression and substrate availability. These could only be elucidated fully by the use of isogenic lines of barley differing only in resistance expression, preferably race non-specific resistance, but some indication is available from these data.

Complexity provides greater possibilities for adaptation to a dynamic environment. In fungi this could be achieved using self-similarity as a key to generating complex structures from minimal genetic coding. Such fractal growth may enable this plant pathogen to optimise nutrient availability by balancing exploratory and exploitative modes of growth in the same way as saprophytic fungi (Ritz and Crawford, 1990) despite the fact that it is resistance expression which may be limiting the nutrient availability. The cultivars Proctor, Prisma, Camargue and Triumph all express low levels of partial resistance under most environmental conditions, but greater than that expressed by Golden Promise. The effect of this resistance on limiting nutrient availability is reduced under the higher nutrient treatment and therefore the MFD increased indicating a change towards exploitation mode of growth. Where the resistance is minimal, as in Golden Promise, the effect of the higher nutrient treatment is also smaller, in fact it was not significant in these data indicating that the mildew was relatively unrestricted by resistance and able to exploit adequate nutrients at low nutrient status.

The MFD values obtained in this study are low compared with saprophytic colonies on rich nutrient sources. This difference might be expected as not only are there resistance mechanisms operating but also the nutrient source is confined within the host and specialised, energetically demanding feeding structures (the haustoria) are required to exploit the substrate. Whilst the theoretical maximum MFD value is 2.0, this is unlikely to be approached very closely in such a system. Necrotrophic pathogens which kill their host

using enzymes and toxins might be expected to achieve higher colony MFDs under high nutrient status as they are immersed in their substrate, but such pathogens are frequently most successful under low nutrient status when host defence mechanisms are less effective.

Jenson and Munk (1997) report that germination of conidia and formation of haustoria are only weakly affected by nitrogen, but it strongly increased formation of secondary hyphae, ie exploitation structure. There were also indications that more infections were successful with higher nitrogen and that more spores were produced, presumably reflecting a denser branching colony structure. They also reported that some susceptible cultivars were less responsive to nitrogen levels than others. Our data indicate similar response to nutrients and genotype dependency.

Partial resistance expression is particularly environmentally labile in barley (Newton and McGurk, 1991) and therefore difficult to assess accurately. Whilst accumulation of additive polygenic resistance is likely to offer better long term benefits, lack of understanding of its genetics together with the environmental expression problems have meant that strategies for its effective handling have not been well developed in breeding programmes. It is therefore frequently discarded in favour of major gene resistance with its short-term benefits but long-term risks. Measurement of MFD may enable selection of thin spreading exploration type growth which may be either an expression of the mildew's inability to form haustoria due to resistance reactions, or encountering poor nutritional quality of the substrate. In either case less damage will be done to the host.

Because of the environmental lability of the barley-mildew pathosystem, as well as the heterogeneity of both the leaf and mildew spores, the technique of measuring MFD is of practical value under only highly controlled conditions. Therefore it could be used to identify cultivars as breeding lines which have low MFDs relative to other cultivars, ie exploration strategies, indicating poor substrate for mildew growth probably due to polygenic resistance expression. A low MFD and lack of MFD increase under the high nutrient treatment would be most desirable and may indicate less environmental lability in disease expression, or practically, suitability for high input agricultural systems. Genotypes with low MFDs only under low nutrient treatments may be valuable for use in low-input agriculture. However, relatively low MFD together with lack of nutrient response was not found in this work and is more likely to be an indication of a non-host response where the host is an unsuitable substrate rather than resistance. Thus it could represent a potentially durable form of resistance if it can be found.

Response either directly to nutrient availability or to the effect of resistance expression limiting nutrient availability may also be expressed in differences in the speed of mildew colony growth. This character was not measured here as considerable variation in the size of colonies was observed on all leaves, whereas colony gross morphology appeared to be more consistent across leaves within cultivars.

MFD measurements may also explain apparent disease tolerance in some genotypes where the visual assessment of mildew corresponds with a higher yield loss than was observed in practice (Newton and Thomas, 1994; Newton et al., 1997). Little and Doodson (1972) showed a correlation between NIAB disease assessment and yield loss due to mildew, highlighting the disease tolerance of the cultivar, Proctor, but Rowe and Doodson (1976) later suggested that it was less tolerant under severe disease pressure. The data presented here indicate that Proctor is a genotype which induces a change in colony morphology in response to nutrient, suggesting an alternative explanation to the apparent disease tolerance under some growth conditions but not others.

The methods used to measure MFD in this work are not automated, time consuming, computationally demanding, and therefore expensive. For practical exploitation a colony labelling technique, such as a fluorescent antibody, needs to be developed so that microscopic images can be processed directly, preferably recorded digitally, and analysed automatically using improved image analysis software. Such developments would enable this technique to become a valuable tool for selection of breeding material and critical analysis of the components of polygenic resistance and the environmental effects on its expression.

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