Project title:	New approaches to microbial control of insect pests in protected crops and their interaction with waste-based growing media
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Project leader:	Dr Dave Chandler, Warwick HRI University of Warwick
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Key staff:	Dave Chandler, Warwick HRI Jude Bennison, ADAS John Buxton, ADAS Gill Prince, ADAS
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Key words:	Ornamental bedding and pot plants, herbs, pests, sciarid fly, shore fly, biological control, insect pathogenic fungi, growing media, composted green waste.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

The following are members of the Consortium for LINK project no HL0193

Bulrush Horticulture Ltd
Bordon Hill Nurseries
Double H Nurseries
Pinetops Nurseries
Brosters Nurseries
Koppert UK
Humber VHB
Fargro Ltd
Swedeponic Ltd

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GROWER SUMMARY

Headline

- Incorporating composted green waste into peat composts can result in significantly higher infestations of sciarid and shore flies.
- Natural fungal infections of sciarid fly larvae and shore fly adults could provide 'free' biological control.

Background and expected deliverables

Sciarid and shore flies are widespread and important pests and contaminants of containerised herb and ornamental crops. There is a requirement for new forms of control that are compatible with Integrated Pest Management (IPM) programmes that enable growers to reduce their reliance on chemical pesticides. Progressive growers prefer to use IPM based on a combination of cultural control, biological control and IPM-compatible insecticides (the latter on ornamentals only; there are no pesticides approved for the control of fly pests on herbs). However, the current IPM options do not always give sufficiently reliable control of sciarid or shore flies. Additional measures are needed therefore.

At present, most of herb and ornamental bedding and pot plant crops are grown in peat based compost. The UK has a Biodiversity Action Plan (BAP) target of 90% replacement of peat by alternatives by 2010. This would be done by incorporating materials into composts such as composted green waste, bark, wood fibre or coir. However, there is evidence from growers that some of these materials are associated with greater fly problems.

In recent years, there has been increasing awareness of natural infections of insect pathogenic fungi in sciarid and shore flies. These infections can result in high levels of ‘free’ natural pest control. If the naturally occurring fungal infections on sciarid and shore flies could be enhanced through conservation or augmentation, they could help reduce fly populations as part of an integrated approach to crop management and provide a novel form of biocontrol.

The project has two aims:

- Develop novel methods of biocontrol for sciarid and shore fly pests on pot herbs and ornamental bedding and pot plants;
- Investigate their interaction with composts containing recycled green material.

The expected deliverables from the project include the following:

- New understanding of the role of naturally occurring fungi in the population dynamics of insect pests that have adverse effects in a complex, integrated plant production system.
- New insights into the methods needed to enhance the effects of the fungi.
- New information on the effect of alternative growing media on the risk of sciarid and shore fly infestation, and on how to mitigate any adverse effects.

Summary of the project and main conclusions

Survey work on fungal infections with growers

Seventeen growers of protected bedding and pot plants or protected herbs were questioned about natural fungal infections of sciarid and shore flies on the crops produced. Fungal infections of sciarid flies were observed on 13 (=76%) of nurseries and fungal infection of shore fly was observed on just one nursery. 70% of growers who observed fungal infections on sciarid flies thought that they were contributing to sciarid fly control on their nurseries.

Fungal infected sciarid fly larvae were found on many sciarid-susceptible bedding and pot plant species including begonia, cyclamen, geranium, lupin, poinsettia, primula and a wide range of cuttings in propagation. On potted herbs, infected sciarid fly larvae were found particularly on mint but also on chives and parsley. Fungal infected shore fly adults were found particularly on thyme but also on parsley.

Infected sciarid fly larvae were found on a range of growing media from various suppliers, including 100% peat, and peat mixed with other materials i.e. composted green waste, wood fibre, bark, coir, perlite. Typical mixes contained 75-85% peat and 25-15% other materials. Peat was sourced from various countries including Estonia, Finland and Latvia. High substrate moisture and relative humidities were considered by most growers to be key factors favouring the incidence of fungal infection of sciarid flies.

Infections on sciarid flies occurred throughout the year, depending on the times various crops are produced, but were more prevalent in the autumn to spring period. Timing of infections was also affected by temperature and humidity e.g. on a nursery growing mint all year round, infected larvae were only seen in the autumn and winter when the glasshouse was cool and the peat remained very wet.

The extent of fungal infection in fly populations was partly dependent on the extent of sciarid fly infestation. Fungicide applications applied against pathogens within the crop did not appear to impact on fungal infections of sciarid flies on ornamental crops.

On site monitoring of natural fungal infections was then done in the glasshouses of six nurseries by project team members from ADAS, Fargro and the host growers. Results confirmed those of the initial grower survey. Over all the sites, the proportion of pots with infected sciarid fly larvae ranged from 1 to over 80%. Frequently all visible sciarid fly larvae were infected.

Isolation of pathogenic fungi from naturally infected sciarid and shore flies

When the project started, it was known that the natural infections of sciarid and shore flies were caused by two different types of insect pathogenic fungi. These fungi needed to be isolated from infected insects and grown on into a 'pure' culture of each fungus, free from contamination by other microorganisms.

The shore fly fungus was isolated from naturally infected adult shore flies associated with thyme plants. The fungus was grown on an agar based medium in Petri dishes. The fungus grew very slowly having an unusual structure, in which hair like projections emerge from the fungus in a Petri dish or from the shore fly adults in natural infections.

The sciarid fly fungus was difficult to isolate. Natural infection cycles were observed which indicated a complex life cycle. The fungus infects sciarid fly larvae which subsequently come up to the surface of the growing media at night and die. The fungus then produces 'primary' spores on the dead bodies overnight, which are actively discharged in order to spread the infection. If the spores hit a sciarid larva, they germinate on it and grow through the insect cuticle and kill it. If the spores do not contact a living sciarid fly larva, they still germinate but grow to produce a small 'secondary' spore that is actively discharged in search of a sciarid larva. The only way to isolate the fungus and grow it in a culture was by collecting spores discharged onto agar from 'fresh' larval cadavers (= dead insect bodies). . In most cases, they produced secondary spores which then died. However, in some cases they germinated to produce a fungus mycelium which could then be grown on in culture.

Fungal identification

Identification of the two fungal species infecting the sciarid and shore flies was important. Traditionally, the identification of fungi relied on visual characteristics, particularly of the spore bearing structures. However, fungi do not vary much in their appearance, meaning that species relationships based on visual characters have a low degree of certainty. While

visual diagnostic characters are still important tools, fungal identification relies increasingly on information collected at the DNA level, such as gene sequencing.

Isolates of the sciarid fungus were compared with a culture of *Furia sciarae* deposited in a herbarium run by the US Department of Agriculture. The two fungi had the same genetic sequence and hence we can be sure the fungus that kills sciarid flies in the UK is *Furia sciarae*.

The gene sequence for the fungus that infects shore flies, combined with an evaluation of its visual characteristics, indicated that it was not a species from the fungal genus *Hirsutella* as originally thought, but from the genus *Torrubiella*. This was highly unexpected because *Torrubiella* is associated with infections of insects in the tropics and SE Asia in particular. *Torrubiella* is the sexual phase of the fungus *Lecanicillium* (previously known as *Verticillium*). The latter is used as a biopesticide in the products Mycotal and Vertalec (Koppert BV) for the control of glasshouse whitefly and aphids respectively. *Lecanicillium* is naturally widespread in the UK. Although it is genetically the same as its sexual form *Torrubiella*, it looks completely different and we suspect that the two forms have very different natural histories.

Fungal bioassay

Laboratory bioassays are currently being developed to quantify the virulence of *Furia* to sciarid fly. The bioassay protocol, at its current state of development, is based on rearing larvae of a known age, and then treating them with *Furia* spores that are actively discharged from plugs of agar and keeping them under controlled conditions to monitor their survival.

Effect of temperature on sciarid fly development

A series of experiments has commenced to determine the effect of temperature on the development of the sciarid fly species *Bradysia difformis* (the most common sciarid fly species occurring in UK glasshouses). This information can then be used to forecast pest activity as a decision support tool in IPM. The development of sciarid larvae will be determined by collecting eggs from laboratory cultures and keeping them at different temperatures under controlled conditions. The dishes will be observed daily and the development of the larvae will be quantified. The results from these experiments will be used to identify the minimum temperature for development and a simple day degree model of sciarid development will be calculated.

Attractiveness of growing media as egg laying sites for sciarid and shore flies

Experiments to quantify the attractiveness to sciarid and shore flies of growing media containing composted green waste were completed. Two experiments were done, the first was in a research glasshouse at ADAS with sciarid and shore flies, the second was done at

a commercial herb nursery with a high population of sciarid flies. In the first experiment, a standard peat based compost was compared with a proprietary compost containing composted green waste and an organic compost. In the second experiment, a standard peat based compost was compared with the same compost supplemented with different amounts of composted green waste or wood fibre. The data from the first experiment indicated that organic compost was more attractive to shore flies for egg laying, while the second experiment indicated that 40% composted green waste was more favourable for the development of sciarid fly populations than the other growing media tested.

