

## Review

**The nutrient supply of pathogenic fungi; a fertile field for study**

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*The Australian Centre for Necrotrophic Fungal Pathogens, SABC, DSE, Murdoch University, Perth 6150, Western Australia, Australia***SUMMARY**

Phytopathogenic fungi must feed on their hosts to propagate and cause disease. Their ability to access the rich nutrient supply offered by living plants is one of the most obvious properties that distinguish pathogens from saprophytes. Successful invasion by pathogens depends as much on their ability to utilize the available nutrient sources offered by plants as on their ability to penetrate plants and evade defensive mechanisms. Here, we review current knowledge on the nature of the nutrient supplies utilized by pathogens during infection.

The available evidence is rudimentary in most cases. There is much evidence to suggest that fungal metabolism can be divided into at least two phases. The first is based on lipolysis and occurs during germination and penetration of the host. The second phase uses glycolysis and predominates during the invasion of host tissue. We also propose, mainly on theoretical grounds, that a third phase of nutrition occurs late in infection during which new spores are produced.

Contrary to early assumptions, the nitrogen sources available to some pathogens appear to be abundant. The idea that nitrogen starvation is a cue that controls fungal gene expression during infection may need to be reassessed. Very little is known about the micronutrient (Fe, S, P) or vitamin supply. The knowledge gained from this research may enable the design of new antifungal strategies targeting potential weaknesses in fungal metabolism and will also impact on agronomic practices.

**INTRODUCTION**

Pathogenicity can be broadly defined as the ability of a pathogen to interfere with the host plant, thus resulting in a disease (Holliday, 1992). It depends on a range of properties that distinguish pathogens from saprobes. Properties such as the production of toxins, cell wall degrading enzymes, host penetration, signal transduction and avirulence genes are well recognized as

pathogenicity factors (reviewed in Idnurm and Howlett, 2001). The ability of a fungus to feed on a plant is no less important, but has been relatively ignored. The purpose of this review is to highlight studies on *in planta* nutrition that suggests that the nutrient supply of fungal pathogens may be as important as the above factors during pathogenesis.

**EFFECT OF THE EXTERNAL ENVIRONMENT OF THE PLANT ON DISEASE DEVELOPMENT****The effect of fertilizer application on pathogenicity**

The interaction of fungal disease and fertilizers is of great practical interest and there have been numerous studies into the interaction of (in particular) nitrogenous fertilizer and disease. Application of nitrogen above the recommended rate has often been shown to significantly increase disease incidence and lesion area. This applies to both necrotrophic (*Magnaporthe grisea*, Long *et al.*, 2000) and biotrophic pathogens (*Blumeria graminis* f. sp. *tritici*; and tomato mildew *Oidium lycopersicum*; Hoffland *et al.*, 2000; Jensen and Munk, 1997).

The nature of the nitrogen also has an effect. Huber and Watson's (1974) review noted that nitrate consistently increased and ammonium decreased the incidence of (hemi)-biotrophs (mildew, *Colletotrichum* and rust) whereas nitrate decreased and ammonium increased the incidence of the necrotrophs *Cochliobolus*, *Magnaporthe* and *Botrytis*.

Sub-optimal nitrogen can also lead to more disease. In the grey mould pathogen *Botrytis cinerea*, the formation of primary lesion on tomatoes was more frequent with low nitrogen levels (Hoffland *et al.*, 1999). These plants have a high physiological carbon/nitrogen ratio and a large amount of soluble carbohydrates in tissue (Hoffland *et al.*, 1999), indicating that the growth of the pathogen was dependent on high soluble carbohydrate levels.

The hypotheses being tested in the above studies can be redefined as follows;

- (A) An insufficiency of fertilizer leads to susceptibility by weakening a plant and promoting invasion and disease.
- (B) Superabundant fertilizer leads to hyper-susceptibility by either:

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- 1 providing excess nutrient that is directly available to support the growth of pathogens,
- 2 promoting the growth of ill-defended plant tissue, or
- 3 diverting metabolic resources into the assimilation of the nutrient.

