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GROWER SUMMARY

Headline

- Incorporating composted green waste into peat based substrates can result in significantly higher infestations of sciarid and shore flies.
- Sciarid fly populations develop faster as temperatures increase. The optimum temperature tested for the development from egg stage to adult is 30°C. Larvae will not develop at 10°C, and development is very slow at 15°C.
- Natural fungal infections of sciarid fly larvae and shore fly adults could provide 'free' biological control and spread of the fungus between flies is probably more effective at lower temperatures.
- Two commercial fungal bio-pesticides tested could be used to give partial control of sciarid fly larvae if approval was sought and obtained for their use in a substrate drench.

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. Some 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 always give sufficiently reliable control of sciarid flies or shore flies. Therefore additional measures are needed.

At present, most of herb and ornamental bedding and pot plant crops are grown in peat based substrates. However, the industry is under pressure to reduce the use of peat and to find alternative, sustainable alternatives. Potential ‘green’ candidate materials for incorporating into substrates include composted green waste, bark, wood fibre and 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 bio-control.

The project has two aims:

- Develop novel methods of bio-control for sciarid and shore fly pests on pot herbs and ornamental bedding and pot plants.
- Investigate their interaction with substrates containing composted green waste.

The expected deliverables from the project include the following:

- 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.
- New understanding of the role of naturally occurring insect pathogenic fungi in the population dynamics of pest insects that have adverse effects in a complex, integrated plant production system.
- New insights into the methods needed to enhance the effects of the fungi.

Summary of the project and main conclusions

Sciarid fly infections on nurseries

In Year 2, the work focused on fungal infections of sciarid flies, as the fungus infecting shore flies was only found on one nursery at very low levels. Two experiments were set up on nurseries to gain information about outbreaks of the insect pathogenic fungus *Furia sciarae* on populations of sciarid flies. One experiment was done on cyclamen and one on mint, on nurseries with a history of sciarid fly infection with *Furia* and they were set up where infected larvae had very recently been observed by the host growers.

Cyclamen and mint plants were grown in pots of standard peat based substrate and a mixture of peat and composted green waste or wood fibre, and the development of sciarid populations and the amount of *Furia* infection were monitored over time. On cyclamen, the sciarid population was monitored over three months. There was a peak in the sciarid population after one month, after which it declined to very low levels and *Furia* infections could not be detected. On mint, no sciarid fly larvae infected with *Furia* were seen, and it is possible that *Furia* had reduced the sciarid fly population to such low numbers that the infection died out just before the experiment was set up. Information on the timing of the onset of *Furia* infection in sequential batches of mint was gained from assessing infected larvae in the grower's crop in mid-December before the experiment was set up. These observations indicated that the onset of visible *Furia* infection was two weeks after the first

larvae were available for infection. At four weeks after the first larvae were available for infection, all the larvae that were visible on the substrate surface were infected with the fungus.

Isolating Furia from naturally infected sciarid flies

Isolating *Furia* from naturally infected sciarid fly larvae is very difficult. Infected larvae migrate to the surface of the substrate, the fungus then kills the larvae and grows out through the cuticle and produces 'ballistospores' that are discharged into the air. This process can occur over the course of a single night, after which the fungus dies. The fungus can only be cultured using ballistospores that are caught from infected larvae, but often these are contaminated with bacteria or other fungi which prevent a 'clean' culture of *Furia* being established. The medium used to grow *Furia* is difficult to prepare and becomes easily contaminated. However, it was found that *Furia* will grow in two liquid media that are used for growing insect cells and which are available from commercial suppliers in pre-sterilized form. The fungus grows as mycelium in these media, and when the mycelium is harvested and washed it goes on to produce ballistospores. The next stage is to quantify the rate of spore production and to see if these will infect sciarid larvae.

Susceptibility of sciarid flies to entomopathogenic fungi

A laboratory experiment was done to investigate whether sciarid larvae were susceptible to three different types of insect pathogenic fungi that are used as 'bio-pesticides' of glasshouse pests: Metarhizium (from Met52, a commercial product undergoing UK registration), Beauveria (from Naturalis, which is registered in the UK) and Lecanicillium (using a fungal strain isolated from a naturally infected sciarid fly, although other strains of this fungus are used in the commercial products Mycotal and Vertalec).