Financial benefits

- Natural fungal infections of sciarid fly larvae and shore fly adults can be exploited by growers.
- An additional, effective and reliable biological control solution to sciarid and shore fly problems will give financial benefits to growers and propagators of a wide range of protected crops, including protected ornamentals, herbs, leafy salads, hardy nursery stock; and cucumbers, tomatoes and peppers in propagation.
- The annual values of UK protected herbs and protected pot and bedding plants is estimated at £25 million and £193 million respectively (Defra Basic Horticultural Statistics, 2002/3).
- Crop losses or marketing problems due to sciarid or shore fly damage or contamination respectively probably cause at least 5% losses in herb and pot / bedding plant crop values annually. This represents a combined loss of £11 million per annum, although losses may increase with wider use of potting media based on composted green waste.
- At present there are no pesticides approved for the control of fly pests on herbs, or for shore fly control on any protected crop. Therefore growers will benefit by reducing the proportion of the crop that is unmarketable as a result of better biological pest control.

Action points for growers

- Look out for natural fungal infections of sciarid fly larvae or shore fly adults when monitoring for pests, particularly from autumn to spring. Infected scairid fly larvae are opaque white, and visible on the compost surface. Infected shore flies have long hairs sticking out of the body and can be found clinging to foliage or stems, or on the growing media surface or side of the pot.

- If help is required recognising infected flies, contact Jude Bennison, ADAS Boxworth (tel. 01954 268225, email jude.bennison@adas.co.uk) for further information.

- Leave infected dead sciarid fly larvae on the surface of the growing media so that the fungal infection can spread.

- If infected flies are present on your nursery and you have not already been contacted about the project, please contact any of us below:

  Jude Bennison, ADAS, tel. 01954 268225, email jude.bennison@adas.co.uk
  John Buxton, ADAS, tel. 01886 822106, email john.buxton@adas.co.uk

SCIENCE SECTION

Introduction

The aims of this project are: (1) to develop novel microbial control strategies for dipteran insect pests of containerised herb and ornamental crops; and (2) to investigate their interaction with growing media (composts) containing recycled green material. Pest problems associated with these materials are a barrier to replacing peat in growing media. The work focuses on the management of sciarid and shore flies, the larvae of which live in or on the compost, and are serious pests and contaminants in a wide range of protected crops including herbs.

The production of containerised herb and ornamental crops is a complex, highly integrated operation. Growers of these crops operate under tight financial margins, and are under significant pressure to make production more environmentally sustainable including replacing peat based growing media with green wastes and other non-peat materials. The UK has a Biodiversity Action Plan (BAP) target of 90% replacement of peat by alternatives by 2010. Approximately 4 million m^3 of growing media is used each year in the UK (Waller & Temple-Heald, 2003; Wallace *et al*., 2005). Most of this is based on peat. Green compost production is done by small enterprises which collectively produce about 300,000 m^3 of the appropriate grade material per annum.

Sciarid and shore flies are widespread and important pests and contaminants of containerised herb and ornamental crops. There is a requirement for new forms of control that are compatible with Integrated Pest Management (IPM) that enable growers to reduce their reliance on chemical pesticides. Progressive growers prefer to use IPM based on a combination of cultural control, biological control and IPM-compatible insecticides (the latter on ornamentals only; there are no pesticides approved for the control of fly pests on herbs). However, the current group of IPM tools do not give sufficiently reliable control of sciarid flies or shore flies. Additional measures are needed therefore.

Growers are under considerable pressure from the major retailers to reduce the amounts of chemical pesticides applied and to reduce the use of peat-based composts in favour of alternatives. However, practical experience shows that alternative materials are associated with greater populations of sciarid and shore flies.

In recent years, there has been increasing awareness of natural infections of insect pathogenic fungi in sciarid and shore flies. These infections can result in high levels of ‘free’ natural pest control. If the naturally occurring fungal infections on sciarid and shore flies

could be enhanced through conservation or augmentation, they could help reduce fly populations as part of an integrated approach to crop management.

The infections in sciarid flies and shore flies are caused by different species of fungi. The infection in sciarid fungi are caused by an entomophthoralean fungus, identified on the basis of morphological characteristics as *Furia* (= *Erynia*) *sciarae* (Zygomycota: Zygomycetes: Entomophthorales) (HDC project PC 277: Protected ornamentals: investigation of fungal pathogens infecting larvae of sciarid and shore flies). At the time of writing this project report, epizootics in shore flies were thought to be caused by a fungus identified as a species of the genus *Hirsutella* (Ascomycota: Sordariomycetes: Hypocreales).

Objectives

The project has 5 Objectives:

O1 Conservation biological control: Determine the extent to which natural fungal outbreaks occur in sciarid (*Bradysia difformis*) and shore flies (*Scatella tenuicosta*) on nurseries and quantify key parameters associated with epizootics.

O2 Quantify fungal-insect population dynamics for conservation and inoculation bio-control.

O3 Inundation biological control: Quantify susceptibility of sciarid and shore flies to selected isolates of entomopathogenic fungi.

O4 Integrated Crop Management: Interactions of sciarid and shore flies with peat-based vs peat alternative growing media and other components of ICM.

O5 Choose biological control strategies with best potential.

O6 Knowledge exchange and technology transfer with industry.

Progress

Objective 1: Conservation biological control: determine the extent to which natural fungal outbreaks occur in sciarid and shore flies on nurseries.

O1/1 Telephone survey (ADAS)

Materials and Methods

Twelve growers of protected bedding and pot plants and five growers of protected herbs were contacted to ask if they would take part in a telephone survey. Nine of the nurseries were selected as known sites for infected sciarid or shore flies and a further eight nurseries were randomly selected. A survey form (see Appendix I) was then sent to the growers. The form introduced the aims of the project and had photographs of healthy sciarid fly larvae and shore fly adults, and of those infected by *Furia sciarae* and *Hirsutella* sp. respectively. The form listed questions designed to determine preliminary information on the following:

- The extent of natural fungal infections of sciarid and shore flies
- The crops on which outbreaks occur
- The timings of outbreaks
- The relationship between outbreaks and production practices e.g. compost types and moistures, temperatures, pesticide use.
- Control measures used for sciarid and shore flies.

All 17 growers agreed to take part in the survey, and were subsequently telephoned or visited to discuss each question.

Results and Discussion

Sites with infected flies

Sciarid fly larvae infected with *F. sciarae* were found at 13 sites, 12 of these having a geographical spread over England and one being in Scotland. Of the 13 sites, three grew pot herbs and 10 nurseries grew bedding and pot plants. Fungal infected shore flies were found at only one site, on pot herbs in southern England.

Plant species with infected flies

Sciarid fly larvae infected with *F. sciarae* were found on many sciarid-susceptible bedding and pot plant species including begonia, cyclamen, geranium, lupin, poinsettia, primula and a wide range of cuttings in propagation. On potted herbs, infected sciarid fly larvae were found particularly on mint but also on chives and parsley.

Fungal infected shore fly adults were found particularly on thyme but also on parsley.

Compost types

Infected sciarid fly larvae were found on a range of composts from various suppliers, including 100% peat, and peat mixed with other materials i.e. green waste, wood fibre, bark, coir, perlite. Typical mixes contained 75-85% peat and 15-25% other materials. Peat was sourced from various countries including Estonia, Finland and Latvia.

Compost moisture

High compost moisture and relative humidities were considered by most growers to be key factors in favouring the incidence of *F. sciarae*. However, wet compost also favours sciarid fly egg laying and larval development, so there will be more available host insects for the fungus in wet, humid conditions. Infected sciarid fly larvae were often seen on pure peat composts only when very wet; peat is difficult to keep at a constant moisture content unless saturated. There were very few reports of seeing infected larvae on dry composts.

Timing of fungal infections

Infected sciarid fly larvae were seen at various times of the year, depending on the times various crops are produced, e.g:

- On poinsettia, infections were seen in the late summer / early autumn during the first few weeks of the crop when the plants are most susceptible to sciarid flies.
- On primula, infections were seen in late autumn to early spring.
- On begonias which are grown all year round, infections were only seen during September and October, on the oldest plants, which were irrigated more than younger plants and thus had higher numbers of sciarid fly hosts.
- On one nursery growing mint, infected sciarid fly larvae (in high numbers) were seen only in the autumn and winter period, when glasshouse temperatures were cool and the peat remained very wet on the surface.
- Infected larvae were usually not seen until 4-6 weeks after potting, although on potted mint they were seen as early as two weeks after potting. This is relevant to potential sciarid fly control by the pathogen on pot herbs, as the summer production cycle is only five weeks.

