FINAL REPORT

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HDC Project PC 277

Protected Ornamentals: investigation of fungal pathogens infecting larvae of sciarid and shore flies

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Grower Summary

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SCIENCE SECTION

Grower summary

Headline

- This project reviewed the available literature on the biology of naturally occurring fungal pathogens of sciarid and shore fly pests of containerised protected crops (ornamentals and herbs). The literature strongly suggests that insect pathogenic fungi have potential as biological control agents of sciarid flies and shore flies

Background and expected deliverables

Sciarid flies (also known as fungus gnats) and shore flies are widespread and important pests of protected ornamental and bedding plants, pot herbs, and nursery stock during propagation. There is a requirement for new forms of control that are compatible with Integrated Pest Management and enable growers to reduce their reliance of chemical pesticides.

In recent years growers have been observing an increase in naturally occurring infections of flies by insect pathogenic fungi. The first such infection on sciarid flies was observed on a grower's holding in 1994 (John Buxton, ADAS). There is good evidence that this infection is caused by the insect pathogenic fungus *Furia sciarae* (=*Erynia sciarae*).

A fungal outbreak on shore flies, caused by a completely different type of insect pathogenic fungus, was observed by Neil Helyer, of Fargro, on commercial pot herbs on the south coast in 2006. For both sciarid and shore flies, the infections appear to cause high levels of natural pest control.

If the naturally occurring fungal infections on sciarid and shore flies could be exploited, they could help reduce fly populations for the grower. This would be a form of biological control, i.e. the use of one living organism (a natural enemy) to control a pest organism.

This project therefore had 2 objectives:

- Identify the fungus that infects shore flies.
- Review what is known about *Erynia sciaridae* and the 'shore fly' fungus.

Summary of the project and main conclusions

Fungal infection of sciarid flies. The fungus infecting sciarid flies is an entomophthoralean fungus ie a parasitic fungus that typically develops in the bodies of insects. Based on initial inspection of material from a Stratford nursery, combined with the host species and the behavioural response to infection, the fungus infecting sciarid flies is likely to be the species *Furia sciarae*. Unfortunately, the taxonomic status of this fungus has not yet been resolved. For this reason, the fungus has one other synonymous genus name and several synonymous species names. Identification is difficult because the fungal spores – which are key diagnostic features - vary in form depending on the host insect species. Molecular techniques will be needed to identify the fungus definitively. We have not yet been able to get this fungus into culture, although recently new types of culture media have become available which may enable us to grow it in the laboratory.

Very little work has been done on this fungus before. However, in both China and the USA it is recorded as a natural control agent of sciarid flies in mushroom houses and it has been investigated as a biological control agent. In the USA, exposing sciarid flies to the fungus caused a 95% reduction in the fly population. In China – which has a long history of using microbial pathogens of insects for pest control - simply introducing fungus infected flies to mushroom houses where it had not been observed before combined with water spraying to raise the humidity caused 60% population control.

Shore flies. The fungus infecting shore flies is likely to be a species of the genus *Hirsutella.* This belongs to a very different taxonomic group to *Furia sciarae*. It has not been recorded on flies before. The genus *Hirsutella* is generally known as a natural control agent of tropical insects and mites.

In the USA, the species *Hirsutella thompsonii* (which is specific to mites and does not infect insects) was developed as a commercial control agent of citrus mites where it could give long lasting control. This fungus is also very difficult to grow in culture. However, a new method of mass producing it has been developed in India. Here, the fungus has been developed into a biological control agent of coconut mite, where it is reported to give levels of pest control that are as good as chemical acaricides.

In the USA, the insect pathogenic fungus *Beauveria bassiana* has also been evaluated as a control agent of shore flies. This fungus has been developed into a number of commercial

products for the control of a range of insect pests in the USA, Europe and elsewhere. When applied to the surface of potting media the fungus caused 100% control of fly populations within 10 days.

The literature suggests strongly that insect pathogenic fungi have potential as biological control agents of sciarid flies and shore flies. There are three strategies that could be used to exploit these fungi:

1. Development of a commercial biocontrol product to be applied to fly populations.

2. Exploit natural infections of the fungi through conservation control. This would entail working out what conditions favour and / or inhibit fungal infection. A successful system for predicting natural fungal infections of aphids is in operation for cotton farmers in the south east USA and could serve as a model.

3. Movement of fungal infected insects to nurseries where natural outbreaks of the fungus have not been found before.

Financial benefits

There are currently no financial benefits to be gained from this work.

Action points for growers

We are not yet in a position to make firm action points for growers, apart from the recommendation to look out for these infections and note when and where they occur. More work will be required before a system of exploiting the fungi can be put into place.

SCIENCE SECTION

Literature review: fungal pathogens infecting larvae of sciarid and shore flies

Introduction

This report discusses the biology of naturally occurring fungal pathogens of sciarid and shore fly pests of containerised protected crops (ornamentals and herbs), and the possibilities for exploiting them for more sustainable pest management and crop production.

