Understanding the evolution and function of entomopathogenic fungi

Improving our understanding of the ecology of entomopathogenic fungi is critical to developing these organisms further as microbial control agents. In this page I will introduce some of the issues that are important to understanding fungal ecology, with particular emphasis on evolution and ecosystem function.

There is a consensus that entomopathogenic fungi could be exploited more effectively as a natural resource. Anamorphic entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, are usually developed as inundative control agents which are applied en masse to a pest population, and there is little expectation that they will persist and reproduce within the biotic environment. As a consequence, research on these fungi has tended to concentrate on technical aspects of biopesticide development (mass production, formulation, application, response to abiotic variables etc) with significantly less work done on understanding their basic ecology. Anamorphic entomopathogenic fungi are naturally widespread, particularly in soil, and yet little is known about the factors that influence their distribution, population structure, econutritional behaviour, and the evolution of virulence related characteristics. In contrast, researchers studying entomophthoralean fungi, which are not easy to mass produce, have focused on the ecology of these organisms and their role as causative agents of natural epizootics.

A factor that may be holding back improving biocontrol with entomopathogenic fungi is the lack of research on the evolution and functional ecology of fungi with biocontrol potential. There is also a need for concepts that unite the very diverse research activities of insect mycopathologists, and underpin both the development of new mycoinsecticides and the exploitation of natural epizootics. One way forward is to place research and thinking on entomopathogenic fungi on more of an evolutionary footing. Identifying fungal fitness traits and the selective forces that act upon them will improve our understanding of how and why entomopathogenic fungi interact with their hosts. Current theories on the evolution of virulence in microparasites, which at present are not being applied to entomopathogenic fungi, could provide fresh insights into their ecology and exploitation for biocontrol. At the same time, the wide array of experiments that can be done with entomopathogenic fungi are ideal for answering basic questions in parasitology and entomology.

'Ecological' and 'industrial' approaches to the exploitation of entomopathogenic fungi

Microbial pest control with entomopathogenic fungi is developing into increasingly contrasting 'ecological' and 'industrial' approaches. The 'ecological' approach is being used for entomophthoralean fungi, key species of which exhibit specific ecomorphological adaptations to host life cycles and cause natural epizootics in pest populations. Biocontrol with these fungi focuses on habitat manipulation to enhance natural epizootics, or on inoculative releases for long term pest management.

In contrast, biocontrol with anamorphic entomopathogenic fungi generally takes an 'industrial' approach. These fungi are rarely associated with natural epizootics but are often straight forward to mass produce, and hence are usually deployed as inundative mycopesticides for short term pest control. Mycopesticide development is based on a chemical pesticide model with little regard to the potential of the fungus as a living organism. This is not to downgrade the significant contribution to fungal biocontrol made by advances in formulation, application, and improving inoculum quality.

There is a risk, however, that wholescale adoption of a chemical pesticide model for entomopathogenic fungi could lead to the perception of these organisms purely as delivery agents of insecticidal toxins, and encourage their use as 'silver bullets'.

It is my belief that both the 'ecological' and 'industrial' approaches to microbial control could be improved through a better understanding of the evolution and function of entomopathogenic fungi. Entomopathogenic fungi show high levels of intraspecific heterogeneity, both they and their hosts have short generation times, and they occupy a wide range of habitats. The interactions between host, fungus and environment are therefore diverse and dynamic.

Insect pathogens are often exceptionally virulent

Trophic relationships form a spectrum from mutualism to lethal parasitism and predation. For our research at Warwick HRI, we are interested in fungi that function as lethal parasites of insects. It is worth pointing out that, compared to other infectious diseases, many insect pathogens are exceptionally virulent. Some entomopathogenic fungi and viruses in particular cause regular epizootics resulting in very high levels of mortality in host populations. For example, epizootics of *Neozygites floridana* have been observed to cause reductions in spidermite populations on US soybean of up to 84% in two days (for further information see the papers by Brandenburg & Kennedy (1982, 1983) and Brown & Hasibuan, (1995)).