Extent of infection in the crops

The extent of *F. sciarae* infection in the crops was partly dependent on the extent of sciarid fly infestation. For example, on one nursery growing poinsettias, infected larvae were only seen in compost where the plants were infected with root disease and thus had high numbers of sciarid fly larvae feeding on the rotting root tissue. The proportion of pots with infected larvae at the 13 sites ranged from 1-5% to over 80% and frequently all visible sciarid larvae were infected. Nine of the 13 growers who had seen infected larvae considered that *F. sciarae* was contributing to sciarid fly control.

The severity of sciarid fly infestations (and thus the extent of infection with *F. sciarae*) was also affected by control measures used for sciarid fly control. These included the following biological control agents or pesticides (the pesticides were only used on the ornamental crops and not on the pot herbs):

- *Hypoaspis* spp. (predatory mites)
- *Steinernema feltiae* (insect-pathogenic nematodes)
- *Atheta coriaria* (predatory beetles)
- imidacloprid (e.g. Intercept drench)
- teflubenzuron (Nemolt)

Effect of fungicides

Very few fungicide applications were used on the pot herbs, only sulphur for powdery mildew control. However, on the ornamental crops, *F. sciarae* seemed to survive a range of

fungicides, applied both as high volume sprays and as compost drenches. Fungicide programmes on ornamental plants with infected sciarid larvae included the following active ingredients (example product names given in brackets):

- azoxystrobin (Amistar)
- benthiavalicarb-isopropyl + mancozeb (Valbon)
- boscalid + pyraclostrobin (Signum)
- carbendazim (Delsene 50 Flo)
- chlorothalonil (Bravo/Repulse)
- copper ammonium carbonate (Croptex Fungex)
- dimethomorph + mancozeb (Invader)
- fenarimol (Rubigan)
- fosetyl-aluminium (Aliette)
- iprodione (Rovral)
- mancozeb + metalaxyl-M (Fubol Gold)
- mepanipyrim (Frupica)
- metalaxyl-M (Subdue)
- myclobutanil (Systhane 20 EW)
- prochloraz (Octave)
- propamocarb hydrochloride (Filex, Proplant)
- pyrimethanil (Scala)
- thiram (Thianosan DG)

01/2 Detailed nursery surveys (ADAS, Fargro and host growers)

Aim

The aim was to monitor selected nurseries on sequential dates in order to determine timing and development of fungal outbreaks.

Sites

The following nurseries were selected for the surveys, where natural outbreaks of either *Furia sciarae* or *Hirsutella* had been confirmed in 1.1 above.

- Double H Nursery, Hants (sciarid fly on begonia)
- Orchard Nursery (part of the Bordon Hill Nurseries group) , Warwicks (sciarid fly on cyclamen)
- Allensmore Nursery, Hereford (sciarid fly on primula)
- Swedeponic, Lincs (sciarid fly on pot herbs)
- Mint propagator, Lincs (sciarid fly on potted mint)
- Humber VHB, West Sussex (shore fly on pot herbs)

Materials and Methods

One glasshouse on each nursery was monitored, either by ADAS, Fargro Ltd. or by the host grower if a member of the project consortium. Dates and frequency of visits depended on the crop grown and the time of year fungal infections occurred on each nursery. On each visit, 50 pots were sampled (five pots at each of 10 sampling points across the glasshouse), unless the host grower was already sampling more plants as part of the nursery pest monitoring system. The sampled pots were taken from pots of the same age in a seasonal crop e.g. cyclamen, or plants of sequential ages in crops grown in cycles e.g. begonia and pot herbs.

Assessments

- Age of sampled plants
- Presence or absence of infected shore fly adults on foliage, pot or compost
- Presence or absence of infected sciarid fly larvae on surface of compost

Results and Discussion

Double H Nursery
Crop: Begonia
Compost: 80% peat, 20% perlite/woodfibre/coir

Monitoring of sciarid fly larvae infected with *Furia sciarae* was done weekly at 171 sampling points by nursery staff between weeks 24 and 51 (early June to late December) as part of the nursery pest monitoring system. Additional monitoring was done on 16 October 2008 at 50 sampling points by Jude Bennison, ADAS.

Healthy and infected sciarid fly larvae were first recorded by nursery staff in early October. Peak numbers of pots with healthy and infected larvae were found one week after initial detection, in 30% and 7% of the pots respectively. Thereafter, numbers of pots with healthy and infected larvae declined, probably due to additional releases of the predatory mite *Hypoaspis miles* by the grower. Healthy larvae were found in 7% of pots in late December, but no infected larvae were found after early November.

Healthy and infected sciarid fly larvae were found mostly on the older begonia plants, 10 weeks after potting, just before the marketing stage. This was probably due to the compost in the older plants being wetter, due to increased watering of the larger plants and to the increased crop canopy shading the compost.

On 16 October, no infected larvae were found by ADAS on 50 sampled pots, but were found on 2% of the 171 pots (one pot) in the same week by the grower. This demonstrated that at very low incidences of infection, 50 pots were insufficient to detect infected larvae.

Orchard Nursery
Crop: mini-cyclamen
Compost: 80% peat, 20% bark

Monitoring of sciarid fly larvae infected with *F. sciarae* was done monthly between 16 October and 9 December 2008 by John Buxton, ADAS. The glasshouse was kept at a minimum 10°C. Infected larvae were seen in more pots (26%) on 16 October than on 12 November (2% pots) and on 9 December (8% pots). No control methods were applied for sciarid fly control. Several fungicides were applied to the crop as high volume sprays for botrytis control during August and September. These were pyrimethanil (Scala), boscalid + pyraclostrobin (Signum) and mepanipyrim (Frupica).

Allensmore
Crop: primula
Compost: 60% peat, 40% bark

Monitoring of sciarid fly larvae infected with *F. sciarae* was done monthly between 16 October and 8 January 2009 by John Buxton, ADAS. The glasshouse was kept cool, with frost protection only. Compost teas were applied to the crop but no conventional fungicides. Infected sciarid fly larvae were seen on all dates: on 14% pots on 16 October, 24% pots on 12 November, 26% pots on 9 December and 36% pots on 8 January, when the crop was being sold. The infection did not seem to be inhibited by cold temperatures in January.

Swedeponic
Crops: potted mint and chives
Mint compost: 100% peat
Chives compost: 80% peat, 20% other components including some green waste

Hypoaspis miles were applied to the crops for sciarid fly control. No pesticides or fungicides were applied to the crops and the glasshouse temperature was maintained at 19°C. Monitoring of sciarid fly larvae infected with *F. sciarae* was done monthly between December 2008 and February 2009, by Kerry Maulden, ADAS and by Steve Helm, the grower. Infected larvae were not detected on the chives on any date and were only detected on the mint on 11 December. On this date, no infected larvae were found on the 50 monitored pots, but were found on a low proportion of additional pots when more were inspected. These pots

had only been on the nursery for two weeks and the grower confirmed that the pots had been received from the propagator with infected larvae. Therefore the mint propagator was included as an additional survey site.

Mint propagator
Crop: potted garden mint
Compost: 100% peat

Monitoring of sciarid fly larvae infected with *F. sciarae* was done in December 2008 and February 2009, by Kerry Maulden, ADAS. Infected larvae were only detected on 11 December, in 28% pots. More pots with infected larvae were seen on the oldest, 6-week old plants, which were ready to be sent to production nurseries including Swedeponic. However, infected larvae were also seen on pots from the 2-week old stage onwards. The crop was grown cool (minimum 12°C) in very wet peat and the grower commented that infections were only seen in the autumn when the compost surface tended to stay very damp.

Humber VHB
Crop: thyme
Compost: 85% peat, 15% other components, not including green waste

Monitoring of shore fly adults infected with '*Hirsutella'* was done by either Neil Helyer, Fargro Ltd. or Simon Budge, the grower, every two weeks from September 2008 until the date of this report. Monitoring will continue until spring 2010. In October 2008, infected shore flies were easily found, but thereafter were very difficult to find. Thus monitoring was done on at least 200 pots from February 2009 onwards. No infected flies have been detected since January 2009.

Conclusions from telephone and nursery surveys in Objective 1

- *S*ciarid fly larvae infected with *F. sciariae* occurred on 13 of the 17 nurseries contacted.
- *F. sciarae* occurred on three of the five pot herb sites and 10 of the 12 bedding and pot plant sites contacted.
- Shore fly adults infected with '*Hirsutella'* sp. were found at only one site, on pot herbs, mainly on thyme but also on parsley. The fungus seems to occur mainly in the autumn/winter period.
- Sciarid fly larvae infected with *F. sciarae* occurred on the compost in which a wide range of plant species were growing, including begonia, cyclamen, poinsettia, primula, chives, mint and parsley.