Studies that might distinguish these possibilities have given conflicting results. Matsuyama and Dimond (1973) showed that the application of nitrogenous fertilizers to rice led to a reduction in the activities of enzymes in the phenylpropanoid pathway and the production of lignin, suggesting that fertilizers compromised the intensity of secondary plant defences. This is consistent with hypothesis B3. The positive effect of ammonium on necrotrophs is consistent with hypothesis B1, whereas the positive effect of low nitrogen on *Botrytis* is consistent with A.

Clearly more work is needed in this area and it is, of course, likely that different fungi will respond in different ways to the application of fertiliser. It is important that future studies combine measurements of fungal growth and symptom production with plant biomass and *in planta* nutrient levels. The use of fungal strains carrying reporter gene constructs linked to nutrient-responsive promoters (for example Bowyer *et al.*, 2000) is a major technical advance that is likely to elucidate this problem.

#### The effect of mycorrhizal symbiosis on pathogenicity

Like fertilization application, arbuscular mycorrhizae (AM) affect pathogenicity. It has been commonly reported that plants inoculated with AM show increased leaf pathogen loads. Dehne (1982) suggested that this increased pathogenicity is probably due to enhanced nutrition and plant growth of mycorrhizal plants. Gernns *et al.* (2001) suggested that improved management of the plant storage pools provided by the AM symbiosis compensates for the deleterious effect of mildew infection on grain yield in barley.

#### The effect of CO<sub>2</sub> levels on plant–pathogen interactions

Studies on plant responses to elevated CO<sub>2</sub> levels have revealed a clear link to pathogen resistance. When barley was grown in elevated CO<sub>2</sub> concentrations, photosynthesis was higher and inoculation with powdery mildew spores produced fewer colonies. However established *B. graminis* colonies grew at a greater rate at elevated atmospheric CO<sub>2</sub> (Hibberd *et al.*, 1996a,b). The authors concluded that plants grown in elevated CO<sub>2</sub> have enhanced defensive capabilities, presumably because of the faster deployment of defensive secondary metabolites and cell-wall strengthening. Once penetrated however, the pathogen was able to grow faster, suggesting that fungal substrate supply was the limiting factor.

## IN VITRO NUTRITIONAL REQUIREMENTS

Many non-obligate fungal pathogens can be grown axenically in minimal media consisting of only a simple carbohydrate, nitrogen (as nitrate), phosphate, and trace metals (see, for example Lau and Hamer, 1996; Newton and Caten, 1988). Phytopathogenic fungi are often capable of using two-carbon compounds such as acetate and ethanol (Bowyer *et al.*, 2000), sugar alcohols (Noeldner *et al.*, 1994), fatty acids (Bowyer *et al.*, 2000; Segers *et al.*, 2001), proteins/amino acids (Jennings and Lysek, 1996; Murphy and Walton, 1996), and plant cell wall materials (Jennings and Lysek, 1996; Lehtinen, 1993). Phytopathogenic fungi can also use many nitrogen sources such as ammonium, nitrate, amino acids and proteins. The non-protein amino acid  $\gamma$ -aminobutyric acid (GABA) is a very efficiently used N source *in vitro* (Solomon and Oliver, 2002). There is therefore good evidence that many phytopathogens can metabolize all the major substrates likely to be available to them during growth *in planta*.

Obligate pathogens cannot, by definition, be grown in culture. Unlike fastidious bacteria, the provision of complex nutrient sources has rarely been shown to enable the growth of such fungi. Instead, it appears that the regulation of nutrient assimilation transporters and enzymes is dependent on factors or signals produced by the plant. Haustoria appear to be the main site of nutrient uptake in obligate pathogens. *In vitro* conditions which induce the appropriate expression of the genes required for haustorial function have rarely been discovered (Hahn and Mendgen, 1997; Thomas *et al.*, 2001, 2002).