In contrast, mammalian pathogens are far less lethal. The majority of directly transmitted human pathogens, for example, cause less than 0.1% population mortality and epidemics are extremely rare, even accounting for medical intervention. Note however that vector borne diseases are significantly more virulent (for more information see Ewald (1994)).

This raises a very fundamental question – why do so many entomopathogenic fungi kill a large proportion of their hosts?

It has long been considered that a well adapted parasite is one which is only moderately pathogenic or not pathogenic at all, because its interest is to preserve its living environment. Hosts and pathogens are engaged in an evolutionary arms race to develop new pathogen defences, and new methods of overcoming those defences, respectively. The conventional premise adopted by parasitologists and some medical micobiologists is that evolutionary adaptation of parasites to their hosts, and the evolutionary arms race, pushes the relationship towards commensalism. Hence highly virulent pathogens, like many of our entomopathogenic fungi, are thought to be in new associations with their hosts.

However, evolutionary analyses by researchers such as Ewald (1994, 1995), Anderson & May (1982), Myers & Rothman (1995), Ebert (1994) and others indicates that virulence (death of the host) can be a highly adaptive trait where the pathogen benefits from large scale multiplication within the host while paying little cost through host immobilisation. Death is a side effect of the intensity of resource utilisation that facilitates pathogen survival and reproduction. Within this overarching strategy, facets of pathogenicity, such as speed of kill, are determined partly by trade offs in the different fitness components of the pathogen.

Transmission is the key to understanding the host pathogen dynamic

These trade offs are essential for understanding the evolution of pathogen virulence. Transmission mode, in particular, plays a key role. Ewald (1994, 1995) has categorised four transmission strategies which influence the virulence (lethality) of pathogens (for more information, see his excellent book 'Evolution of infectious disease' (Paul W Ewald, Oxford University Press, 1994)):

Type	Transmission strategy	Effect of host death on transmission	Survival in environment	Virulence
Vertical		Reduces	-	low
Horizontal	mobile host	Reduces	poor	low
	Vectors	Slight reduction	-	high
	Sit & wait	Enhances	good	high

It should be obvious that it is highly adaptive for a vertically-transmitted pathogen not to kill its host before it has reproduced. Similarly, for pathogens that are transmitted by direct contact between hosts, there should be selection pressure against immobilising the host through the effects of the infection such that transmission is prevented. For example, human rhinoviruses (the cause of the common cold), are very short lived outside of the

host and require human to human contact for transmission, and as a result these disease agents are relatively benign and do not cause significant incapacitation. For entomopathogenic fungi, the Laboulenials could fit into this category. These obligate pathogens are transmitted primarily by direct contact between infected and susceptible individuals. It is worth noting that they are found mainly in adult stages (they are primarily associated with the cuticle, which means they would be shed when larvae moult {so it is adaptive not to infect larvae} although some penetrate deeper into the host) and infections tends to reduce host life-span (i.e. low virulence compared to some other fungi) although heavy infections of some species results in high host mortality.

'Sit & wait' transmission

The most virulent insect pathogens, which include a range of species of entomopathogenic fungi, function according to what Ewald calls a 'sit & wait' strategy. These organisms are primarily transmitted horizontally, and produce infective stages which are released into the environment when the host dies. The infection cycle is continued when a new susceptible host acquires the infective stages from the environment. The efficiency of transmission in this system is affected very strongly by the density of susceptible hosts. This is a critical issue for insect pathogenic fungi, as many insect species have patchy distributions, and hence the probability of contacting new hosts is often low. Under such circumstances, there is a strong selection pressure in favour of traits that increase the probability of successful transmission. Hence the longer the infective stage lasts in the environment, the more likely it is to be contact a susceptible host.