Sciarid flies (*Bradysia difformis; Diptera, Sciaridae*) (also known as fungus gnats) and shore flies (*Scatella tenuicosta; Diptera, Ephydridae*) are widespread and important pests of protected ornamental and bedding plants, pot herbs, and nursery stock during propagation. The literature on sciarid fly biology and economic significance has been reviewed by Harris *et al.* (1996) and on shore flies by Foote (1995). Both species have four larval stages. They have short generation times and reproduce rapidly.

The larvae of sciarid flies feed on organic material in the growing medium, and they also feed on plant roots and inside the stems. This reduces crop quality and can cause the death of susceptible plants such as poinsettias. The adult flies can act as vectors of a range of plant pathogens, such as *Pythium* and *Phytophthora.* Shore flies feed on algae, and can also transmit plant diseases. (Keates *et al.,* 1989). They are a particular problem on herbs because of the lack of approved chemical pesticides. Fly populations can be extremely high during the spring and summer, causing considerable nuisance to workers, and both ornamental and herb plants contaminated with flies can be rejected by supermarkets. The sleeving of ornamental pot plants such as lilies also makes contamination by the adult flies a problem, as shipments with flies visible inside the sleeve can lead to customer complaints. Large numbers can also leave frass spots on foliage. (Jacobson, 1995; 1998).

Some growers rely solely on pesticides to control sciarid flies, applying high volumes of insecticide sprays to knock down the adult flies, and insecticide drenches to control larvae in compost. However, control can be ineffective; numbers may be reduced for a short time but they usually recover quickly as pupae in the compost emerge into new adults. If control breaks down, very high populations of larvae can develop. For example, in 2006, crops of poinsettias (especially the variety Monreal) were infested with up to 10 larvae per stem (John Buxton, ADAS, personal communication). Research has shown that there is a direct correlation between the numbers of sciarid flies and the crop vigour of poinsettias (Lindquist, 1992). Growers are under considerable pressure to reduce the amounts of chemical

pesticides applied. Progressive growers of ornamentals and growers of herbs prefer to use IPM to control sciarid flies and related pests. (Lindquist *et al*., 1994). These programmes use a combination of cultural control (traps and crop hygiene), biological control and IPM-compatible insecticides (on ornamentals only; as explained above there are no pesticides approved for the control of fly pests on herbs). At present, biological control of sciarid flies is done with insect parasitic nematodes, *Steinernema feltiae,* or predatory mites, *Hypoaspis* spp. These biological control agents are not effective against shore flies at commercially acceptable rates (Vanninen, 1991). The predatory beetles, *Atheta coriaria*, are available for the control of both sciarid and shore flies; current HDC-funded work is investigating how best to use this commercially (PC239). The three biological control agents do not always give reliable control of sciarid flies or shore flies, either alone or in combination. Improved integrated strategies are needed therefore.

In addition to having to cope with the economic damage caused by sciarid and shore flies, growers are under considerable pressure from the major retailers to reduce the amounts of chemical pesticides applied, in order to respond to consumer demands. Furthermore, there is a general drive for growers to develop methods that reduce their environmental footprint and increase the sustainability of the crop production process, which includes the reduction of chemical pesticides. Another change in grower practice driven by the sustainability agenda is reduced use of peat compost in favour of alternatives such as wood fibre or composted green waste. However, it is possible that these alternative materials may be associated with greater populations of flies. Lindquist (1992) found that the highest number of sciarid flies occurred in composts with higher microbial activity, such as peat/bark mixes where the bark was not fully composted, whereas numbers were lower in media composed solely of older peats, which had lower microbial activity.

In recent years growers have been observing an increase in naturally occurring infections of flies by insect pathogenic fungi. The first such infection on sciarid flies was observed on a grower's holding in 1994 (John Buxton, ADAS, personal communication). However the infections do not occur on all nurseries. Initial discussions held by John Buxton with colleagues in Europe indicate that the fungal infections on sciarids have not been seen or recorded, although infection may have been overlooked. A fungal outbreak on shore flies was observed by Neil Helyer, of Fargro, on commercial pot herbs on the south coast in 2006. The infections are caused by a different species to those causing infections on sciarid flies. This type of infection has not been reported before. It too appeared to cause high levels of mortality.

If the naturally occurring fungal infections on sciarid and shore flies could be exploited, they could help reduce fly populations for the grower. This would be a form of biological control, i.e. the use of one living organism (a natural enemy) to control a pest organism.

Biological control

Two basic strategies for biological control with natural enemies are worth considering with respect to the fungal infections of sciarids and shore flies.

1. **Augmentation biological control** involves the application of natural enemies (i.e. therapeutic control). Augmentation generally uses control agents supplied as commercial products and has two forms:

    a. **Inoculative applications** are based on pest control through the action of individuals of the released agent and their progeny (Hajek, 2004). The agent is expected to persist within the pest's environment, although without permanent establishment. Sometimes, inoculation control involves introducing a natural enemy into new areas. In the specific case where the control agent is not endemic to the country of introduction, and where the pest is also non endemic (i.e. an alien, invasive species) this is called 'classical control'. A long standing hypothesis is that invasive species become pests because they have escaped their natural enemies as a result of introduction into a new area (Torchin *et al*., 2003). The introduced agent is expected to establish permanently and spread within its new environment.

    b. In contrast, **inundative applications** achieve pest control by the mass application of individuals of the released agent only, with no expectation of control by their progeny. The efficacy of inundative control agents is dose dependent. Inundative control using microbial agents, such as insect pathogenic fungi, is akin to the use of chemical pesticides, which may explain why it is the most widely used form of microbial control. Inundative biological control agents are often referred to as biopesticides.