## METHODOLOGIES TO STUDY IN PLANTA NUTRITION

### Expression of metabolism-related genes *in planta*

Expressed sequence tag (EST) studies of pathogens would seem to have great potential in identifying fungal nutritional pathways expressed during infection. At first sight, the identification of pathogen genes involved in a given metabolic pathway would appear to indicate that the pathway operates during infection. However, for this conclusion to be drawn, several criteria need to be addressed. Firstly, it must be shown that the gene is expressed at an appreciable level *in planta*. Secondly, the function of the gene product must be ascertained; some form of functional characterization is needed to confirm the properties of a gene product. Thirdly, if the encoded enzyme catalyses a reversible reaction, it is necessary to determine whether its metabolite is being synthesized or consumed.

Isolating fungal genes from infected tissue is often problematic. Differential screening was used to identify plant-induced fungal genes in the rice blast system (Talbot *et al.*, 1993), leading to the identification and characterization of a hydrophobin. It is

possible in some special cases to isolate pure fungal tissue from within plants (Hahn and Mendgen, 1997). Haustoria of *Uromyces fabae* can be purified and used as a source of mRNA for library construction. This approach has given a direct window into fungal gene expression with many surprising results (see below).

A number of groups have isolated genes from *in vitro* grown tissue and subsequently analysed their expression *in planta* (Coleman *et al.*, 1997; Oliver *et al.*, 2000; Stephenson *et al.*, 2000). The conditions under which the fungus is grown *in vitro* can be altered in an attempt to mimic *in planta* conditions. The use of starvation as a potential mimic of *in planta* expression was suggested by the observation that the avirulence gene *avr9* was induced *in vitro* only by nitrogen limitation (van den Ackerveken *et al.*, 1994). Another approach also uses pure fungal material but utilizes cells that are undergoing infection-specific morphological changes, such as appressorial maturation (Hwang and Kolattukudy, 1995; Thomas *et al.*, 2001, 2002). These studies are based on the assumption that the genes expressed during infection-specific morphological changes would also be expressed during appressorial infection on plants.

### Real-time monitoring of gene expression during infection

An alternative way of assessing the role of a gene product in fungal nutrition expression is to generate promoter-reporter fusions and to follow expression of the reporter during the infection cycle. This approach, using confocal microscopy and GFP-expressing (green fluorescent protein) constructs, is well suited to uncovering the spatial and temporal details of gene regulation *in planta*. Several caveats also apply to this technique. Since gene expression is measured qualitatively, it can only provide a rough indication of the amount of the enzyme and of the flux through the pathway. GFP is a relatively slowly synthesized and long-lived reporter molecule (Cubitt *et al.*, 1995). Therefore, it is best suited to measuring slow (several hours to days) changes in gene expression rates. Nonetheless, this method has thrown new light on the glyoxylate shunt in *Tapesia yallundae* (Bowyer *et al.*, 2000) and could be used to study many other pathways.

### Auxotrophic mutational analysis

In contrast to the many caveats associated with genomic methods, the generation of mutants impaired in their ability to utilize a particular nutrient is accepted as a powerful way of studying pathogen nutrition. Traditionally, mutants were selected after treatment with (chemical or radiation) mutagens and screened for auxotrophy. Ideally, genetic analysis would have indicated that a single mutation was present and feeding studies determined the affected enzyme. More recently, mutations have been generated using site-specific gene disruption (reviewed in

Ildnurm and Howlett, 2001). These studies have enhanced our understanding of all aspects of fungal nutrition.

### Measurement of nutrient concentration fluxes during infection

A direct way of studying fungal nutrition is to measure the changes in the concentration of potential nutrient sources in the plant during infection. This approach is best suited to pathogens that are restricted to the apoplast; the prime example is *Cladosporium fulvum*. Apoplastic washing fluids can be recovered and their contents measured. The interpretation of these results is not straightforward, as both the plant and the fungus might be contributing to both the synthesis and consumption of each compound. This method has been used to study both carbohydrate and nitrogen metabolic profiles (Joosten *et al.*, 1990; Solomon and Oliver, 2001). Microscopical stains for lipid and glycogen can give valuable spatial, if qualitative, information on the presence and accumulation of nutrient sources (Thines *et al.*, 2000).