It is probably for this reason that entomophthoralean entomopathogenic fungi often produce resting spores, which are able to survive over winter when hosts are particularly scarce. Since the chances of successful transmission are positively correlated with the numbers of infective stages released into the environment, there is also selection pressure for producing large numbers of these stages on susceptible hosts. In fact, under the sit and wait strategy, the chances of transmission are maximised if the pathogen can convert as much host biomass as possible into new pathogen infective stages, which will result in the death of the host. Hence sit and wait pathogens are usually highly virulent.

Having said that, we should not overlook the fact that complete eradication of a host population by horizontally transmitted, sit & wait pathogens will incur a severe fitness cost (disease 'burn out') unless the pathogen can survive in the environment until new susceptible hosts arrive.

Natural selection, of course, works on existing traits. A basic characteristic of the Fungi is the production of spores, which are resistant propagules released into the environment for dispersal. Perhaps, then, many species of entomopathogenic fungi were predisposed to sit and wait transmission because of their ability to produce spores.

High virulence fungal pathogens – the Entomophthorales

Many species in the Entomophthorales, such as *Pandora neoaphidis*, *Entomophthora muscae*, *Neozygites fresenii* etc., conform to the sit and wait model. These fungi produce sexual zygospores through the fusion of hyphal bodies in the haemocoel, and these function as true resting spores (i.e. they have dormancy) to enable the fungus to survive in the absence of the host or during unfavourable weather conditions. Some species and strains are able to overwinter by other means, for example within cadavers or by infection cycling on 'reservoir' hosts.

In our *Neozygites* example (mentioned above), resting spores are formed late season (October to November) in populations of *T. urticae* in Alabama USA, but in N. Carolina the fungus is thought to pass the winter in mummies or by infection cycling. In addition, these fungi exhibit some highly evolved ecomorphological adaptations to host life cycle that enable them to respond quickly to favourable host density and thereby initiate epizootics rapidly under favourable conditions. Transmission at high host densities (epizootic phase) is facilitated by the production of ballistospores (primary conidia) which are actively dispersed from cadavers and which may or may not be the infective stage (in some cases, the primary conidia are not themselves capable of infecting the host, but instead germinate and develop into structures that are infective).

In a generalised entomophthoralean life-cycle, if primary conidia do not contact a host, they germinate to form a secondary, actively discharged infective conidium and so on until the protoplasm is depleted. Although these are not strictly 'sit & wait' propagules, neither are they primarily reliant upon host mobility for infection. Hence, as before, conversion of host into pathogen (i.e. host death) is a highly adaptive trait because it improves the chances of transmission.

These adaptations can be seen readily in our *Neozygites floridana* example. *Neozygites floridana* is highly adapted to its hosts, and an understanding of its life cycle is proving central to its exploitation as a biocontrol agent. The fungus develops within the haemocoel of diseased mites as hyphal bodies which multiply by budding (Carner & Canerday, 1968). The first symptoms of disease occur three to four days post infection, depending upon the host, when mites become sluggish, pale, and swollen with hyphal bodies, and death follows shortly thereafter (Carner, 1976). Under dry conditions, the cadaver becomes mummified, but in a humid environment, fungal conidiophores grow through the cuticle and a single pyriform conidium is formed on each (Remaudiere & Keller, 1980). The cadaver quickly adopts a 'glass beaded' appearance at this point with conidiophores tightly packed upon the integument (Dick *et al*., 1992). These primary conidia are forcibly discharged over the first 24 h after death, forming an aureole 1 – 3 mm from the cadaver (Brown & Hasibuan, 1995). The primary conidia are not infective to mites, and the majority germinate to form a slender capillary, c. 60 μm high, terminating in an almond-shaped, infective capilliconidium (Carner, 1976). The capilliconidia are passively disseminated, attaching to passing mites by means of an adhesive haptor on the distal terminus (Dick *et al*., 1992). Some of the primary conidia may germinate to form identical, but smaller, secondary conidia on conidiophores which are, again, actively discharged. Tertiary conidia can also be produced in the same way. The ratio of capilliconidia to secondary conidia may be determined by the availability of water (Oduor *et al*., 1995a, see also Oduor *et a*l., 1995b).