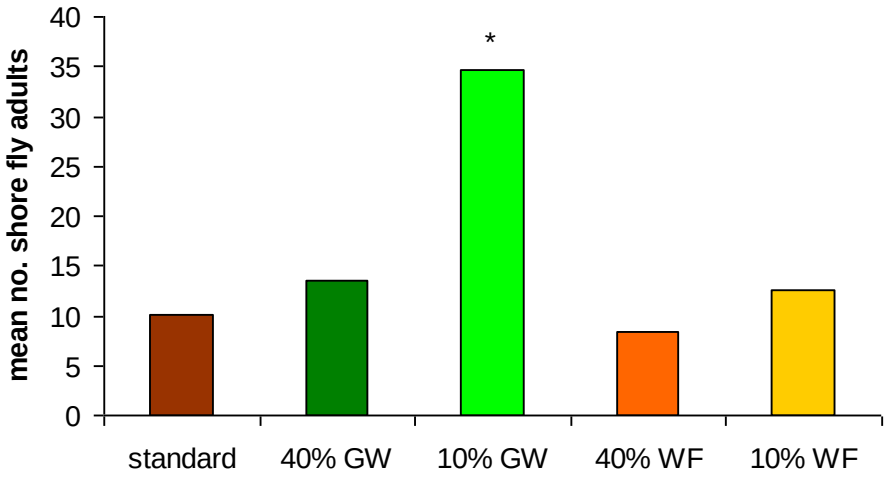
These types of fungi are not normally associated with fly infections, and unlike *Furia* they do not cause 'outbreaks' that have the ability to reproduce on insects and spread rapidly through an insect population. Instead, they are used in a similar way to a chemical insecticide. In the laboratory experiment, spores of these fungi were applied as a drench to the surface of the substrate containing sciarid fly eggs. The spores were applied at three different times, namely (1) before sciarid fly egg hatch; (2) when first and second instar larvae were present; (3) when second and third instar larvae were present. All the fungi reduced the sciarid fly populations although the overall level of control was low. As a general rule, the level of control increased with the age of the larvae. Insect pathogenic fungi infect insects using spores that grow through the insect cuticle, and therefore larvae that moult before the spores have grown sufficiently may be able to rid themselves of infection. Hence applications to early stage larvae present more opportunities for larvae to

escape infection. From previous studies it was found that spores applied to substrates tend to be concentrated in a band in the top few cm of the substrate. It may be that the later stage larvae are migrating more often to the top of the substrate surface and hence exposing themselves to more fungal inoculum.

Attractiveness of growing media to sciarid and shore flies

An experiment was done on a commercial nursery to measure the attractiveness to shore flies of five different kinds of substrate: (1) A standard peat-based substrate suitable for growing bedding plants or pot herbs; (2) a mixture of 40% composted green waste and 60% peat (3) a mixture of 10% composted green waste and 90% peat; (4) a mixture of 40% wood fibre, 60% peat and (5) a mixture of 10% wood fibre and 90% peat.

Significantly more shore fly adults emerged from the substrate with 10% composted green waste than from the other substrates tested. The results indicate that either shore fly females were more attracted to the 10% composted green waste than the other substrates for egg laying, and/or this substrate was more suitable for shore fly larval development.



Mean number of shore fly adults emerging per pot. GW = composted green waste, WF = wood fibre. * Significantly more in composted green waste than other substrates, $P<0.05$.

The substrates containing composted green waste had consistently higher moisture readings than the other substrates, with the 40% composted green waste substrate having a higher moisture volume than the 10% composted green waste substrate on some assessment dates which may have been more conducive to sciarid fly development?. The two wood fibre substrates and the standard peat based substrate had similar readings throughout.

Olfactometer studies indicated that sciarid fly adults are attracted to the smell of 40% composted green waste. This result is consistent with those of glasshouse experiments in Year 1 and with grower observations that substrates based on composted green waste lead to greater problems with sciarid flies. Olfactometer studies with shore flies were less conclusive, possibly because of the absence of algae from the fresh substrates tested; this will be further investigated in future work.