## IN PLANTA NUTRITIONAL STUDIES

### Carbon

Carbon catabolism is the source of the energy used by the fungus during infection. Prior to penetration, the fungus must rely on stored sources of carbon. These are likely to include glycogen, trehalose, sugar alcohols and lipids (Jennings and Lysek, 1996; Thines *et al.*, 2000; Weber *et al.*, 2001). Genomic studies using ESTs and SAGE (serial analysis of gene expression) have indicated that the barley powdery mildew pathogen expresses genes required for glycogen breakdown, glycolysis, lipolysis,  $\beta$ -oxidation and the TCA cycle (Thomas *et al.*, 2001, 2002). Temporal analysis suggests that lipid catabolism persists throughout the germination and penetration phases but that glycolysis may decline in relative importance as the appressorium matures. Expression of the glyoxylate shunt enzyme isocitrate lyase (ICL) is characteristic of lipid metabolism. Using a GFP fusion, Bowyer *et al.* (2000) showed that the expression of ICL in *T. yallundae* was highest in spores and hyphae prior to penetration into the host, suggesting that lipids were being consumed during penetration. Histochemical (Thines *et al.*, 2000; Weber *et al.*, 2001) studies on *M. grisea* indicated that the breakdown of lipid is the main source of the glycerol required to generate turgor pressure in the appressoria (de Jong *et al.*, 1997). The generation of targeted mutations has also implicated lipid metabolism in the penetration phase. Kimura *et al.* (2001) created mutants deficient in *ClapEX6* in the cucumber anthracnose pathogen *Colletotrichum lagenarium*. The product of *ClapEX6* is required for peroxisome biogenesis where enzymes for  $\beta$ -oxidation of fatty acids reside. Mutants of *clapex6*

failed to grow in a medium supplied with fatty acids as carbon and lacked the ability to penetrate the host.

After penetration, the presumably rich resources within the plant become available. As *C. fulvum* only grows within the apoplast of the tomato leaf, it is possible to extract the intercellular fluid and determine which nutrients are present and presumably available to the fungus. Joosten *et al.* (1990) examined the sugar composition in the leaf apoplast of tomato infected with *C. fulvum* over a 14-day infection period. Sucrose levels accumulated during the early stages of infection but declined later. Glucose and fructose levels increased as sucrose declined. The levels of glucose and fructose fell in the later stages of infection and were replaced by mannitol. These results suggest that plant and/or fungal invertases cleave photosynthetic sucrose into fructose and glucose, which are in turn converted to mannitol by the fungus (Noeldner *et al.*, 1994). The role of mannitol is not clear but may include defence against reactive oxygen species (Jennings *et al.*, 1998). It was surprising to note that the total sugar concentration remained high until late in the infection cycle (Joosten *et al.*, 1990). Fungal demand for nutrient increases as fungal biomass accumulates. This implies that there must be a net transfer of carbohydrate from plant source cells into the apoplast that is more than enough to supply the fungus, at least until close to the end of the infection cycle. A high effective sugar concentration within the leaf is also indicated in *T. yallundae*-infected wheat. GFP fluorescence of ICL declined rapidly after penetration, indicating the availability of sugars within the host. (Bowyer *et al.*, 2000). Direct measurements of powdery mildew-infected barley leaves indicate high levels of fructose, glucose and sucrose (Scholes *et al.*, 1994). Genomic studies have also confirmed that sugars act as the predominant carbon source during infection (Voegelé *et al.*, 2001). A hexose transporter gene, HXT1 from *U. fabae*, is specifically expressed in the haustorium during infection. An elegant study using asymmetrically labelled sucrose has further indicated that wheat mildew haustoria take up glucose, but not sucrose (Sutton *et al.*, 1999).

A large number of depolymerases have been isolated from pathogenic fungi. These include cell wall degrading enzymes and proteases. It has been difficult to establish a role in pathogenicity for many of these enzymes because of genetic redundancy. It is furthermore unclear whether these enzymes have specific roles in penetration or nutrition for the same reason.