Under some circumstances, which appear to be related to geography and may also be linked to the weather, the hyphal bodies of *N. floridana* remain within the haemocoel of the host where they form resting spores. Mite cadavers that contain *N. floridana* resting spores may be anchored to the plant surface by fungal rhizoids (Keller, 1991). Resting spores are formed late season (October to November) in populations of *T. urticae* in Alabama USA (Carner, 1976), but in N. Carolina the fungus is thought to pass the winter in mummies or by infection cycling (Brandenburg & Kennedy, 1981).

Dissemination and infection of *N. floridana* happens mainly at night when relative humidity exceeds 97% (Brown & Hasibuan, 1995) and is then probably regulated by subtle shifts in temperature. Although the requirement for such high humidity seems restrictive, conditions conducive to infection may be more common than first expected, because tetranychid mites are situated well within the boundary layer of the plant surface, where the microclimate humidity is significantly higher than ambient. When humidity is not limiting, transmission is rapid and could occur within a single night, although transmission over successive nights appears more likely. Under non-limiting conditions, the growth of conidiophores through the host cuticle and the formation of primary conidia can occur within 6 h of death, while the median time for the germination of primary conidia and the subsequent formation of capilliconidia takes approximately 9 h (Carner, 1976; Dick *et al*., 1992; Oduor *et al*., 1996b). Capilliconidia start to germinate rapidly, for example within 2 h of attachment to the integument of *M. tanajoa* (Oduor *et al*., 1996a) suggesting that, host density aside, it is the formation of primary and capilliconidia that is the rate limiting step to disease transmission. Although the formation of primary conidia occurs at night, light is not physiologically damaging to the process (Oduor *et al*., 1996b), and it is possible that the fungus detects nightfall as a cue for an impending rise in humidity or a change in temperature.

Such adaptations to exploiting periods of high host density are a common feature of sit and wait insect pathogens and are not confined to fungi. For example, nucleopolyhedroviruses (NPVs) are also sit and wait pathogens, and can survive for many years in the environment and have a high level of virulence. Some NPVs block larval

moulting to the adult stadium, hence the host dies in the larval microhabitat where succeeding generations of susceptible larvae will be present.

Pathogen modification of host behaviour

Note that for some host-fungal pathogen systems (e.g. *Lecanicillium longisporum* on aphids), hosts behave normally until a relatively short time before death, and hence have potential to disseminate the pathogen to new hosts before death. There is of course a selective advantage in releasing your conidia where uninfected susceptible hosts are likely to be located. For entomophthoralean fungi that initiate epizootics in hosts that have aggregated populations, the majority of spores are discharged locally. For example, *Neozygites floridana* discharges primary conidia in an aureole 1 – 3 mm from the cadaver. *Pandora neoaphidis* – which infect aphids (which form colonies though parthenogenesis) – also characteristically discharges its conidia in a halo close to the cadaver and of sufficient distance to contact cohorts.

Other entomophthoralean fungi that infect insects that do not form colonies manipulate the behaviour of their hosts to aid dispersal over wider distances during epizootic phase. For example, *Entomophthora muscae* induces its fly hosts to climb to exposed positions on grass stalks etc to facilitate dispersal of conidia over a wider area (summit disease). Ewald (1994) has stated that such manipulative behaviours are not constrained by lethal parasitism, and may even be promoted by it. However, the benefits of inducing such behaviour would have to be balanced against the cost of increased exposure of the infected host to predation. It would be a disadvantage to the pathogen to increase the availability of the host to predators before sporulation, for example.

Exploitation of the Entomophthorales for 'ecological' microbial control

Microbial control with entomophthoralean fungi is based primarily on ecological exploitation, for example being able to predict or enhance natural epizootics for pest control.