- Sciarid fly larvae infected with *F. sciarae* occurred in a range of commercial composts including pure peat and peat mixed with other materials i.e. green waste, wood fibre, bark, coir or perlite.
- High compost moisture was considered to be a key factor favouring the incidence of *F. sciarae*.
- High compost moisture also favours sciarid fly egg laying and completion of the life cycle thus wet, humid conditions will lead to higher numbers of host insects for infection.
- Sciarid fly larvae infected with *F. sciarae* occurred at various times of the year, according to crop production times, e.g. in late summer / early autumn on poinsettia, late autumn to early spring on primula. Timing of infections was also affected by temperature and humidity e.g. on a nursery growing mint all year round, infected larvae were only seen in autumn and winter when the glasshouse was cool and the peat remained very wet. However, the fungus seems to occur at a wide range of temperatures, from very cold conditions in glasshouses with only frost protection in winter (e.g. on primula), to summer glasshouse temperatures (e.g. on begonia and poinsettia).
- Although many growers commented that infected larvae were not usually seen until 4-6 weeks after potting, on pot herbs they were seen as early as two weeks after potting.
- The proportion of pots with infected sciarid fly larvae ranged from 1-5% to over 80%. Frequently all visible sciarid fly larvae were infected.
- Nine of the 13 growers who had seen infected larvae considered that *F. sciarae* had contributed to sciarid fly control.
- The extent of sciarid larval infection with *F. sciarae* was affected by the biological or chemical control measures being used against the pest.
- *F. sciarae* seemed to survive fungicide programmes commonly used for disease control on protected ornamentals.

O1/3 Isolation of pathogenic fungi from naturally infect sciarid and shore flies (WHRI)

Note: Because of the nature of this type of work, it is being presented as a combined section that includes Materials and Methods, Results and Discussion

When the project started, it was known that the natural infections of sciarid fly (*Bradysia difformis*) and shore fly (*Scatella tenuicosta*) were caused by two different types of insect pathogenic fungi. Sciarid fly infections were caused by a fungus identified, on the basis of spore morphology, as *Furia sciarae*. Shore fly infections were cause by a fungus identified –

again on the basis of spore morphology – as a species from the genus *Hirsutella*. These fungi needed to be isolated from infected insects and grown in axenic culture (i.e. a 'pure' culture of each fungus, free from contamination by other microorganisms). This will provide material for species identification based on gene sequencing, and also act as a source of fresh material to infect insects in laboratory and glasshouse experiments.

'*Hirsutella*'. Analysis of nucleotide sequence data showed that the fungus identified as '*Hirsutella*' on the basis of spore morphology was in fact a different species (see O1/4 below). The fungus was only found on one location, on a herb nursery on the south coast, on shore flies associated with thyme plants. The fungus was isolated into culture successfully using sections of synnemata (spore bearing structures composed of an aggregation of fungal hyphae, about 0.5 mm in diameter and 5mm in length and looking very much like a piece of human hair. Shore flies killed by '*Hirsutella*' typically have one or two synnemata growing vertically from the thorax). Synnemata were excised from infected cadavers and placed on Sabouraud dextrose agar (SDA). Fungal mycelium grew slowly on the agar out from the synnemata, typically reaching a colony diameter of c. 2 cm in 4 weeks at 25ºC. Two different types of culture morphology were observed: some culture produced more synnemata from a mat of mycelium on the agar, while others consisted just of a grey mycelial mat, velvety to downy in texture. Spores were harvested from these cultures and placed into storage at -80 ºC but subsequent recovery from the frozen material was poor. Cultures are currently being stored on SDA slopes at 5 ºC with the aim of developing a better method of long term storage in the future, such as freeze drying mycelium.

Furia sciarae. This fungus is a member of the order Entomophthorales (phylum Zygomycota). Many species of entomophthoralean fungi have evolved specific eco-morphological adaptations to the life cycles of their insect hosts. They can also have very particular nutrient requirements for growth and some species cannot be grown at all in the absence of a host insect (i.e. they are physiologically obligate pathogens). In the case of *F. sciarae*, we established by observation of naturally infected sciarid larvae that the fungus infects its hosts using actively discharged 'ballistospores' fired from infected cadavers. The spores are released into the air for dispersal and to contact and infect new host individuals. This mode of dispersal and infection is typical of many entomophthoralean species. A range of methods was investigated for isolating the fungus into agar based culture. This kind of investigation is very time consuming (it took us 9 months continuous experimentation before finally isolating the fungus into culture) and it does not provide much at all in the way of quantitative data. Moreover, there are no reports of standard methods for *Furia sciarae* isolation in the literature. For the purposes of this report we summarise the main isolation methods investigated here and the results obtained. The methods used were adapted from general techniques used with other entomophthoralean fungi (Papierok & Hajek, 1997).

- Result 1: Isolation required larval cadavers collected <24h post death. *Furia* isolations were attempted from infected sciarid larvae received from 9 different nurseries (7 pot and bedding, 2 herbs) including the following: Pentland Plants, Bordon Hill (Welford), Double H, Yoder Toddington, Coletta & Tyson, Brosters of Peover, Swedeponic, and Allensmore. Most of the samples were received as infected cadavers on potted plants sent by the grower. However, we also made regular collecting trips to Bordon Hill Nurseries at Welford, where infected sciarid larvae were collected on site or plants with large numbers of infected cadavers were taken back to the laboratory. Through observation of infected material collected at Bordon Hill, we concluded that the discharge of spores on cadavers occurred over a short time window, normally 24 – 48 h. Spore production occurred mostly at night: when we cleared pots of dead larvae at the end of the day, we observed 'new' sporulating cadavers infected by *Furia* the next morning. Thus fungus-infected larvae came up to the surface of the compost at night, died, and then *Furia* spores were produced on them and actively discharged in the space of a single night. Fungal infected sciarid larvae sent to us from nurseries tended to produce very few spores or none at all, indicating that this material was > 48h old and not a good choice for fungal isolation (see Result 2). Towards the end of this part of the project, we therefore concentrated on isolating the fungus from cadavers collected from Bordon Hill in the morning, where we could guarantee that the cadavers were 'fresh' and would yield a good supply of spores.
- Result 2: the fungus could not be isolated from mycelium that had colonised sciarid larvae. We concentrated on culturing the fungus from spores, as we found that it was not possible to culture it from fungal mycelium growing in infected larvae. When an insect is killed by a fungus, you can often isolate the fungus by simply placing the cadaver on an agar based medium and the fungal mycelium in the insect will grow out onto the medium. However, when infected larvae were placed on plates of agar-based medium, there were high levels of contamination by bacteria and other species of fungi. This is not surprising given that the larvae live and feed in compost. We investigated surface sterilising infected cadavers by washing them in ethanol / sodium hypochlorite solution, but the cadavers were highly fragile. In all cases they disintegrated and hence any *Furia* mycelium within the cadavers would have been killed therefore. Furthermore, there is published evidence from other species of entomophthorlaean fungi that the mycelium dies soon after spores have been actively discharged.
- Result 3: the fungus could be cultured from captured ballistospores and grown on YSMA medium. It was possible to grow the fungus on agar-based media from ballistospores captured from sporulating larval cadavers using a 'spore showering' technique. Larval cadavers that had died overnight were placed on the lids of Petri

dishes containing agar based media. Spores were then released and showered upwards onto the surface of the medium. 'Showering down' (i.e. holding cadavers above the agar based medium) resulted in large amounts of bacterial and fungal contamination caused by material dropping from cadavers. Each cadaver was placed on a tower made of small pieces of damp filter paper, so that they were c. 5mm from the agar surface. This ensured that large numbers of spores were captured. Large numbers of spores (c. 10^3) were required in order to get them to germinate myceliogenically. In most cases, the spores would germinate and grow to produce additional small, secondary spores (one on each primary spore) that were actively released from the conidiophores but which did not, to the best of our knowledge, then germinate and form mycelium. However, in some cases, the ballistospores germinated to form fungal hyphae, which then coalesced to form mycelium. We tried a number of methods to increase the success rate of myceliogenic germination, including covering spores with cover slips or placing them in liquid medium (since secondary spore production is thought to be stimulated when a primary spore does not contact a susceptible host but instead lands on an non-host substrate in air). However these did not guarantee myceliogenic germination. The fungus would not germinate on SDA, but it would germinate on a 'YSMA' medium comprising of sterilised, yeast extract, sucrose cow's milk and egg yolk (albumin).

- We have now successfully isolated a number of cultures of *Furia* from Bordon Hill which are growing on YSMA medium. However, isolation remains something of a hit and miss affair. We also obtained a slope taken from the only registered culture of *F. sciarae*, provided to us by Dr Richard Humber, curator of the USDA ARSEF (Agriculture Research Service collection of Entomopathogenic Fungi) culture collection at Cornell University, New York. This strain (ARSEF 1870) was isolated from an adult sciarid on soil. This is being used as a reference for molecular phylogeny studies and lab experiments.