Deletion of a gene encoding ccSNF1, a transcription factor from *Cochliobolus carbonum*, abolished pathogenicity. ccSNF1 is an orthologue of a yeast gene that controls the expression of carbon catabolite repressed genes. Amongst the genes controlled in *C. carbonum* is a suite of cell wall degrading enzymes. The mutants have a reduced penetration efficiency and pathogenicity on maize but also have impaired ability to catabolize arabinose, xylose, xylan and cellulose, the main constituent of the monocot cell wall. Thus, whilst the primary role of the genes controlled by

ccSNF1 is to penetrate the leaf, a role in nutrition is not ruled out (Tonukari *et al.*, 2000).

What emerges from these studies, from different pathogens and using different techniques, is a paradigm that lipids are the primary sources of energy that are used during germination and penetration. Lipids also appear to provide the materials that are used to generate the osmotic pressure for penetration. Carbohydrates (and proteins) are used sparingly at this early stage. After penetration, sugars are available and become the main energy source, whilst any further lipolysis is inhibited.

## Nitrogen

All nitrogen for fungal growth is derived from plant sources. These sources could include nitrate, ammonia, amino acids and other small molecules and proteins. Genomic studies of barley powdery mildew showed that many of the genes expressed during germination and appressorial formation encode enzymes with putative roles in proteolysis and amino acid recycling (Thomas *et al.*, 2001, 2002). Thus, some of the nitrogen for early development may well be derived from stored proteins.

The discovery that the *C. fulvum* avirulence gene *Avr9* was only expressed *in vitro* under nitrogen-limiting conditions suggested that the fungus is starved for nitrogen during growth *in planta* (Coleman *et al.*, 1997; van den Ackerveken *et al.*, 1994). Indeed, it has been suggested that nitrogen starvation is a cue for symptom development (Snoeijsers *et al.*, 2000; Talbot *et al.*, 1997). To address this issue directly, Solomon and Oliver (2001) exploited the *C. fulvum*–tomato system to measure the nitrogenous content of the apoplast during infection. The fluids contained a remarkable diversity of compounds and the total concentration of nitrogen was much higher than expected. Only two of the 20 protein amino acids (Cys and Trp) were undetectable. Evidently these must be synthesized by the fungus during growth. A second group (Met, Arg, His and Pro) were present at very low levels, suggesting that they would also need to be synthesized. The suggestion that the fungus must synthesize Met was supported by studies showing that the biosynthetic gene *Met6* was expressed by *C. fulvum* during infection (Solomon *et al.*, 2000). Furthermore, a *M. grisea* methionine auxotroph (Balhadere *et al.*, 1999) as well as a *Fusarium oxysporum* arginine auxotroph (Namiki *et al.*, 2001) were non-pathogenic. We can conclude that synthesis of Met, Arg and probably Trp, Cys, His and Pro, are likely to be general features of pathogenesis. Indeed, commercially successful fungicides such as cyprodanil and pyrimethanil are now believed to target Met biosynthesis (Hewitt, 1998). Other amino acids, notably Ala, Asp, GABA, Glu, Gly and Ser as well as nitrate were present at mM concentrations in the *C. fulvum* apoplast. These concentrations would be sufficient to support growth *in vitro* and can account for the nitrogen used by the fungus during the early and middle

stages of infection. The levels are sufficient to repress *Avr9* expression in *C. fulvum* *in vitro* (van den Ackerveken *et al.*, 1994). This suggests that signals other than low nitrogen concentration induce the expression of pathogenicity genes such as *Avr9*. It will be interesting to determine the nature of the inducing conditions.

The most surprising finding was that the concentration of most amino acids actually increased during infection. One might expect a pathogenic fungus to drain nutrient from a plant. These results therefore suggest that the fungus somehow manipulates plant metabolism to maintain and even increase the apoplastic concentration of nitrogen compounds. This is particularly striking in the case of GABA, a non-protein amino acid which *in vitro* acts as amongst the most efficient nitrogen sources for the fungus. Furthermore, fungal GAT1, a homologue of GABA transaminase and the tomato glutamate decarboxylase gene are up-regulated during infection, strongly suggesting that the plant is synthesizing and the fungus is catabolizing GABA (Solomon and Oliver, 2002).