In the southern USA, for example, research on *Neozygites floridana* has focused on dissecting the life cycle of the fungus and understanding the factors that trigger epizootics in spidermites on field crops. Disease outbreaks are periodic and are triggered by a drop in the maximum daily temperature to below 29^{O}C and an elevation in humidity to greater than 80% for 8 – 10 h per day sufficient for the formation of capilliconidia. When initiated, epizootics of *N. floridana* can cause precipitous falls in spider mite populations. Reductions in spider mite populations have been observed of up to 84% in two days on soybean. Epizootics of *N. floridana* are delayed and / or suppressed by regular sprays of fungicides and farmers have been encouraged to modify their spray programmes appropriately to take full advantage of the fungus. Although *N. floridana* undoubtedly plays an important role in spidermite population dynamics, epizootics may occur too late to prevent crop damage on some crops such as maize. However, epizootics in maize may well restrict the migration of spider mites to other crops such as peanut. Moist conditions can also sometimes stimulate early season epizootics in peanut which keep mite populations at basal levels all season.

Anamorphic entomopathogenic fungi

So far, I have talked about sit & wait strategies in general, and entomophthoralean fungi in particular, which tend to be exploited for pest control using 'ecological' approaches.

In contrast, mycopesticides for nonpersistent inundative control are nearly always based on anamorphic fungal entomopathogens, because these tend to be easy to grow in the absence of a host and hence can be mass produced readily. These fungi are not frequently associated with epizootics but they might still conform to the sit & wait model – unfortunately we don't know enough about their ecology to make a definitive statement yet. At the species level they have potentially wide host ranges, although they exhibit significant intraspecific heterogeneity with respect to host preference. They produce conidia, which are resistant life stages but are not true resting spores. Many anamorphic entomopathogenic fungi are common residents of the soil biota and conidia are able to survive for extended periods in soil. A consequence of the sit & wait strategy should be

selection of increased persistence of free living stages (conidia) so data on the longevity of conidia in the absence of hosts is important. A long lived free living stage should lower the rate at which the cost of immobilisation is increased as a function of the level of pathogen reproduction. This reduces dependence on host mobility.

Because these fungi can be grown readily outside the host, they have often been described as facultative pathogens, and indeed some authors claim that anamorphic soil dwelling fungi such as *Beauveria bassiana* grow saprotrophically in soil. This is a key issue, because the presence of a true saprotrophic phase has significant implications for the evolution and function of these fungi. Many fungi are not fixed in a single nutritional mode and can show various degrees of flexibility during their life cycle.

However, the ability to grow in axenic culture, or the growth of mycelium though soil from an infected and colonised cadaver, does not mean that a fungus is a facultative pathogen. For example, *Coniothyrium minitans*, used as a biocontrol agent of sclerotia-forming plant pathogens, can be grown readily in culture and yet does not grow in soil in the absence of a host because of inhibition through microbial competition, and in nature it functions as an obligate pathogen. The same should be expected for Mitosporic entomopathogenic fungi. These fungi have evolved many highly specialised adaptations to the infection of insects and mites (e.g. overcoming host immune systems, production of specific cuticle degrading enzymes such as Pr1 in *Metarhizium*). These adaptations would be expected to be traded off with other characteristics that constrain their simultaneous evolution, such as the ability to compete with other micro-organisms for non-living organic substrates.