    In reality, inoculation and inundation form a continuum, with the control agent persisting for various amounts of time depending on its biological characteristics, the availability of hosts, the ecological stability of the environment and the cropping system. The best way to utilise biopesticides is through Integrated Pest Management (IPM) and Integrated

Crop Management (ICM), i.e. combined use of a range of complementary pest control methods to reduce a pest population below its economic threshold with minimum impacts on other component of the crop ecosystem (Kogan, 1998). Experience shows that biopesticides can make important contributions to ICM and help reduce reliance on chemical pesticides with minimal risk to the environment or operators.

2. **Conservation biological control** is based on exploiting natural enemies that are already resident within the environment of the pest. It involves modifying the environment to enhance the natural pest control activity of the natural enemy. This can be done either by introducing new practices (e.g. increased plantings in areas that act as refugia for natural enemies) or stopping practices detrimental to natural enemy function, for example stopping fungicide use, or only using compatible fungicides, in order to promote the activity of naturally occurring insect pathogenic fungi.

The type of biological control strategy used largely depends on the type of pest and the biology of the natural enemies available, including their suitability for mass production and ability to persist and reproduce within the host environment. It is also influenced by the economic cost of the control options and regulatory barriers (for example the registration procedure required for microbial biopesticides).

The insect pathogenic fungi that are the subject of this paper are microbial control agents (MCAs). MCAs are based on types of bacteria, protozoa, fungi and viruses that are natural enemies of particular groups of phytophagous invertebrates, plant diseases or weeds. They can be pathogens, antagonists or competitors. They are naturally widespread in many environments and contribute to the natural regulation of populations of their hosts. As stated above, they can also be used as therapeutic agents for pest management and have a range of properties that make them desirable for IPM (Hajek, 2004). They do not naturally infect vertebrates, and so are considered safe to humans, livestock and vertebrate wildlife. They produce little or no toxic residue and are relatively inexpensive to develop. MCAs that can be mass produced can be applied to crops using the same apparatus used to apply chemical pesticides, and formulated in similar ways to pesticides to enhance their efficacy. It is their potential for self-perpetuating pest control that distinguishes them from chemical control agents.

Insect pathogenic fungi

Fungi are important natural enemies of insects and can be used for biological control. Approximately 750 species of fungi in 56 genera are known to be pathogens of insects

(usually referred to as entomopathogens) although the true number is likely to be significantly higher (Hawksworth, 1991; Hawksworth *et al*., 1995). They occur in all four divisions of the true fungi but most species reside in the divisions Zygomycota (in particular the Entomophthorales) and the Ascomycota. These groups also contain the most virulent fungal pathogens of insects, all of which are transmitted horizontally (i.e. between individuals of the same cohort, not from parents to offspring).

- The fungus that infects sciarids is in the division Zygomycota, class Zygomycetes, order Entomophthorales. The Entomophthorales contain many species that are obligate pathogens of insects and cause natural epizootics in a range of agricultural pests (an epizootic is an overt disease outbreak in an animal population). However many of these species cannot be grown readily *in vitro* (McCoy *et al*., 1988). For these reasons they have tended to have been investigated and exploited for biological control using conservation strategies. There is some disagreement about the evolutionary relationships, and hence taxonomy and nomenclature, of some species and genera of entomophthoralean fungi, especially for those infecting flies. This is partly because, for a single species, the morphology of the spores (which are key taxonomic features) can change depending on what host species is being infected (Jensen *et al.*, 2006). However these are likely to be resolved soon with the increasing use of gene sequence data for taxonomy. Genera of entomophthoralean fungi that have been investigated for conservation biological control include: *Erynia* (= *Pandora*) against aphids; *Entomophthora* against muscid flies, such as blow fly (*Musca domestica*) and cabbage root fly (*Delia radicum*); *Zoophthora*, against diamondback moth; and *Neozygites* species against aphids and mites.

- The fungus that infects shore flies is in the division Ascomyctoa, class Sordariomycetes. This particular fungus is asexual (i.e. it reproduces by producing asexual spores). A large number of fungi in the Ascomyctes belong to species which are asexual or – more correctly – in which a sexual phase has not yet been discovered. For this reason these fungi are referred to as anamorphic Ascomycetes or mitosporic fungi. Members of the anamorphic Ascomycetes are associated less commonly with natural epizootics than the Entomophthorales, but they are popular choices for biopesticides because many of them can be mass-produced easily. About 20 – 30 products are available commercially, mainly for the management of Homoptera (e.g. aphids and whiteflies), Coleoptera (e.g. black vine weevil, Colorado potato beetle, cockchafers), Lepidoptera (for example pine moths), and Orthoptera (African locusts) (Shah & Goettel, 1999). The majority of

products are based on the anamorphic Ascomycete fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, and *Verticillium lecanii*.