It appears that *C. fulvum*'s nitrogen requirements are at least partially met by the uptake of amino acids from the apoplast. The rust *U. fabae* expresses amino acid transporters in haustoria, that take up histidine and lysine (Hahn *et al.*, 1997; Mendgen *et al.*, 2000) suggesting a direct uptake from the extra-haustorial space.

Nitrate was present in mM concentrations in the apoplast of *C. fulvum*. Nitrate non-utilizing mutants have been found to be fully pathogenic in *C. fulvum*, *M. grisea* and *Stagonospora nodorum* (Cutler *et al.*, 1998; Lau and Hamer, 1996; Talbot, 1990) suggesting that the fungi do not access nitrate stores during infection. Nitrate assimilation is highly energy-requiring and subject to repression by reduced nitrogen sources. It is likely that amino acids and other reduced nitrogen sources are used in preference to nitrate and these appear to be available.

### Vitamins

Thiamine pyrophosphate (TPP) is used as a co-enzyme in pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase and transketolase. These are all enzymes associated with carbohydrate utilization. Two of the 31 genes identified as being expressed in haustoria in *U. fabae* (Hahn and Mendgen, 1997) are involved in TPP synthesis. Sohn *et al.* (2000) showed that both genes were abundantly and specifically expressed in haustoria. This observation indicates that the fungus must devote considerable resources to the synthesis of this coenzyme.

A pyrimidine auxotroph was selected in *C. fulvum* by selection for 5-fluoroacetate resistance. Complementation with a *Ura3* gene confirmed the site of the mutation. The mutant was non-pathogenic whereas pathogenicity was restored in the complemented mutant. This suggests that fungi must synthesize all the

pyrimidine from simple precursors during infection (Marmeisse *et al.*, 1993).

Polyamines are a group of positively charged molecules essential for normal cellular functions such as growth and replication (Walters, 2000). Plants subjected to stresses, such as infection, accumulate polyamines. An enzyme crucial to the biosynthesis of polyamines is ornithine decarboxylase. This enzyme catalyses the conversion of ornithine to putrescine and is present in both plants and fungi. Bailey *et al.* (2000) disrupted the ornithine decarboxylase gene of the wheat blotch pathogen *Stagonospora nodorum*. As a result, polyamine auxotrophic mutants could only grow if putrescine was supplemented. Furthermore, the authors demonstrated that ODC negative mutants were less pathogenic on wheat, indicating that the fungus cannot utilize polyamines from the host. In contrast, deleting the same gene in *Tapesia yallundae* did not affect pathogenicity, although infection plaque formation was impaired (Mueller *et al.*, 2001). Clearly, the requirement for polyamines varies.

### Sulphur

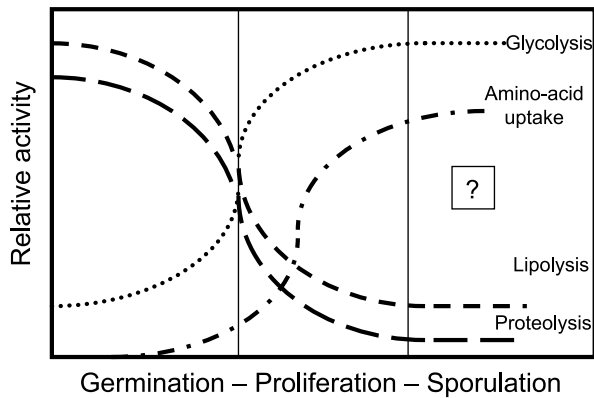
Arylsulphatase is synthesized in response to sulphur starvation to increase internal sulphate availability by degrading sulphur-containing compounds such as tyrosine-O-sulphate. The expression of this enzyme is repressible when sulphur becomes abundant (Marzluf, 1997). The expression of an arylsulphatase gene, *cgars*, from *Colletotrichum gloeosporioides* f. sp. *malvae* was monitored by RT-PCR during infection on round-leaves mallow (Goodwin *et al.*, 2000). The expression of *cgars* was highest during penetration but gradually declined during growth *in planta*. This suggests that the pathogen was sulphate-starved during the early stages of infection.