Other fungal species. We also have seven other isolates of two species of entomopathogenic fungi obtained from sciarid and shore flies: *Lecanicillium* sp. (1 isolate) and *Beauveria bassiana* (6 isolates) (Table 1). These were isolated from infected adult flies sent to us by colleagues over the last 16 years. Both *Lecanicillium* and *Beauveria* are ascomycete fungi. They are not reported in the scientific literature to cause natural epizootics (= outbreaks) in sciarid fly or shore fly populations, and so they do not have the potential to provide 'free' biocontrol like *Furia* or '*Hirsutella*'. However, isolates of these species are being used as commercial biopesticides against other insect pests. Later in the project, selected isolates of *B. bassiana* and *Lecanicillium* will be evaluated as potential biopesticides against sciarid and shore flies.

Table 1. Isolates of *Lecanicillium* sp and *Beauveria bassiana* obtained from naturally infected adult sciarids and shore flies

Isolate no	Fungal species	Host	Source
399.93	*Lecanicillium sp.*	*Scatella tenuicosta*	John Buxton (ADAS)
461.99	*Beauveria bassiana*	*Bradysia difformis*	John Buxton
462.99	*Beauveria bassiana*	*Scatella tenuicosta*	Rob Jacobson (Crop Protection Consultant)
514.00	*Beauveria bassiana*	*Scatella tenuicosta*	Rob Jacobson
1341.05	*Beauveria bassiana*	*Scatella tenuicosta*	Jude Bennison (ADAS)
1730.08	*Beauveria bassiana*	*Bradysia difformis*	Russ Woodcock
1731.08	*Beauveria bassiana*	*Scatella tenuicosta*	Rob Jacobson

O1/4 Fungal molecular phylogeny (WHRI)

Materials and Methods

It is important to be able to identify the species of the two fungi naturally infecting sciarid and shore flies. Published information about other, closely related species of fungi – which we assume are likely to have similar a biology - can then be used to inform the design, execution and analysis of experiments in this project. Traditionally, the taxonomy and phylogeny of fungi relied on morphological characteristics, particularly of the spore bearing structures. However, fungi do not vary much in their morphology, meaning that many of the species relationships based on morphology have a low degree of certainty. While morphology is still an important tool, fungal taxonomy and phylogeny relies increasingly on information at the DNA level which provides phylogenetically meaningful information with a high degree of resolution. This includes nucleotide sequence data, single nucleotide polymorphisms (SNPs) and polymorphisms in hypervariable noncoding regions such as microsatellites. In this study, nucleotide sequence information of the rRNA gene repeat unit (Internal Transcribed Spacer (ITS) I, 5.8S gene, ITS II) was obtained using PCR amplification with universal fungal primers (White *et al*.,1990) followed by sequencing. This method is used commonly in fungal phylogeny studies as it tends to give good resolution at the species level (Sung *et al,* 2007). Sequence data was generated for the two different fungi isolated from infected sciarid and shore flies and provisionally identified before the start of the project, on the basis of spore morphology, as *Furia sciarae* (sciarid flies) and *Hirsutella* sp. (shore flies).

DNA was extracted from fungal mycelium harvested from axenic cultures grown on agar based media. Extraction was done using the Qiagen 'DNeasy' plant mini kit (Qiagen, Crawley, UK) and quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop

Technologies, Wilmington, USA). Fungal DNA (1ng) was amplified by PCR using ITS primers 1 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 Gel Red 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 4 to generate forward and reverse products. Sequence data was produced by an ABI 3130xl genetic analyser (Applied Biosystems, Warrington UK). These sequences were compared and consensus versions were constructed. A multiple sequence alignment programme (MegAlign, DNASTAR Inc., Madison, USA) was used to compare these sequences and others downloaded from DNA databases available on the internet (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/). Unrooted phylogenetic trees (neighbour joining) were constructed in *MEGA* version 4 (Tamura et al, 2007).

Results and Discussion

Furia sciarae. Ribosomal RNA sequence data were generated for two fungal isolates: (1) one isolate of fungus cultured from an infected sciarid larva at Bordon Hill nurseries, provisionally identified by us as *F. sciarae* on the basis of spore morphology; and (2) the fungal culture obtained from the ARSEF collection and identified by Dr R. Humber of the USDA as *F. sciarae* on the basis of morphology. There is no rDNA sequence information for *F. sciarae* published on the NCBI database portal to compare with our two isolates from sciarid flies. However, the two isolates showed high sequence identity. In an unrooted phylogenetic tree, the two isolates formed a deep rooted cluster separate from five other species of entomophthoralean fungi (*Zoophthora radicans*, *Pandora kondoiensis*, *Pandora neoaphidis*, *Conidiobolus coronatus*, and *Entomopthora muscae*; sequence data taken from NCBI) and two species of ascomycete entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*; sequence data taken from NCBI). This gives us confidence that the fungus that we have isolated and cultured from naturally infected sciarid fly larvae in the UK is a member of the species *Furia sciarae*. Given that *F. sciarae* has not been widely studied, there is a requirement to generate sequence data from other taxonomic molecular markers in order to more fully resolve its phylogenetic relationships with other entomopathogenic fungi, particularly the entomophthoraleans, as our data suggest that *Furia* is phylogenetically distinct from them. This will have to include a fungal species to be used as a suitable outgroup to calculate rooted phylogenetic trees.

'*Hirsutella*'. Ribosomal RNA sequence data were generated for two isolates of fungi obtained from naturally infected shore flies at the nursery on the south coast. An unrooted phylogenetic tree was constructed based on this data plus RNA sequence data on ascomycete fungi submitted to NCBI from reliable sources (i.e. academics specialising in fungal phylogeny). This included eight species of *Hirsutella* (*H. unicata*, *H. kirchneri*, *H. sinensis*, *H. rhossiliensis*. *H. rostrata*, *H. proturicola*, *H. thompsonii*, *H. guyana*) plus other ascomycete entomopathogenic fungi: *Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium longisporum*, *Lecanicillium muscarium*, and *Torrubiella confragosa*. Unexpectedly, our '*Hirsutella*' fungus formed a deep clade with *L. longisporum*, *L. muscarium*, and *T. confragosa* (Figure 1). It did not group with species of *Hirsutella*, which formed a distinct cluster. All nucleotide sequence profiles obtained were 'clean' and the same sequence was obtained from fungi isolated from different shore flies collected in 2008 and 2009 from the same nursery. The fungal cultures used were checked regularly for contamination by other fungi. Therefore, we can be confident that the sequence data represent the rRNA sequence from the shore fly fungus and are not a contaminant of *Lecanicillium* acquired in the lab or that grew as a hyperparasite on shore flies.

As explained above, we originally classified the shore fly fungus as a species of *Hirsutella*, based on spore morphology. However we are very confident now that it is not a member of the genus *Hirsutella*, but instead it is a species of *Lecanicillium* / *Torrubiella*. The ascomycete fungi exist in two different forms: a sexually reproducing phase (termed a teleomorph) and an asexual phase (= anamorph). These two forms are usually completely different in terms of their morphology, and prior to the use of DNA data for phylogenetic studies, they used to be considered as separate species. The teleomorph / anamorph connections of the ascomycete entomopathogenic fungi are still being resolved and codified. Eventually, anamorphs and teleomorphs of the same species will be given a single species name. But at present, it is common for anamorphs and teleomorphs from the same phylogenetic species to have different species names. In the case of *Lecanicillium* / *Torrubiella*, the anamorphs are labelled as *Lecanicillium* and their teleomorph forms are called *Torrubiella*.