Mode of action

Entomopathogenic fungi exhibit a wide range of ecological adaptations to exploiting their hosts, reflecting the host-pathogen co-evolutionary relationship. However the basic mechanisms by which entomopathogenic fungi infect their hosts are essentially similar. Infection occurs using specialised spores which attach to, germinate on, and penetrate the integument. Infection does not occur through ingestion. The penetrating fungus multiplies within the haemocoel (the central pool of blood that is contained within an insect's thorax) and soft tissues of the host, using the host as a nutritional resource, and death occurs usually within three to ten days after infection by water loss, nutrient deprivation, gross mechanical damage and – for Ascomycetes – the action of toxins. Under favourable conditions, the fungus sporulates extensively on the cadaver to facilitate further infections in the host population and thus continue the disease cycle. Comprehensive accounts of the infection processes, pathology and epizootiology of entomopathogenic fungi are given by Tanada & Kaya (1993), St. Leger (1993) and Hajek & St. Leger (1994).

Species within the Entomophthorales probably exhibit the widest array of adaptations to the life cycles of their hosts of all the entomopathogenic fungi. In the summer, these fungi usually reproduce by producing asexual spores. For many entomophthoralean species, such as *Pandora neoaphidis* (which infects aphids) and *Entomophthora radicans* (which infects muscid flies such as the house fly), spores are produced on the host body immediately after death and are actively discharged into the area surrounding the dead body. This is thought to be an adaptation to increasing the chances of the fungus being transmitted to new hosts. The production of spores on the host cadaver can often give it a 'glass bead' texture (growers and practitioners should look out for this on infected sciarid flies as a diagnostic feature alongside 'summit disease'). Spores discharged from the cadaver often form a halo around it. Some other species, such as *Strongwellsea castrans*, produce spores on the insect host before it dies (again, this is thought to be an adaptation to enhance transmission). Entomophthoralean fungi are thought to be biotrophs, i.e. they keep their hosts alive until all nutritional resources have been used up. Behavioural modifications in the host caused by the fungus are common (see below). In the autumn, as environmental conditions change and the supply of hosts starts to decline, they produce sexual spores. These are true resting structures that have evolved to help the fungus survive adverse environmental conditions in the absence of an insect host. However, resting spores are not

produced in all species, some of which are thought to survive the winter as hyphal bodies within cadavers.

The strategy used by the anamorphic Ascomycete entomopathogenic fungi is to act as hemibiotrophs: the host insect is first infected biotrophically and then the fungus switches to a saprotrophic phase in which it grows and sporulates on the cadaver (Roy *et al*., 2006). Host death often involves that action of secondary metabolites. These fungi do not produce resting spores and the asexual spores are not actively discharged from host insects. With the exceptions of *Hirsutella* spp. and *Verticillium lecanii* , these fungi tend to operate more opportunistically for soil dwelling insects in temperate regions (Samson *et al*., 1988; Roy *et al.*, 2006).

Environmental requirements for infection

Both infection and sporulation of all entomopathogenic fungi require the presence of free water or high humidity, the lower limit for the germination of spores *in vitro* being c. 93 % RH (Andersen *et al*., 2006). In some cases, entomopathogenic fungi are able to cause infections at seemingly lower humidities than those required for germination *in vitro*, because the microclimate humidity of the host is higher than ambient (Milner *et al*., 1997). For inundative biopesticide products, formulating the spores in oils, or oil-in-water emulsions, also facilitates infections at lower humidities (Burges, 1999).

Many isolates of entomopathogenic fungi require moderate temperatures (15 – 27ºC) for optimal infection, although the maximum temperatures for growth vary extensively with isolate, e.g. 33 – 36ºC for *V. lecanii*, 33 – 40ºC for *Conidiobolus coronatus,* and more than 37ºC for *Beauveria* spp. (Burges 1981). The genus *Metarhizium* appears to exhibit the widest range of temperatures for growth (Fargues *et al*., 1992), and isolates active at high temperatures have been identified. For example, an isolate of *Metarhizium flavoviride*, originating from Madagascar, grew optimally at 34ºC and had a maximum temperature for growth of 38ºC (Welling *et al*., 1994). Heat-active strains of *M. anisopliae* have also been obtained that germinate rapidly at 37ºC (McCammon & Rath, 1994).

Fungal infection in sciarid flies from UK nurseries

The diseased sciarid flies on UK nurseries are being infected with an entomophthoralean fungus. As described above, these fungi are well known for causing epizootics in a range of insect species. Reports have been received about fungal infections in sciarids from a range of nurseries, and samples have been sent in to Warwick HRI from Findons nursery Stratford upon Avon. However we have not yet been able to culture the fungus in the laboratory and

this has impaired attempts to identify it definitively. The fungus infects sciarid larvae and causes a characteristic behavioural change during infection: just before they die, the larvae crawl from the roots to the surface of the compost or even onto the plant. This phenomenon, known as summit disease, occurs with many host species infected by entomophthoralean fungi and is thought by some authors to be an adaptation to increase the transmission of the fungus to new hosts, although it may also be a response by infected hosts to remove themselves from closely related individuals (Roy *et al*., 2006). In classic summit disease, infected hosts crawl to elevated positions a few hours before death. The mechanisms behind the phenomenon are unknown, and few experimental investigations have been done with it. Most observations of summit disease concern insects that feed on above ground parts of plants. However, in their review of behavioural modifications on insects by entomopathogenic fungi, Roy *et al*. (2006) mention two examples where soil dwelling insects crawl onto the soil surface to die: (1) infections of sugarbeet root aphids by the fungus *Pandora neoaphidis*; and (2) infections of sciarid fly larvae in compost on potted plants by *Erynia sciarae*.