### Iron

Iron acquisition is an important pathogenicity factor in the bacterial and fungal pathogenesis of animals. It was surprising that mutants of *Ustilago maydis*, which lacked a siderophore gene, retained their pathogenicity (Mei *et al.*, 1993). Clearly the fungus must have other means of acquiring iron that still need to be elucidated.

### CONCLUSIONS

The above review summarizes our current level of knowledge on the relationship between fungal phytopathogenicity and the nutrition provided by the infected host. Given the key role that fungal nutrition must play in phytopathogenicity, surprisingly little is known, in particular, about micronutrient levels. From the evidence gathered above, a model describing the nutrient availability for phytopathogenic fungi during the development of a



**Fig. 1** Schematic diagram of fungal nutrition during infection. The relative importance of various sources of nutrient and/or energy is depicted on the *y*-axis. The *x*-axis represents a composite fungal infection arbitrarily divided into three phases: germination of the spores and penetration of the host; proliferation within the host tissue including, in some cases, the elaboration of haustoria; and the generation of spores and/or resting structures. The *x*-axis can be taken to represent both time and distance in different pathosystems.

life-cycle within the host was constructed (Fig. 1). This model can be divided into three stages.

The first stage of the fungal parasitic life-cycle is the germination of the spore and the development of structures required for host penetration. During this phase, external nutrient sources are likely to be scarce. Growth at this stage must involve the degradation of storage compounds. Genomic, histological and genetic studies all suggest that lipid catabolism is critical. There is less information relating to nitrogen reserves. Genomic studies show that many proteases are expressed, suggesting that protein turnover may be the main source of amino acids prior to penetration (Thomas *et al.*, 2001, 2002).

The second stage, 'proliferation', occurs after penetration, where the pathogen establishes within the host and can access the nutrients within the plant tissue. Early genomic studies (Coleman *et al.*, 1997) assumed that fungi were starving during infection. However the presence of high concentrations of hexose and amino acids during infection and the decline in ICL expression (Bowyer *et al.*, 2000) lead us to question this assumption. The main source of energy is likely to be glycolysis. Biotrophs such as mildews, rusts and *C. fulvum* appear to utilize hexoses, mostly derived from sucrose, that are taken up either from the apoplast or (in the case of pathogens possessing haustoria) via haustorial membranes. Necrotrophs are also likely to take up free sugars. As they all secrete cell wall degrading enzymes, the sugars released from plant polymers would be available for absorption.

Both gene expression studies (Hahn and Mendgen, 1997) and direct measurements of mycelial bathing fluids (Solomon and Oliver, 2001) have shown that biotrophs utilize free amino acids at least as a partial nitrogen source. Some amino acids appear to

be absent from the *in planta* environment. Enzymes synthesizing these amino acids are therefore attractive fungicide targets.

The evidence for a third stage is largely conjectural. This stage occurs late in infection where the host tissues, especially in the case of necrotrophic pathogens, are increasingly incapacitated by the pathogen. As a result, nutrients are depleted, leading to starvation of the pathogen. During this stage, the pathogen produces spores for progeny dissemination. No reports have been published on nutrient availability during this final stage of infection.

Much of our knowledge comes from a small number of pathogens that have diverse unique features. To take two examples, the *C. fulvum*–tomato interaction is confined to the apoplast whilst *U. fabae* haustoria are tough enough to be isolated intact. We must ask if these results are representative. It is clear that the most powerful way to approach these questions is genetic, by isolating and characterizing well-defined mutants strains, but that this approach is problematic for all obligate pathogens, including most biotrophs for which non-pathogenic mutants are non-viable. It is clear that more research into the genetically tractable and economically significant interactions is required.

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