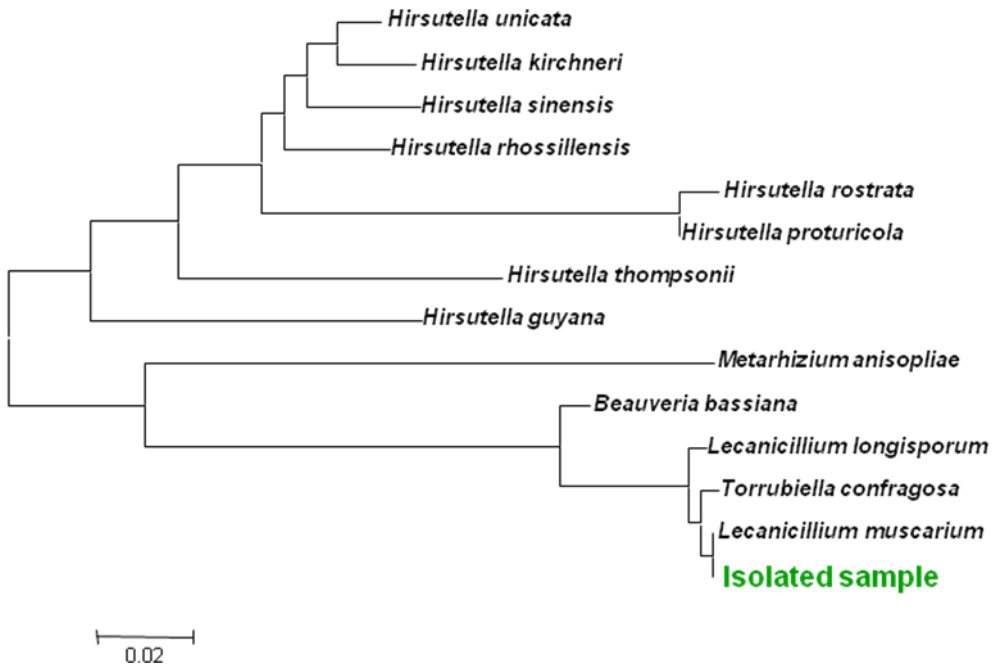

Figure 1. Unrooted phylogenetic tree showing the relationship of the fungus provisionally indentified as '*Hirsutella*' on the basis of spore morphology and obtained from shore flies ('Isolated sample' in this diagram) with other ascomycete fungi.

Torrubiella / *Lecanicillium* is not normally associated with causing infection in Diptera. As a rule, the teleomorphic entomopathogenic fungi are confined to the tropics, and their centre of origin is thought to be SE Asia. There are no reports of *Torrubiella* from temperate Europe, although it is common in Japan, tropical China, Thailand etc. Therefore, finding it in the UK is highly unexpected. We should add that the anamorphic fungus *Hirsutella*, which resembles the shore fly fungus morphologically, does occur naturally in temperate regions.

Lecanicillium species are also common in temperate regions. But *Lecanicillium* has no morphological similarity at all to the shore fly fungus. *Lecanicillium* does not form synnemata, and produces masses of sticky conidia on conidiophores in a characteristic whorl or 'stair rod' configuration on single hyphae, and the mycelium grows reasonably quickly; whereas the shore fly fungus produces synnemata that contain conidiophores along its length, and it grows very slowly. Morphologically, the shore fly fungus bears some resemblance to *Torrubiella dimorpha* (http://web.kyoto-inet.or.jp/people/ignatius/pages6/T_d.htm) but does not look like other *Torrubiella* species.

In conclusion, we are confident that the shore fly fungus is a species within the genus *Lecanicillium* / *Torrubiella*. At this stage, we think that it is a teleomorphic (sexual) form. This is a highly unexpected finding. Further work is required, specifically to obtain sequence data

from additional loci, and to compare sequence data and morphological characteristics with a wide range of *Torrubiella* fungi.

Other fungal species. We also obtained rRNA sequence data for the isolates of *Beauveria bassiana* in our culture collection from sciarid and shore flies (Table 1). The taxonomic classification of *Beauveria bassiana* is currently undergoing revision based on DNA data. It consists of two deep rooted clades that probably constitute two different species, although they are indistinguishable morphologically. At present, researchers are referring to these as 'clade A' and 'clade B'. In comparison to reference sequences for *B. bassiana* on NCBI, the *B. bassiana isolates* from sciarid and shore flies in our culture collection clustered into *B. bassiana* 'clade A'. This clade includes the isolates of *B. bassiana* that are used in the commercial biopesticides Naturalis (Intrachem (It); and which has just been approved for use in the UK) and BotaniGard (Laverlam (USA); which is currently undergoing UK approval).

Isolation from growing media. Experiments were done to investigate whether *Furia sciarae* could be detected in samples of growing media. 100% peat samples from which infected sciarids were observed was collected and samples homogenized in a Mini Beadbeater-8 cell disrupter for 3 minutes prior to DNA extraction using the FastDNA ®SPIN kit for soil. DNA samples were amplified with universal fungal primers in a PCR reaction. The thermocycling conditions consisted of an initial denaturation of 95^{O}C for 3 min followed by 30 cycles of 95^{O}c for 30 s, 55^{O}C for 60 s, 72^{O}C for 60 s. The final extension was at 72^{O}C for 10 min. The PCR products were cloned using the QIAGEN PCR cloning plus kit. Plasmid DNA from 41 colonies underwent Templiphi™ amplification. Sequencing was carried out using the vector targeted PCR primers M13 F and M13 R on an automated sequencer, ABI PRISM1 3130xl Genetic Analyzer, using the BigDye® version 3.1 sequencing chemistry. Sequences were assembled and trimmed to the primer sites using DNA star and compared to sequences at NCBI (http://www.ncbi.nlm.nih.gov/). The resultant fungal clone library consisted of uncultured zygomycetes (48.6%) and ascomycetes (51.4%) namely *Gibberella* sp. (10.9%) and *Gibberella moniliformis* (40.5%). *Furia sciarae* was not detected by this method: it is probably present in the substrate but in insufficient numbers to be detected against the background of other fungi identified in the clone library. Hence we conclude that a more specific method will be needed to detect it reliably within media.

Objective 2: Quantify fungal-insect population dynamics for conservation and inoculation bio-control.

O2/1 Fungal bioassay (WHRI)

Sciarid bioassays are being developed to quantify the virulence of entomopathogenic fungi to sciarid fly (*Bradysia difformis*). The laboratory bioassay protocol, at its current state of development, is as follows: selected larval instars are collected from fixed age cultures, and placed on damp filter paper within a Petri dish. The larvae are inoculated with primary conidia from agar plugs using a spore showering technique. Treated insects will be transferred to pots, 30 larvae per pot, containing compost and soya flour. The bioassays will be done at 25^{o}C and high relative humidity with a light:dark regime of 16;8H and examined daily for 21 days. Dead larvae will be removed and placed on moistened filter paper within Petri dishes and incubated at 25^{o}C to examine for the presence of *Furia.*

Objective 4: Integrated Crop Management: Interactions of sciarid and shore flies with peat and peat-alternative growing media and other components of IPM

O4/1 Selection of peat-alternative growing media (project consortium)

The growing media to be used for the experiments in Objective 4 and for the remainder of the project were selected by the project consortium at a meeting on 4 June 2008. The two selected peat alternatives to be used in the substrate mixes were green waste and wood fibre. Most growers using green waste in substrate mixes use a maximum of 20% green waste, with 10% being the most commonly used proportion. This is due to substrate structure and nutrition being adversely affected by higher proportions of green waste. However, to compare the effects of the two peat alternatives on sciarid and shore flies, it was decided to use 10% and 40% mixes in initial experiments, compared with a standard peat-based substrate suitable for growing ornamental bedding and pot plants and pot herbs.

O4/3 Attractiveness of growing media as egg-laying sites for sciarid and shore flies (ADAS)

Pilot experiment with sciarid and shore flies

Aim

The aim of the pilot experiment was to help determine the numbers of sciarid and shore flies needed to quantify the relative attractiveness of selected growing media to ovipositing flies. The number of flies should be sufficient to achieve significant differences between treatments, whilst avoiding ‘swamping’ all pots with flies and thus masking the results.

Site

The experiment was set up on 22 September 2008 in a research glasshouse at ADAS Boxworth.

Materials and Methods

Treatments

Three readily available composts were used in the pilot experiment, prior to doing the main experiments with the selected 10% and 40% compost mixes:

1. Standard 'M2' peat-based substrate
2. Klasmann green waste compost
3. Klasmann organic compost

Preparation of pots of compost

Sixteen replicate plastic plant pots (6x6 cm) were filled to 2 cm below the rim with each of the three substrates. Four replicate pots of each substrate were placed in four replicate plastic trays in a randomised design (12 pots in each of four trays). The base of the trays were lined with capillary matting. The pots of compost were watered to full water-holding capacity.

Experiment layout

The four trays of pots were placed on the floor in the middle of the glasshouse, in between trays of cyclamen plants infested with both sciarid and shore flies. The pots were left in the glasshouse for 18 days. The compost and matting were checked daily and watered as necessary to keep the composts damp. The glasshouse temperature was set at 19°C, venting at 21°C, with natural daylength.

Assessments

After 18 days, numbers of sciarid and shore fly eggs and larvae on the surface of the compost in each pot were recorded, after examining the surface of the compost under a low power microscope. The data was analysed using analysis of variance (ANOVA).

Results and Discussion

Numbers of sciarid and shore fly eggs per pot

Significantly more (P<0.001) shore fly eggs and larvae were recorded on the green waste substrate (a mean of 8.5 per pot) than on the standard peat-based or organic substrates (means of 0.8 and 1.4 per pot respectively) (Table 2). It was observed that the green waste substrate had more algal growth on the surface than the other two substrates, which will have attracted shore fly females to lay eggs and will have provided a food source.