Anamorphic fungi and natural epizootics

We should not lose sight of the fact that anamorphic entomopathogenic fungi can and do cause epizootics in host populations of sufficient density, for example *Hirsutella* spp. in phytophagous mite populations, and *Lecanicillium* spp. in some homopteran hosts. One dramatic example is infection of the salt marsh aphid, *Pemphigus trehernei*, by *Metarhizium anisopliae*, a relationship that was investigated by William Foster at Cambridge Unversity UK. This aphid feeds on the roots of sea aster on salt marsh off the coast of Norfolk, UK. It forms parthenogenetic colonies on plant roots that are infected by *Metarhizium flavoviride* var *pemphigum,* which is also the principle mortality factor of the aphid. The fungus infects up to 50% of populations. There is no correlation between aphid density and disease incidence, suggesting that the fungus is very widespread in the soil. The ability of the fungus to survive for prolonged periods in the absence of the host has not been investigated but from our current knowledge of its natural history, it certainly appears to conform to the sit & wait model.

Host availability and pathogen evolution

It is worth speculating as to the extent that host availability influences the evolution of pathogen specialisation. In what circumstances is it highly adaptive to be a specialist or to have a broad host range? In the case of *Metarhizium anisopliae*, research by St. Leger and co workers indicates that while most pathotypes are contained within a limited number of genotypic classes and have potentially broad host ranges, a minority have evolved with a clear specialisation towards a particular group of hosts. There are costs and benefits to specialisation. Selection for evasion mechanisms from immune systems which are adapted to a large range of host species must be particularly costly. Specialisation also reduces interspecific competition but also depends on host availability. However, selection of narrow specificity is possible only if the host species has stable populations through time. This condition appears to be met for the *Metarhizium flavoviride* var. *pemphigum* – salt marsh aphid system, with a large stable host population. Molecular data suggests this fungus is not closely related to other *Metarhizium* groups – perhaps this is evidence that the relationship is not new. Other adaptations to the salt marsh habitat should also be expected, and these will also influence the evolution of the fungus.

Anamorphic fungi, asexuality and evolution

We cannot leave anamorphic entomopathogenic fungi without remarking on the significance of their asexual life history to both function and evolution. Is asexuality of adaptive benefit to anamorphic fungi – if so, how and why? The ability to produce large

numbers of conidia is certainly of value to sit & wait fungal pathogens, and indeed we have already seen how entomophthoralean fungi produce asexual spores during their 'epizootic phase'. In addition, the restriction on gene flow between populations that occurs in anamorphic fungi allows them to diverge by any evolutionary force, regardless of its nature or strength. However, it should be remembered that not every trait is an adaptation to be explained by natural selection.

For anamorphic fungi, the absence of an overt sexual phase could be an adaptation to entomopathogenicity, but equally it may have occurred before entomopathogenicity evolved. Secondly, it is worth noting that molecular studies indicate that populations of anamorphic entomopathogenic fungi are not clonal, indicating larger amounts of gene flow than might be expected for asexual organisms. This is not to say that asexuality will not have a profound influence on gene flow within populations of entomopathogenic fungi, and deserves greater attention.

How, then, is variability generated and maintained within species of anamorphic entomopathogenic fungi and to what extent does genetic mixing occur between populations? Adaptive mutations can of course spread quickly in clonal populations, leading to effective eradication ('purging') of diversity in the population. Models of bacterial evolution suggest that genetic exchange mechanisms which are infrequent but promiscuous allows adaptations to be transferred between divergent groups. To what extent do gene capture events occur in asexual fungi? Parasexual recombination may also be important, and has been demonstrated in laboratory experiments but not in the field. Finally, we have the thorny issue not only of how to define a 'species' of anamorphic fungus in biological terms (as opposed to purely taxonomic or categorical terms) but to understand if and how 'speciation' occurs, and the importance of competition between reproductively isolated, clonal populations to evolutionary change.

Trade offs and their consequences

So far, we have seen that there is a very strong interaction between pathogen transmission mode and virulence. Pathogens tend not to be lethal when incapacity of the host significantly disrupts transmission. Killing the host is an adaptive trait for sit & wait pathogens, including entomopathogenic fungi used for biocontrol. We must now go a stage further, and for the sit & wait fungal pathogens we need to draw the distinction between the high virulence strategy *per se*, and the degree to which different life history traits (including pathogenicity-related characteristics) are expressed by different species and strains of fungi.