Effect of temperature on the development rate of sciarid flies

Research was done to measure the rate of development of sciarid fly, *Bradysia difformis*, from egg to adulthood in peat-based substrate at different temperatures from 10°C to 30°C. The effect of temperature on the rate of development of *B. difformis* has not been measured before, although some information is available for other *Bradysia* species. Total development time was inversely proportional to temperature, with the shortest development time being at temperatures above 30°C. At the lowest temperature studied, 10°C, eggs hatched slowly and did not develop to complete the larval stage. There was no emergence of adults at 10°C even after 70 days of incubation. At all the temperatures used, larvae were seen moving close to the substrate surface prior to pupation. At lower temperatures (less than 20°C) larvae were observed on the substrate surface for greater periods than at the higher temperatures. This may indicate a mismatch between the optimum temperature for sciarid development and the best conditions for the transmission of *Furia* infections: the fungus is likely to be transmitted more effectively at lower temperatures because transmission occurs on the substrate surface. Thus, the longer a larva remains at the surface, the more likely it is to contact *Furia*.

Effect of substrate type on the development rate of sciarid flies

An experiment is being run to measure the effect of different types of substrate (standard bedding peat, composted green waste and wood fibre, obtained from Bulrush Horticulture) on the development of sciarid fly populations. This is ongoing at the time of writing. The substrates are maintained at the same total wet weight via manipulation of the irrigation regime and infested with batches of sciarid fly eggs. The production of adult flies that emerge from these eggs is being recorded over time.

Financial benefits

- Natural fungal infections of sciarid fly larvae and shore fly adults occur ‘free of charge’ to 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 *Furia* infections of sciarid fly larvae when monitoring for pests, particularly from autumn to spring. Infected sciarid fly larvae are opaque white, visible on the substrate surface and are relatively easy to spot.

Sciarid fly larva infected with *Furia sciariae* © ADAS. The shiny black head capsule is still visible at one end and the body is opaque white and covered with tiny spores

- Leave infected dead sciarid fly larvae on the surface of the substrate so that the fungal infection can spread.
- As the infection probably spreads better at lower temperatures (20°C), adjust the environmental growing conditions, if possible, to both favour development of the insect pathogenic fungi and delay development of any sciarid fly.
- If you need help recognising healthy or infected infected flies, contact Jude Bennison, ADAS Boxworth (tel. 01954 268225, email jude.bennison@adas.co.uk) who can send you more photographs and discuss them with you.
- If you have seen infected flies and we have not already contacted you about the project, please contact any of us below:

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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 (substrates) containing composted 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 growing media, 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 composted green wastes and other non-peat materials. 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 waste 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) and 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 substrates 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 is caused by an entomophthoralean fungus, identified on the basis of morphological characteristics as *Furia* (= *Erynia*) *sciarae* (Zygomycota: Zygomycetes: Entomophthorales).

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

01/2 Nursery experiments (ADAS and host growers)

The aim of these experiments was to gain information on key parameters of fungal outbreaks including time of infection onset in plant production batches, cumulative rate of increase of dead flies during an outbreak and any evidence of correlation between the onset of infection and environmental variables including time of year, temperatures, substrate type and moisture. As surveys in year 1 had found that infection of shore flies only occurred at one nursery and very few infected shore flies had been observed by the grower or by the Fargro consultant, the work focused on infections of sciarid fly larvae with *Furia sciarae*, which occur more commonly.

Two experiments were set up, one on cyclamen and one on mint, on nurseries with a history of sciarid fly infection with *Furia* and where infected larvae had recently been observed by

the host growers. In order to gain most information, natural sciarid fly infestation was encouraged by using substrate containing composted green waste as one of the treatments, as work in Year 1 had indicated that these substrates led to greater sciarid fly populations than peat-based substrates.

Experiment A, Materials and Methods

This experiment was done at Bordon Hill Nursery, Stratford on Avon, on a crop of cyclamen. The experiment was set up on 4 August 2009. There were 2 treatments: (1) Standard peat-based substrate; and (2) 25% composted green waste, 75% standard peat based substrate. Both substrates were supplied by Bulrush. The experimental plots were set up as follows: 100 replicate 10 cm pots were filled with each substrate and planted with cyclamen plants. The pots were watered using overhead irrigation as in the commercial crop. The experiment was set up as a randomised block design, with five blocks, each containing 20 replicate pots of each treatment. The experiment was set up amongst the commercial cyclamen crop. At monthly intervals from 28 August to 26 November, 20 pots per treatment (four randomly selected pots from each of the five blocks) were selected and numbers of infected sciarid larvae visible on the surface of the substrate were recorded. On the same 20 pots per treatment, % substrate moisture was recorded using a portable meter (Digital instruments Ltd., model PMS-714). Using the same 20 pots, each pot was placed inside a larger empty pot with a small yellow sticky trap attached to the inside rim and each larger 'emergence' pot was covered with muslin secured with a rubber band. Emerged adult sciarid flies in each pot were counted on the sticky traps after four weeks. The data was analysed using a t-test.