Table 2. Mean numbers of sciarid and shore fly eggs and larvae 18 days after experiment set up. *** *P*<0.001

Substrate	Mean no. sciarid fly eggs + larvae per pot	Mean no. shore fly eggs + larvae per pot
'M2'	0.5	0.8
Green waste	0.3	1.4
Organic	8.6	8.5***

Although mean numbers of sciarid fly eggs and larvae were higher on the green waste substrate (a mean of 8.6 per pot) than on the standard or organic substrates (0.5 and 0.3 per pot respectively), these differences were not significant due to the large variation between replicates. Unlike shore fly eggs and larvae which are always found on the surface of the substrate, sciarid fly eggs and larvae occur both on and below the substrate surface. Thus, visual counts of sciarid fly eggs and larvae were less reliable than those of shore flies. It was concluded that the main experiment(s) should be done in higher densities of sciarid flies and that eggs and larvae should be reared through to the adult stage before assessments are made, in order to account for all the flies in the substrate.

Nursery experiment with sciarid flies

Sites

The experiment was done at a commercial pot herb nursery and in a research glasshouse at ADAS Boxworth.

Materials and Methods

Treatments

All substrates were supplied by Bulrush Horticulture Ltd. and were made up in the following proportions:

1. Standard peat-based substrate suitable for growing bedding plants or pot herbs
2. 40% green waste substrate, 60% standard
3. 10% green waste substrate, 90% standard
4. 40% wood fibre, 60% standard
5. 10% wood fibre, 90% standard

Compost analysis

The standard substrate, wood fibre and composted green waste were analysed for density, pH, conductivity and various mineral contents immediately before sending to ADAS. Once

the composts had been mixed for the experiment, similar analyses were done on samples of the mixes by Warwick HRI. Microbial analysis of the substrate mixes were also done at Warwick HRI.

Preparation of pots of substrate

Twenty replicate plastic plant pots (6x6 cm) were filled to 2 cm below the rim with each of the five treatment substrates. Two replicate pots of each compost were placed in 10 replicate plastic trays in a randomised design (10 pots in each of 10 trays). The bases of the trays were lined with capillary matting. The pots of substrate were watered to full water-holding capacity immediately before transferring the pots to the pot herb nursery on 19 May 2009.

Experiment layout at herb nursery

The trays of pots were placed amongst pots of basil heavily infested with sciarid flies. Two trays were placed on each of five adjacent benches of basil. In order to maintain all substrates at full water-holding capacity in the prevailing hot weather, the pots and capillary matting in the trays were watered again and the grower was asked to add water to the matting and to mist the surface of the substrates with water as necessary. The trays of pots were left on the nursery for two days.

Assessments

After two days, the pots of substrate were returned to ADAS Boxworth and the following assessments were made:

1. Although visual assessments of sciarid fly eggs was known to be unreliable, counts were made in order to determine what proportion of the fly population is missed using this method. The surface of the substrate in each pot was examined under a low-power microscope, then the surface of the substrate was gently disturbed with a mounted needle to find any further eggs laid below the surface.
2. Numbers of sciarid fly adults emerging from the substrate in each pot were made, after keeping the pots in a research glasshouse in individual, larger 'emergence' pots covered with a 'lid' of insect-proof mesh. The pots were laid out in a randomised block design and kept for five weeks in the glasshouse to allow all sciarid fly adults to emerge. The compost in each pot was kept damp by standing each pot on a piece of capillary matting in the base of each 'emergence' pot and by daily overhead watering using a watering can with a fine rose. Emerged sciarid fly adults were caught on a small yellow sticky trap secured onto the inside of each emergence pot with a paper clip.

The data was analysed using analysis of variance (ANOVA).

Results and Discussion

Numbers of sciarid fly eggs per pot

Mean numbers of sciarid fly eggs per pot were very high in all substrates, ranging from 29 to 66 (Figure 2). The eggs were found on both the surface of the composts and just below the surface. It was very difficult to determine whether all eggs in each pot were found. Due to the large variation between replicate pots for each treatment, there were no significant differences between the numbers of eggs laid in any of the composts.

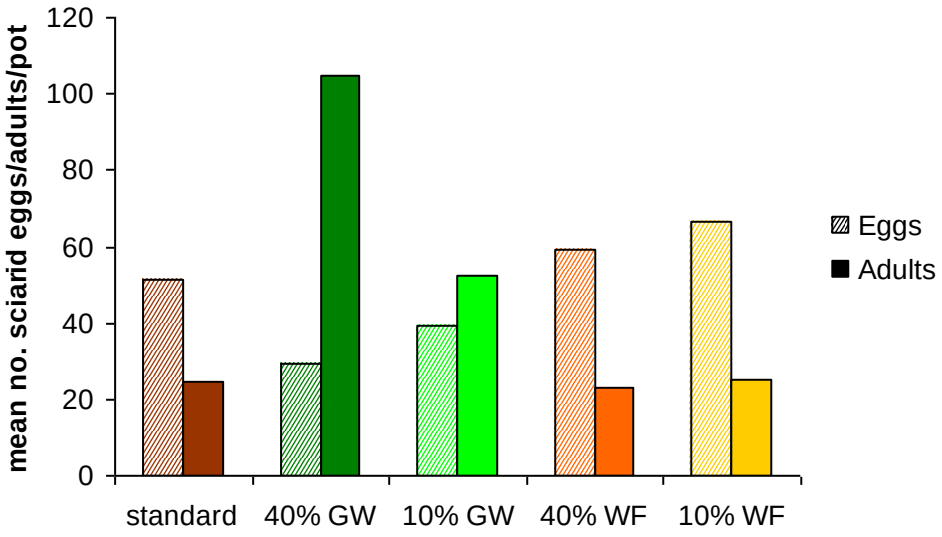

Figure 2. Mean numbers of sciarid fly eggs and emerging adults per pot. GW = green waste substrate, WF= wood fibre.

Mean numbers of sciarid fly adults emerging per pot

Mean numbers of sciarid flies emerging per pot are shown in Figure 2. Although there were no significant differences between treatments at the 95% level, more sciarid fly adults emerged from the 40% green waste substrate at the 90% level ($P<0.1$). There was a trend for numbers of emerged adults to be higher than the numbers of eggs recorded per pot in both green waste mixes, and for numbers of adults to be lower than numbers of eggs in the standard substrate and both wood fibre mixes (Figure 2). When the data was analysed as percentage of adults developing and emerging from the eggs recorded in each pot, a significantly higher proportion (656%) of adults were recorded in the 40% green waste mix ($P<0.05$, Figure 3). This indicates that although egg counts were unreliable, the 40% green waste substrate may have been more favourable for sciarid fly larval development and adult emergence than the other composts, possibly as a result of having a high moisture content. This is consistent with grower observations that higher sciarid fly infestations occur in substrates containing green waste. As fewer adults emerged from pots with the standard

peat-based substrate and those with the wood fibre mixes, mortalities must have occurred at the egg and/or larval stages.

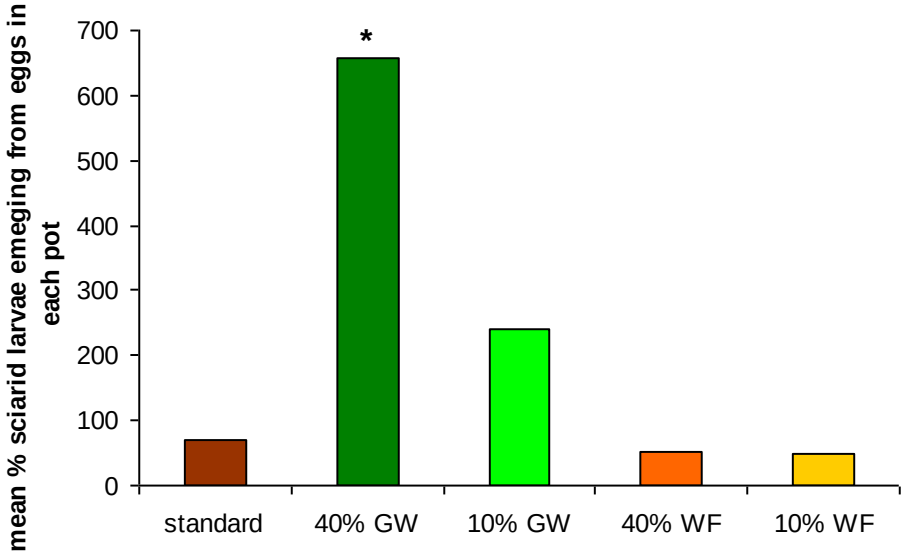

Figure 3. Mean % sciarid adults emerging from eggs in each pot with * significantly more in the 40% green waste substrate, *P*<0.05.