Trade-offs occur between life-history traits that constrain their simultaneous evolution and hence have a profound influence on a pathogen's biocontrol potential. In entomopathogenic fungi, for example, correlation data from our lab suggests that speed of kill is traded off with spore production on host cadavers. A trade off between speed of kill and propagule formation appears to be common in horizontally transmitted pathogens. Correlation data, however, is not proof of cause and effect. Direct evidence for a trade off between speed of kill and propagule formation in entomopathogens comes from baculoviruses, where manipulation of the viral genome to increase speed of kill simultaneously reduces virion production in the host (see Myers and Rothman, 1995).

Entomopathogenic fungi show high levels of intraspecific variation with respect to pathogenicity related characteristics. It is becoming apparent that entomopathogenic fungi have a very large number and diversity of virulence genes The trade offs that occur between these and other traits could partly account for fungal intraspecific variation.

Other factors

This article has been deliberately pathogen-centred. However, other key evolutionary influences include the abiotic environment and host-factors, such as host abundance (we have mentioned this briefly already) and the ability of hosts to evolve resistance mechanisms. Work by Bidochka and co workers (1998, 2000, 2001) for example, has shown that the occurrence of genetic groupings in *B. bassiana* and *M. anisopliae* are influenced by habitat type. These kinds of factors should be expected to strongly influence the evolution and ecology of entomopathogenic fungi.

A number of tantalising questions arise in relation to identifying the factors affecting the evolution of entomopathogenic fungi other than the host organism. Why, for example, are the teleomorphs of the ascomycete entomopathogens (*Cordyceps*, *Torrubiella*) rare in temperate regions? Why are anamorphic entomopathogens more common in temperate regions or in disturbed habitats in the tropics rather than primary tropical forest habitats? Microclimatic and host influences come together in a dramatic way when insects behaviourally thermo-regulate to fight infection, for example behavioural fever in locusts or grasshoppers in response to infection with *Metarhizium*. Consideration of the evolutionary arms races between host and pathogen brings us back to our original point: the conventional premise has been that the evolutionary relationship between host and pathogen evolves towards commensalism, whereas recent insights reveal that high pathogen virulence can be a highly adaptive trait. By extension, strong selection pressure in pathogen populations for causing host death should also have a profound and significant effect on the evolution of hosts. Appreciating how these factors affect natural selection can improve our understanding of the functional diversity of entomopathogenic fungi and lead to better strategies for microbial control.

Practical applications for biocontrol

Recognition of both the sit & wait strategy, and of trade offs between life history traits within it, could have practical benefits for biocontrol. Key issues are as follows:

1) Identification of parameters that must be manipulated to improve the impact of natural epizootics.
2) Selection of the most suitable pathogens for use as biopesticides.
3) Prolonging pest control with microbial biopesticides.

I have already mentioned that there is an adaptive benefit for sit & wait pathogens to sit and wait in the same location as their hosts. Could ploughing, for example, bury resting spores of entomophthoralean fungi and thereby disrupt the initiation of epizootics? Similarly, does crop rotation affect epizootics?

Trade offs are a key issue for the selection of isolates of fungi for use in microbial control. For example, if pathogens are to be released with the intention of inducing an epizootic, then isolates that kill the host more slowly might be beneficial since they allow the production and release of more-infectious particles in the environment. Similarly, strains of pathogen engineered for faster speed of kill should be expected to persist less well in the environment.

I have highlighted the fact that anamorphic fungi can cause epizootics, and there may be opportunities to prolong pest control by biopesticides. Selection of fungal isolates with attributes that favour secondary cycling could improve biocontrol in other pest systems. Finally, it should also be recognised that because a wide array of experiments can be done with entomopathogenic fungi and their hosts, they make excellent models for addressing basic questions in parasitology, ecology and evolution.

Dave Chandler, March 2009

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