Experiment A, Results and Discussion

The results are shown in Table 1. Infected sciarid fly larvae were only seen on one assessment date on 1 October, when four infected larvae were recorded in the 20 pots of the composed green waste substrate and one infected larva was recorded in the pots of peat-based substrate (Table 1). As numbers were so low there were no differences between the two substrate treatments. Substrate moisture readings indicated that the green waste substrate had a significantly higher % moisture than the peat-based substrate during August and September ($P<0.05$, Table 1), but the two substrates had similar % moistures during October and November once the plant canopy had closed over. However, the moisture meter was thought to under-estimate substrate moisture as readings were very low in both substrates (ranging from 13-22%). On the first assessment date on 1 October, high numbers of adult flies per sticky trap were recorded, with significantly more (76.0 per pot) in pots with the composted green waste substrate then in pots with the standard peat-based substrate (49.1 per pot), $P<0.05$, see Table 1. This result was consistent with those in experiments in

Year 1 of the project, and with grower observations that inclusions of composted green waste in substrates leads to greater sciarid fly problems compared with standard peat-based substrates. However, on subsequent assessment dates, numbers of adult flies per trap were much lower, with no significant differences between the two substrates.

Table 1. Mean numbers of pots with *Furia*-infected sciarid fly larvae, mean numbers of adult sciarid flies emerging per pot and mean % substrate moisture in pots of cyclamen with composted green waste or standard peat-based substrates. * significantly different than the other treatment, P<0.05.

Date	Mean no. pots with *Furia* infection		Mean no. adult sciarid flies emerged per pot		Mean % substrate moisture	
	Green waste	Peat-based	Green waste	Peat-based	Green waste	Peat-based
28 Aug	0	0	N/A	N/A	22.2*	16.2
1 Oct	4	1	76.0*	49.1	21.5*	14.0
30 Oct	0	0	3.7	1.5	20.8	18.6
26 Nov	0	0	4.2	2.8	12.8	13.8

Experiment B, Materials and Methods

An experiment on a crop of garden mint was set up on the nursery of a mint propagator in Lincolnshire on 16 December 2009. There were 2 treatments: (1) 100% peat based substrate as used by the grower; and (2) 40% composted green waste, 60% peat substrate. Both substrates were supplied by Bulrush. For each of the two substrate treatments, 120 replicate 7 cm pots were filled with substrate and planted with mint plugs. The pots were watered using overhead irrigation as in the commercial crop. The experiment was done according to a randomised block design, with five blocks, each containing 24 replicate pots of each treatment. The experiment was set up in the middle of a bench amongst the mint crop to avoid edge effects. The grower aimed to keep the substrate wet throughout the growing period (six weeks from potting to selling on to a herb nursery for final production). Glasshouse temperatures were 15.5°□C day, 13.5°□C night, with supplementary lighting between 3 and 7 a.m. No pesticides or fungicides were applied to the crop. The following assessments were done three, four, five and six weeks after experiment set-up, during the 6-week production period of the crop: (1) 25 pots per treatment (five pots from each of the five blocks) were collected and numbers of infected sciarid larvae visible on the surface of the substrate were recorded. (2) On the same 25 pots per treatment, % volume of substrate moisture was recorded using a portable meter (Delta-T, model SM200). (3) An additional five pots per treatment (one from each of the five blocks) were collected to compare % substrate moisture volumes when measured with the SM200 meter and % substrate moisture weights

when measured using wet and dry weights. (4) Using the same 25 pots used for 1 and 2 above, after assessment of infected sciarid larvae and substrate moisture, each pot was placed inside a 3-litre empty pot with a piece of wet capillary matting in the bottom. A 6x10 cm piece of yellow sticky trap was attached to the inside rim of each 'emergence' pot using a paper clip. The emergence pots were covered with horticultural fleece secured with a rubber band. The pots were placed onto wet capillary matting in a research glasshouse at ADAS Boxworth. The capillary matting was kept wet with automatic drip irrigation lines and the glasshouse temperatures and lighting were the same as used on the host nursery. Each pot was assessed at weekly intervals for six weeks and any infected sciarid fly larvae on the surface of the substrate recorded. Emerged adult sciarid flies in each pot were counted on the sticky traps after six weeks.