Further experiments planned include a similar compost choice experiment with shore flies on a commercial nursery, in which compost moistures will be monitored to identify any differences between composts and the effect on subsequent shore fly infestation. Olfactometer studies with the different compost mixes will determine any olfactory responses of sciarid and shore flies.

Substrate analyses

Selected results of the Blrush analysis of the standard substrate, green waste and wood fibre before mixing are shown in Table 3. The composted green waste had a higher conductivity and higher levels of chloride and potassium than the standard substrate and the wood fibre. Similar high conductivities and potassium in the green waste mixes were confirmed by analyses carried out at Warwick HRI (Table 4).

Table 3. Bulrush analysis of substrates before mixing for the sciarid fly compost choice experiment on herb nursery

Substrate	**Conductivity (µs/cm)**	**Cl (mg/l)**	**K (mg/l)**
Wood fibre	22	5.6	4.3
Green waste	686	547.5	956.8
Standard bedding	327	19.9	145.5

Table 4. Warwick HRI analysis of compost mixes for the sciarid fly compost choice experiment on herb nursery

	40% wood fibre	**10% wood fibre**	**40% green waste**	**10% green waste**
Conductivity (µs/cm at 20^0c)	181	205	675	355
K (mg/l)	98.6	102	909	343

Warwick HRI microbial biomass analysis of the compost mixes was estimated from CO_2 respiration measurements as shown in Table 5 (an increase in CO_2 levels is indicative of an increase in microbial growth). There looked to be no link between compost composition and microbial activity but there was evidence of a that media with a higher water content had a high microbial biomass (data not shown).

Table 5. Warwick HRI microbial analysis of substrate mixes

Compost	**Respiration µg CO_2/g wt/min**
Standard bedding mix	0.140
M2	0.050
10% green waste	0.098
40% green waste	0.198
10% wood fibre	0.103
40% wood fibre	0.175

Conclusions from nursery experiment

- Visual counts of sciarid fly eggs on the surface of the compost is unreliable, even if carried out using a low-power binocular microscope. Using this method, there were no significant differences between sciarid fly eggs laid onto standard peat compost or compost mixes with 10% or 40% green waste or wood fibre.
- Rearing sciarid fly eggs laid onto composts through to the adult stage and assessing numbers of emerged adults on sticky traps in individual enclosed pots is a more reliable method of determining numbers of sciarid flies in pots.
- More sciarid fly adults emerged from the 40% green waste compost than from the other composts (P<0.1).
- Numbers of emerged sciarid fly adults were higher than the number of eggs recorded in both green waste mixes, and for numbers of adults to be lower than the number of eggs in the standard compost and both wood fibre mixes. A significantly higher proportion (656%) of emerged adults were recorded in the 40% green waste mix (P<0.05). This indicates that the green waste substrate may have been more

favourable for sciarid fly egg survival, larval development and adult emergence than the other composts. This is consistent with grower observations that higher sciarid fly infestations are seen in composts containing green waste.

- Further experiments will include monitoring of compost moisture to identify any effects on fly infestation. Olfactometer studies will determine any olfactory responses of sciarid and shore flies to the different substrate mixes.
- Analysis of the different composts demonstrated that the composted green waste had a higher conductivity and higher levels of chloride and potassium than the other experimental composts.

O4/4 Effect of temperature on sciarid fly development

A series of experiments are being developed to determine the effect of temperature on the development of *B. difformis.* These include the development of immature stages, female fecundity, ovipostion period and adult longevity. All of the experiments will be run at five temperatures (10, 15, 20, 25 and 30^{O}C) and at two photoperiods (16:8 and 8:16 Light :Dark). The development of immature stages will be determined by collecting eggs from laboratory *B. difformis* cultures and placed on moistened compost mixed with soya flour within a Petri dish. The dishes will be observed daily and because there are no simple morphological changes during the development of sciarid larvae (Binns, 1981ab) instars will be identified from the size of their head capsules. Female fecundity will be determined by pairing newly emerged adult female *B. difformis* with males within an oviposition chamber which consists of a cylindrical plastic frame (5 x 6cm) covered in fine mesh nylon gauze, placed on a base of a Petri dish containing 25 g of compost. Every 24h the chamber will be transferred to a new dish until the death of both male and females and the total number of eggs recorded. The results from these experiments will be used to identify the minimum (i.e. lower threshold) temperature for development and a simple day degree model of sciarid development will be produced.

Conclusions

- Natural fungal infections of sciarid fly larvae are caused by *Furia sciarae*. These infections are widespread on growers' nurseries and appear to be contributing to natural control of sciarid fly pests on bedding and pot plants and pot herbs.
- The Furia fungus infects sciarid larvae. Infected larvae move up onto the compost surface at night, die, and then the fungus produces infective spores which are actively discharged as a way of dispersing the infection. This process can occur over the course of a single night.
- Natural fungal infections in shore flies are caused by a species of Torrubiella, which is a sexual phase of Lecanicillium. It has only been observed on one nursery at present.
- There is evidence that composts containing green waste material can be more favourable to the development of sciarid and shore fly populations than standard peat based compost.

Technology transfer

- Protected herbs: best practice guidelines for integrated pest and disease management (Defra/HDC). BHTA 'Herb News' article September 2008
- HDC IPM Seminar 'Whats new in IPM' October 2008
- HDC herbs Crop Walkers Guide October 2008
- HDC website protected herbs Best Practice Guide updated Feb 2009 http://www.hdc.org.uk/herbs/
- Consortia meetings held on 4th June 2008, 3rd December 2008, 14th January 2009, 24th July 2009.

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APPENDIX: Grower Survey Form

Horticulture Link

As part of a new Horticulture LINK project, we are investigating the incidence of natural fungal infections of sciarid and shore flies in protected crops of ornamentals and herbs.

We will be telephoning you to ask if you have seen infected flies on your nursery, and a few further questions about your production practices that could affect the incidence of infected flies. The questions are listed on the following pages with space for you to make notes. Pictures of sciarid and shore flies are shown below.

Recognition of healthy and infected sciarid fly larvae

1) Sciarid fly adults are small, black, gnat-like flies with long antennae, © ADAS UK Ltd.

2) Healthy sciarid fly larvae are usually found around the roots in the compost. They have a black head (left) and a transparent body, showing the gut contents, © ADAS UK Ltd.

3) Sciarid fly larvae infected with *Furia* are visible on the surface of the compost. © ADAS UK Ltd.

4) They still have a black head but the body is opaque white, so the gut contents are not visible, © ADAS UK Ltd.

Recognition of healthy and infected shore fly adults

5) Shore fly adults are small, robust flies with short antennae and pale spots on the wings, © Nigel Cattlin / FLPA.

6) Shore flies infected with *Hirsutella* have very long rod-like hairs sticking out of the body, see arrows, © Neil Helyer, Fargro Ltd.

ADAS Warwick HRI HDC defra

- In recent years, we have been seeing naturally infected sciarid fly larvae and shore fly adults on several nurseries.
- This is biological control for free!
- Our project aims to understand what factors affect the incidence of natural infections, so that you might encourage them to occur.

Questions to be discussed when we telephone you:

GENERAL QUESTIONS:

Name of nursery	
Main crops grown	
Type of compost? (type and make, % peat, bark, green waste, coir or other amendments) Irrigation method	
Is IPM used?	

QUESTIONS ABOUT SCIARID FLIES

Which crops do you get sciarid problems on?	
Have you ever seen sciarid larvae infected with Furia? (see figures 3 & 4).	
What crops were the infected larvae seen on?	
Did you buy in the plants or plant material, if so, from where?	
What time of year did you first see infected larvae?	
How widespread was the infection in the crop(s)? For how long were infected larvae seen?	
Do you think any particular factors affect the first appearance and incidence infected larvae? (e.g. time after potting, compost moisture)	
Do you think that the natural infection has helped control of sciarid flies?	

QUESTIONS ABOUT SCIARID FLIES - continued

What control measures do you use for sciarid flies? (biological or chemical)	
Which fungicides do you commonly use on crops susceptible to sciarid flies? (these may affect the Furia)	

QUESTIONS ABOUT SHORE FLIES

Which crops do you get shore fly problems on?	
Have you ever seen shore fly adults infected with Hirsutella? (see figure 6).	
What crops were the infected adults seen on?	
Did you buy in the plants or plant material, if so, from where?	
What time of year did you first see infected adults?	
How widespread was the infection in the crop(s)?	
For how long were infected adults seen?	
Do you think any particular factors affect the first appearance and incidence of infected flies?	
Do you think that the natural infection has helped control of shore flies?	
What control measures do you use for shore flies? (biological or chemical)	
Which fungicides do you commonly use on crops susceptible to shore flies? (these may affect the Hirsutella)	

MANY THANKS FOR YOUR HELP

Jude Bennison & John Buxton, ADAS Entomologists