There is very little published information on fungal infections of sciarid flies, so inference about fungal biology and exploitation for biological control will have to be done in part using information and accumulated experience with other, related fungi. Unfortunately, the taxonomic status of many of the entomophthoralean species – including the fungal species most likely infecting sciarids - has not yet been fully resolved, which makes inference more complicated.

Records of the entomophthoralean fungal species infecting sciarid flies include *Erynia sciarae* (Ben Ze'ev & Kenneth, 1982; Roy et al., 2006); *Erynia byfordii* (Keller, 2002); *Erynia montana* (Betterley, 1989); and *Erynia ithacensis* (Huang *et al.*, 1992). Given the problems and uncertainties over the nomenclature of the Entomophthorales it is very possible that these are all the same species. The genus *Erynia* is also variously named as *Furia* and *Empusa*. These genus names are also synonymous. For the purpose of this paper, the most recently revised genus name, *Furia*, is preferred.

Given that the symptoms of fungal infection on sciarids in the UK appear to be identical on different nurseries, I hypothesise that only one species of fungus is causing these infections (but work in the future will be required to confirm or refute this). Based on initial inspection of material from Findons, combined with the host species, the behavioural response to infection (summit disease), and the fact that larvae are being infected, it is likely to be the synonymous species *Erynia sciarae* / *Erynia byfordii* / *Erynia montana* / *Erynia ithacensis*.

For the purpose of this review, the fungus infecting sciarid larvae in UK nurseries will be referred to as *Furia sciarae*: this uses the most recently revised genus name (*Furia*), and the simplest species name (*sciarae*).

Unfortunately, we have not been able to culture the fungus on the standard media recommended for entomophthoralean fungi. Many species of entomophthoralean fungi grow slowly in culture and have complex nutritional requirements for growth. However, Leite *et al*. (2005) have investigated complex media which are claimed to be suitable for mass production of *Furia* species. These media are worth investigating for the culture of fungi isolated from sciarid flies.

Huang *et al*. (1992) observed natural epizootics of *F. sciaria* (which they referred to as *Erynia ithacensis*) in populations of the yellow legged fungus gnat *Phoradonta flavipes*, a sciarid fly pest of mushroom houses in south east China. The fungus caused up to 40% mortality in fly populations. The paper only refers to infections in adult flies: these died in humid microhabitats in elevated positions, attached to substrates by their hind legs. Fungal holdfasts developed to anchor the cadavers post mortem. The authors successfully introduced the fungus to areas where epizootics had not been observed before (a type of inoculation control, see above). This was done by placing 50 – 60 infected cadavers in mushroom houses every day for one week. The main fly habitat areas were sprayed with water to elevate humidity as a strategy for enhancing the fungal infection (although the authors report that it was common commercial practice anyway to spray houses with water at certain stages of cropping). This strategy resulted in 60% adult fly mortality 12 days after application of the fungus. However, the initiation of infection required humidity above 80% RH. This strategy of introducing fungal infected cadavers into 'non-epizootic areas' has been used widely in China with a range of insect pests and appears to work well.

In the USA, Betterley (1989) investigated *F. sciarae* (which they referred to as *E. montana*) as a control agent of sciarid flies (*Lycoriella mali*), also in mushroom houses. Infected flies were collected from a mushroom farm in northern California, and the fungus was successfully cultured on a specialised medium. Fungal spores or mats of mycelium were then added to trays of spawned mushroom compost followed by the addition of *L. mali*. The symptoms induced in adult flies were the same as those described by Huang *et al*. (1992). Inoculation of the mushroom compost and casing caused infection and mortality levels of 85 – 95% in larvae, and 40 – 80% in adults.

Fungal infection in shore flies

The fungus infecting shore flies (Figure 1) is an anamorphic Ascomycete fungus. I believe it is a member of the genus *Hirsutella* based on its morphology. It may well be a new species of fungus in this genus. The fungus had caused a natural epizootic in adult shore flies. Natural epizootics are unusual for anamorphic Ascomycete fungi but not unheard of for *Hirsutella* species. In contrast to the fungus infecting sciarids, the fungal pathogen of shore flies was observed infecting adult flies. It produced hyphal protuberances on the insect cadaver, called synnemata, which are characteristic of *Hirsutella*. Recently, a similar fungus described as *Hirsutella* has been observed on shore flies in Australia (Nigel Hywel Jones, University of Bangkok, pers. comm.). Most anamorphic Ascomycetes are relatively easy to culture and grow quite quickly *in vitro*. However we have found that the species infecting shore flies grows very slowly in culture. This is typical of *Hirsutella*.