Experiment B, Results and Discussion

The results are shown in Table 2. After the experiment had been set up, no sciarid fly larvae infected with *Furia* were seen on any of the assessment dates. The incidence of *Furia* infection had followed a similar pattern in the winter of 2008/09, i.e. visible in November and December, then no occurrence from January onwards. It is possible that in both years, *Furia* reduced the sciarid fly population to such low numbers that the infection died out. This is supported by the numbers of sciarid fly adults emerging from the experimental pots with both substrate treatments in February and March 2011 being very low (Table 2). Some information on the timing of the onset of *Furia* infection in sequential batches of mint was gained from a brief assessment of infected larvae in the grower's crop in mid-December before the experiment was set up. Pots one, two, three, four and six weeks after potting were assessed to determine the number of weeks after potting that infected larvae were first visible on the substrate surface. Results were as follows:

First week after potting: No sciarid larvae visible.

Second and third weeks after potting: Occasional live larvae visible.

Fourth weeks after potting: Both live and infected larvae visible.

Sixth week after potting: All visible larvae infected.

The first live larvae were seen two weeks after potting, which reflects the life cycle of *Bradysia difformis* at the temperatures in the glasshouse, i.e. at temperatures of 14-16°C, eggs laid soon after potting would take a week to hatch into larvae (see Table 5). These observations indicated that the onset of visible *Furia* infection was two weeks after the first larvae were available for infection.

Another observation made on *Furia*-infected larvae in the mint pots brought back to the laboratory before the experiment was set up, was that healthy larvae consumed infected ones on the surface of the substrate. It is possible that healthy larvae become infected

during this cannibalistic behaviour, due to coming into close contact with the infective spores on the dead larva.

Substrate moistures measured by wet and dry weights were very similar on each of the four dates for each substrate type (Table 2). When analysed using a t-test, substrate % moistures were significantly higher in the pure peat substrate than in the 40% composted green waste on each date ($P<0.05$). Mean % substrate water volumes (measured with the SM200 meter) for each substrate were much lower than the % substrate moistures by weight, on the same pots of substrate and were similar for both peat and composted green waste substrates.

Table 2. Mean numbers of adult sciarid flies emerging per pot, mean % substrate moisture and mean % water volume in pots of mint with composted green waste substrate or with standard peat-based substrate. * significantly higher in peat substrate than in composted green waste substrate, $P<0.05$.

Date of collection	Mean no. adult sciarid flies emerged per pot 6 weeks after collection		Mean % substrate moisture (using wet & dry weights)		Mean % substrate water volume (using SM200 meter)	
	Green waste	Peat	Green waste	Peat	Green waste	Peat
6 Jan 2011	2.20	4.45	73.32	84.51*	-	-
14 Jan	2.85	1.45	70.36	82.39*	44.15	44.36
20 Jan	1.15	3.60	70.56	81.82*	39.56	37.60
28 Jan	4.55	4.65	68.74	81.89*	32.08	37.66

O1/3 Isolation of fungi from naturally infected sciarid flies (WHRI)

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

Isolation of the fungus Torrubiella from shore flies. When the project started, it was thought that the natural infections of shore fly (*Scatella tenuicosta*) were caused by an entomopathogenic fungus from the genus *Hirsutella*. This identification was based on the morphology of the fungus. However, analysis of ribosomal RNA gene sequence data in Year 1 showed that, although the fungus was morphologically similar to *Hirsutella*, it actually was a member of the genus *Torrubiella*. *Torrubiella* is a sexually reproducing ascomycete fungus. In fact, our shore fly fungus does have some morphological similarities to *Torrubiella*, but we thought that it was unlikely to be *Torrubiella* because sexually reproducing ascomycete

insect pathogenic fungi have not been recorded before in the UK. The genus *Torrubiella* includes species of the insect pathogenic fungus *Lecaniciullium*, such as *L. muscarium* and *L. longisporum* which are used as commercial biopesticides against greenhouse pests. *Lecanicillium* species are morphologically very different to *Torrubiella* species, and before the advent of molecular phylogenies they were thought to be members of a completely separate genus, which explains why they are described as '*Lecanicillium*' rather than '*Torrubiella*'. As the phylogenetic relationships of insect pathogenic fungi becomes more complete, with new molecular biology sequence data, then it is likely that '*Lecanicillium*' will be replaced with '*Torrubiella*'.

So far in the project, three shore flies infected with *Torrubiella* have been received from a single nursery on the south coast. The fungus has been isolated from these insects and placed into culture. Of an assessment of 300 thyme plants on the same nursery only 3 infected insects were found. Shore flies are not currently a particular problem at this nursery. *Torrubiella* has not been observed infecting shore flies in any other nursery, and hence it is almost certainly a rare and unusual infection. As a result, the project consortium (i.e. the nine industry partners) concluded at the consortium meeting held on 11 February 2010, that *Torrubiella* is not contributing to natural biological control of shore flies and this should be taken into consideration when planning the remaining work in the project. They recommended that it may be worth stopping research on *Torrubiella* infections of shore flies in order to concentrate more on natural fungal infections of sciarid flies.

Furia sciarae. This is the fungus that causes natural outbreaks in populations of sciarid flies (*Bradysia difformis*). It is a member of the Entomophthorales, a specialized group of insect pathogenic fungi. In Year 1, we developed a method for isolating the fungus from naturally infected sciarid flies. The fungus produces specially adapted spores, termed 'ballistospores' that are actively discharged from diseased sciarid larvae that move onto the substrate surface to die. The fungus cannot be cultured easily from the fungal mycelium growing within infected larvae, because it starts to die as soon as the ballistospores are produced. Entomophthoralean fungi that infect large insects, such as aphids or caterpillars, can be isolated by surface sterilizing the insect cadaver in alcohol or sodium hypochlorite solution and then breaking it open and placing it on a mycological medium. However, we have found that this is not possible with sciarid larvae, because the cadavers are not large enough to withstand surface sterilization intact (they break easily when placed in a sterilizing solution and this kills the fungus). Instead, we have been isolating the fungus by 'showering' ballistospores from individual infected cadavers onto a specialized 'YSMA' growth medium comprised of yeast, sucrose, milk and egg yolks (albumin). A small proportion of the captured spores will grow myceliogenically, but most form 'secondary' spores that are

actively discharged into the air. Because the infected sciarid larvae are obtained from substrate, the spores are often contaminated with bacteria or spores of saprotrophic fungi such as *Penicillium*, *Aspergillus* and *Mucor*. We have not been able to control these contaminants by adding antibiotics to the growth medium. Hence isolation of the fungus remains a hit and miss affair, we estimate that less than 3% of isolation attempts are successful. As described in the previous report, *Furia* outbreaks occur sporadically on nurseries, which means that isolation attempts cannot be planned in advance. Our grower partners inform us when an outbreak is occurring which allows us to start isolation attempts. We have found that it is better to travel to the grower to perform isolations on the nursery rather than have samples of infected larvae sent to us. We have observed that infected larvae produce ballistospores for a very short time period (c. 24 hours) and then the fungus decays rapidly. Hence one of the challenges in isolating *Furia* from naturally infected sciarid larvae is to obtain cadavers at the best time for collection of ballistospores.

One of the objectives of the research is to be able to grow *Furia* to a morphological stage where it produces ballistospores in culture. These could then be showered onto sciarid flies to investigate their effects on fly survival in laboratory experiments. The basic premise for obtaining these spores is to first grow mycelium of the fungus on a specialized, nutrient rich culture medium, then 'starve' the fungus by reducing its supply of carbon and nitrogen in order to induce sporulation. To do this, the fungus must be grown in a liquid medium (i.e. not on agar) and then the growing mycelium harvested, washed and placed on a low carbon-nitrogen substrate. The YSMA medium used to isolate the ballistospores from naturally infected sciarid larvae could be used as a liquid to grow *Furia* mycelium, however it is difficult to make, prone to contamination, and it is opaque (and hence it is not possible to observe the growth of mycelium within it). We have now identified alternative liquid media that are suitable for *Furia*: Grace's insect medium, and Hink's medium. These media are used to culture insect cells and consist of amino acids, 9 vitamins / growth factors, plus sugars and various salts. The media are available from commercial suppliers pre-sterilized and at a reasonable price. They have been used previously to culture the entomophthoralean fungi *Neozygites tanajoae*, *Neozygites floridana* and *Entomophthora planchoniana* (Delalibera et al., 2003; Freimoser et al. 2001). In experiments using an isolate of *Furia* isolated from Bordon Hill nursery, Warwickshire, we found that the fungus will grow myceliogenically on Grace's and Hink's media in medical flats (i.e. in 'still' culture). When mycelium was harvested, washed, and placed onto damp filter paper, it produced ballistospores. Based on this result, we have designed a set of more detailed experiments to compare different isolates of *Furia*. These have just started (at the time of writing) and we are investigating the following: (1) quantify rate of mycelium growth (dry weight) in liquid media in shaking and still culture (2) quantify production of ballistospores from harvested

and washed mycelium placed on different 'spore producing' substrates, specifically mycelium placed on a low nutrient agar (0.5 % sucrose) versus mycelium placed on water agar. We will then quantify the effect of ballistospores on the mortality of sciarid flies in laboratory bioassays.