The taxonomy of *Hirsutella* is complex and not fully understood (Samson *et al*., 1988). Most species are pathogens of tropical insects or mites (McCoy, 1981) which makes the occurrence of a species in the UK unusual. The most widely studied member of the genus is *Hirsutella thompsonii* (Fisher, 1950), a specific pathogen of mites (McCoy, 1981). The fungus was first reported as the causative agent of natural epizootics in the citrus rust mite, *Phyllocoptruta oleivora* in Florida, and it was first cultured and its pathogenicity confirmed by McCoy & Kanavel (1969). *Hirsutella thompsonii* was developed by McCoy and co-workers, in collaboration with Abbott Laboratories, as a biopesticide for control of *P. oleivora* and other eriophyoids in the 1970s, and was registered under the trade name Mycar™ in the USA in 1981 (McCoy, 1996). In Florida citrus groves, the fungus was applied early in the growing season to suppress mite population development at low densities. In field experiments under favourable weather conditions, control of *P. oleivora* with Mycar was comparable to that achieved with chemical acaricides (McCoy, 1996). Epizootics were often initiated within two weeks of application and mite populations remained low for six months to a year after application (McCoy, 1981). In commercial practice, the product gave variable results, attributed to poor product stability in storage and difficulties in its mass production, and sales were terminated in 1985 (McCoy, 1996). Recently researchers in India have developed a new system for mass production of *H. thompsonii* for use against coconut mite, where the fungus also causes natural infections (Kumar & Singh, 2000; Sreerama Kumar, personal communication). A product based on this system, Mycohit, was evaluated in farm scale experiments in 2000 – 2005. Mycohit was able to reduce mite populations by up to 90%, and biopesticide producers are now showing strong interest in commercialising it for use throughout India.

Infections of other fly species by entomopathogenic fungi

Natural epizootics by the entomophthoralean fungi *Entomophthora muscae* and *Strongwellsea castrans* occur among populations of adult root feeding *Delia* spp. (Berisford and Tsao 1974, Carruthers *et al.* 1985, Eilenberg 1991), while *E. muscae* is also an important natural control agent of cattle flies, causing 50% mortality in populations of some species such as yellow dung fly (Steenberg *et al*., 2001). Although natural infections by anamorphic Ascomycete fungi of dipterous flies appear to be rare (Majchrowicz *et al.* 1990, Steinkraus *et al.* 1990), *Beauveria bassiana* and *Metarhizium anisopliae* have been shown to be highly pathogenic when tested as biopesticides against adult *Delia radicum* (cabbage root fly) and *Delia antiqua* (onion fly) (Rizzo 1977; Meadow *et al.* 2000; Chandler & Davidson, 2005; Davidson & Chandler, 2005) as well as horn flies, *Hematobia irritans* (Lohmeyer & Miller, 2006), sugar beet maggot, *Tetanops myopaeformis* (Campbell *et al*., 2006), and fruit fly, *Ceratitis capitata* (Quesada-Moraga *et al.*, 2006). In general, from the literature, there appears to be an increasing interest in the use of these fungi as biopesticides for the biological control of fly pests. However there is no evidence in the literature that these fungi have been investigated as potential inundative biopesticides against sciarid flies.

Stanghellini & El-Hamalawi (2005) investigated *B. bassiana* as a biopesticide against the shore fly *Scatella stagnalis* in California. Spores of the fungus were mass produced on autoclaved millet seed (this is a standard way for mass producing this fungus). Dried colonised millet seeds were then broadcast on to the surface of potting medium in pots infested with adult flies or larvae and pupae. The fungus caused c. 90% mortality in larvae and pupae after 15 days, and 100% mortality of adult flies in 10 days.

Exploiting fungi for the control of sciarids and shore flies

The literature suggests strongly that entomopathogenic fungi have potential as biological control agents of sciarid flies and shore flies. The strategies that could be used to exploit them are:

- Development of a commercial biopesticide product to be used for augmentation biocontrol, either using inundation or inoculation approaches;
- To exploit natural infections of the fungi through conservation control.
- Movement of fungal infected cadavers to nurseries where epizootics do not occur.

<u>Augmentation control: development of a biopesticide product for the control of sciarid and shore flies</u>

If a fungal pathogen is to be supplied as a commercial product then it must be able to be produced in large quantities in an economic way. It must also be amenable to formulation to

make sure it is stable during storage and to enhance its efficacy. Ideally, the potential of the fungus for self replicating control should be exploited as part of the control method, i.e. it should reproduce, spread and persist within the fly population. The disadvantage of the biopesticide approach is that any commercial product used would have to be registered through the Pesticides Safety Directorate using their Biopesticides Scheme. This has a financial cost: a minimum of £12000 for dossier assessment, not including the costs of developing the product, safety testing and running efficacy trials. However the costs for developing a biopesticide are significantly less than those for a conventional chemical pesticide. If a fungal control agent were to be commercialised, it would have to be for a sufficiently large market in order to make the product profitable. Hence the fungal control agent would probably have to be efficacious against sciarids, shore flies and related pest species on a range of crop types. The options available are as follows:

Sciarid flies

1. *Furia sciarae*. This fungus can be grown in culture, although is not straightforward to grow. Experiments using it as a control agent applied against sciarid flies in mushroom houses in China and the USA showed that it can cause high levels of infection, although it is not clear how long these infections persisted. In principle, however, there appears to be biological potential for using this fungus as a biopesticide. Because it is not possible to produce infective spores of this fungus in culture, any product would have to be based on fungal mycelium. This mycelium is not in itself infective – it will have to sporulate first to produce infective spores. It might be possible to apply the fungus by incorporating mycelium into compost or applying it to the compost surface. If the summit disease behaviour seen in sciarid larvae (in which infected larvae crawl to the compost surface to die and sporulate) is an adaptation by the fungus to increase its transmission, then this suggests that the fungus should be applied to the compost surface. The mycelium could be formulated with nutrients to encourage its sporulation (this method was used successfully for a mycelium based formulation of *M. anisopliae* produced by Bayer in the 1990s for control of vine weevil on ornamentals).

2. Other fungal species. Research has shown that adult diptera of a range of species are very susceptible to the anamorphic Ascomycete fungal pathogens *B. bassiana* and *M. anisopliae*. These fungi have not been assessed against adult sciarid flies, although I have seen adult sciarids naturally infected with *B. bassiana*, albeit rarely. Research would be required to test the susceptibility of sciarid fly adults to *B. bassiana* and *M. anisopliae*. In my experience with anthomyiid flies, larvae have a significantly lower susceptibility to these fungi than adults (Chandler & Davidson, 2005; Davidson &

Chandler, 2005). These fungi are easy to mass produce, and a number of commercial products are already available based on them in the EU, USA, South America and Australia for the control of Lepidoptera (caterpillars), Homoptera (aphids, whiteflies), Coleoptera (weevils and beetles), and acridids (locusts and grasshoppers). However, when used as biopesticides against these pests, they do not reproduce and spread within host populations with great efficiency. However, there is evidence with houseflies and cabbage root fly that these fungi can be spread effectively from fly to fly (Barson *et al*., 1994; Meadows *et al*., 2000). It would also be worth testing the species of *Hirsutella* infecting shore flies against sciarid flies to look for cross activity and the ability to persist and spread within populations, as occurs evidently in shore flies.

Shore flies

1. *Hirsutella*. The basic principles outlined above for augmentation control with *F. sciarae* of sciarid flies also apply to *Hirsutella* and shore flies. It should be able to mass produce the fungus, using the systems developed for *H. thompsonii* in India. It is not known whether the mass production system yields infective spores or is confined to the production of fungal mycelium. Based on the observations of the fungal outbreak in shore flies, the aim would be for the fungus to persist, spread and replicate in the pest population to give control that lasts for an extended period.

2. Other fungal species. The same principles apply here as for sciarid flies: investigation of the susceptibility of shore flies to other fungal species is warranted, in particular *B. bassiana* (Stanghellini & El-Hamalawi, 2005).

Conservation control; exploiting natural epizootics of sciarid and shore flies

If natural fungal outbreaks could be predicted in advance, then growers would have the option to withhold insecticide applications for sciarid and shore fly control. Moreover, if the factors controlling the infection outbreaks could be understood, then they could be manipulated to make the natural outbreaks more effective. For example, Huang *et al*. (1992) demonstrated that fungal epizootics in mushroom sciarid fly populations were enhanced by water spraying to raise humidity levels. A highly effective system of forecasting natural outbreaks of the entomophthoralean fungus *Neozygites fresenii* in cotton aphids was developed by Steinkraus and co workers for cotton farmers in the south east USA (Hollingsworth *et al*., 1995; Steinkraus *et al.*, 1999). The outbreaks are regular, and appear synchronously in each region of occurrence, although they do not occur on all cotton plantations in a region. The fungus causes an 80% decline in cotton aphids within 5 days of

the start of an epizootic, and it can be predicted reliably up to 10 days in advance of the start of the epizootic. This is done by farmers sending samples of cotton leaves with aphids on them by courier to the Steinkraus lab at the University of Arkansas. The lab employs two part time technicians in the summer to analyse the samples, the system is offered across 8 states and is thought to save farmers around $30 million p.a. in insecticide sprays (Steinkraus, personal communication).

The research questions that would need to be addressed in order to evaluate conservation control as an option for sciarid and shore flies includes the following:

1. How widespread is the phenomenon?
2. What fungi are causing the outbreaks? Definitively identify the fungal species causing epizootics in sciarid and shore flies using a combination of conventional taxonomy (based on morphology) and molecular techniques (Note that a partial sequence for the small subunit ribosomal RNA gene is available for *F. sciarae* within GenBank, providing the basis of a molecular taxonomic comparison).
3. To what extent do fungal infections reduce pest populations? When do outbreaks occur, and how quickly do populations decline?
4. What insect life stages are infected?
5. What are the environmental conditions (particularly temperature and humidity) associated with fungal epizootics? Are they associated with particular plant species or types of potting medium or crop production practice?

<u>Inoculation control based on the Chinese model: Moving infected plants from nursery to nursery</u>

In China, Huang *et al*. (1992) introduced cadavers of the yellow legged fungus gnat naturally infected with *Erynia ithacensis* to mushroom houses in areas where epizootics had not been observed before. This strategy resulted in reasonably high (60%) levels of fly mortality. As stated above, this strategy has been used widely in China for the control of a range of insect pests with different species of entomopathogenic fungi. The same strategy could be considered for the UK, i.e. introducing *F. sciarae* and *Hirsutella* to nurseries where these fungi have not been found before for control of sciarid and shore flies respectively. If it proved to be successful in pilot studies, it could be an inexpensive form of biological control, although its success would depend on first addressing the same kind of questions outlined above for the conservation biocontrol strategy. A key issue is whether the fungi would need to be registered as biopesticides with the Pesticides Safety Directorate. I originally thought that registration would be required. However, PSD is developing their thinking in this area,

caused in part by the need to develop a strategy for the regulation of non endemic microbial control agents for classical control of invasive weed species, which is being investigated by colleagues at CABI Bioscience, Ascot UK. It may well be that an inoculation strategy for sciarid and shore fly control could be regulated as a plant health issue rather than as an issue for PSD. The arguments contributing to this include the following: (a) the regulation of biopesticides by PSD is done under the auspices of legislation covering plant protection products. The inoculation control strategy for sciarids and shore flies would not involve the use of a product; (b) there is no use of a mass produced agent, and hence exposure to operators is minimal; (c) the fungi already cause natural outbreaks, so exposure of operators and non target organisms following an inoculation strategy would be no greater than that which occurs already in other nurseries; (b) the entomopathogenic fungi concerned are not pathogens of vertebrates; (c) there is no evidence that *F. sciarae* or *Hirsutella* pose an infectious hazard to humans; (d) these fungi are endemic to the UK, and hence would not be construed as posing a threat as an invasive species.

Use of molecular tools for the identification of an entomopathogenic fungus causing natural epizootics in shore flies

DNA based techniques offer a method for the detection and identification of fungi with high sensitivity. They are particularly valuable for entomopathogenic fungi, as different species within a genus are often have very similar morphologies and thus are difficult to separate using conventional taxonomic methods. In this study, the intention was to use nucleotide sequence information of the rRNA gene repeat unit (ITS I, 5.8S gene, ITS II) using Polymerase Chain Reaction (PCR) amplification with universal fungal primers (White *et al*.,1990) followed by sequencing.

Fungus infected shore flies (*S. tenuicosta*) were obtained from Neil Helyer, Fargro, as described previously. Infected insects were placed on dampened filter paper within Petri dishes and maintained at 23ºC for up to 10 days in darkness until fungus structures had fully developed on insect cadavers. These included in particular synnemata (an erect aggregation of hyphal strands containing fungal conidia). Synnemata were excised from insect cadavers and cultured on Sabouraud's dextrose agar (SDA) at 23ºC for up to 6 weeks (the fungus grew very slowly in culture). Provisional observation of synnemata, together with colour of the mycelium and the slow growth on SDA placed the fungus within the genus *Hirsutella* (Ascomycota, Hypocreales). DNA was extracted from c. 100mg fresh mycelium grown on SDA using a Qiagen DNeasy plant genomic DNA miniprep kit (Qiagen, Crawley, UK). The concentration of DNA was measured using a Nanodrop ND-1000

spectrophotometer (Nanodrop Technologies, Wilmington, USA). Fungal DNA (1ng) was amplified by PCR using ITS primers 1, 3, 4, and 5 in the following combinations: (a) 1 and 4; (b) 3 and 4; (c) 1 and 4; (d) 5 and 4 (White *et al*.,1990). The thermocycler conditions were as follows: (a) Initial denaturing 94ºC for 2 min, annealing 55ºC 30s: (b) 35 cycles of extension 72ºC 30s denaturing 94ºC for 30 s, annealing 55ºC 30s; (c) final extension conditions of 72ºC 5 min. PCR products were then separated on a 1.5% agarose gel at $6V.cm^{-1}$ for 1h and visualised using ethidium bromide staining and exposure to UV light. PCR products were purified using a QIAquick PCR product purification kit (Qiagen, Crawley, UK) then a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Warrington, UK) was used together with ITS primers 1 and 3 (forward) and 4 and 5 (reverse) to generate forward and reverse products. Sequence data was produced by an ABI 3130xl genetic analyser (Applied Biosystems, Warrington UK).

The experiment was repeated three times. Unfortunately, however, yields of DNA were low and PCR yielded low amounts of product compared to other species of entomopathogenic fungi that are tested routinely using this method. This was an unexpected result. Poor sequence data was obtained and it was unsuitable as a source for querying DNA databases available on the internet (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) in order to provide a definitive identification of the fungus. Further research is required, therefore, to develop a method that yields high yield DNA suitable for good quality sequence information. The following areas need to be addressed: (1) possible use of liquid culture to provide fungal biomass for DNA extraction (since it is possible that fungal mycelium grown on SDA is resistant to physical disruption for DNA extraction); (2) modification of PCR conditions, possibly using conditions of lower stringency for improved primer binding.

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Fig 1 A, B, C : Fungus infected *Scatella* species (Neil Helyer)

A

B

C