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

O2/1 Furia population dynamics

The aim of this work package is to quantify the effect of *Furia* on the survival of populations of sciarid flies (*Bradysia difformis*) in laboratory experiments under controlled conditions. Work was also done to investigate whether Furia infection could be induced into a sciarid fly culture using naturally infected larvae. One hundred infected larvae collected from a nursery were added to the substrate surface of a laboratory reared sciarid culture of third and fourth instar larvae. The sciarid culture was maintained in a vented plastic box (28x16x10cm) containing 250g of moistened coir substrate mixed with soya flour (5%) at 20^{O}C, 16:8 Light : Dark. This was repeated on two occasions. On the first occasion, 10 newly infected larvae were observed 4-5 days after inoculation but no further infection was observed in future generations of sciarid flies. On the second occasion no transfer of infection was observed.

Research was also done to investigate whether the *Furia* infection originated from the substrate. Substrate, which was used to seed a cyclamen crop in which Furia infection was observed in the commercial nursery, was brought back to WHRI. The substrate was added to 20 pots. Ten of the pots were inoculated each with 100 sciarid eggs and all 20 pots were maintained within a glasshouse (12-14°C) with a low level sciarid population. The pots were watered daily to provide ideal conditions for sciarid development. Sciarid adults emerged from both the inoculated and the non-inoculated pots but no infected sciarids were observed in any of the pots over the 8 week period of the experiment.

Objective 3: Inundation biological control: Quantify susceptibility of sciarid and shore flies to selected isolates of entomopathogenic fungi

O3/1 Susceptibility of sciarid flies to selected isolates of entomopathogenic fungi

Materials and Methods

Because the isolation and culture of *Furia* has been problematic and is taking longer than planned to complete, we decided to bring forward the work to investigate the susceptibility of sciarid flies to selected isolates of other entomopathogenic fungi. The aim of this work package is to determine if isolates of asexual ascomycete fungi (i.e. fungi other than *Furia*

and related species in the Entomophthorales) also have potential for the control of sciarid flies. Asexual ascomycete entomopathogenic fungi are generally much easier to culture than entomopthoralean fungi. We used two isolates of fungi that are used in commercial biopesticides available in the UK / EU for the control of other pests of protected crops: *Metarhizium anisopliae* strain Met52, which is used in the fungal biopesticide Met52 produced by Novozymes. Inc. and which is currently undergoing registration in the UK as a biopesticide for control of a range of soil dwelling horticultural pests; *Beauveria bassiana* strain ATCC 74040, which is used in the biopesticide Naturalis-L (Intrachem) and is sold in the UK for the control of a range of pests of protected crops (isolate code 275.86). In addition we also used an isolate of *Lecanicillium* sp. obtained from a naturally infected sciarid fly in Year 1.

Fungi were cultured on Sabouraud's dextrose agar at 23°C for 10 days, then conidia harvested and a suspension prepared in a wetting agent (0.05% Triton X-100). Batches of sciarid (*Bradysia difformis*) eggs (100 per pot) were placed on the surface of pots (10 x 8 cm) containing 100g coir substrate filled to a depth of 7.5 cm with soya flour placed on the surface to act as a protein source (see below for details of sciarid fly culturing). Pots were maintained at 25°C for 21 days. The surface of each pot was drenched with a fungal conidia suspension (40mls, 10^8 conidia.ml^{-1}) applied at a different time (2 replicate pots per treatment), based on estimates of when different sciarid life stages would be present: (i) prior to egg hatch (day 0), (ii) presence of L1 – L2 larvae (day 7) ; (iii) presence of L3 – L4 larvae (day 12). Pots were examined daily for the presence of dead larvae on the surface. Emerged adults were caught and counted on yellow sticky traps placed within the lid of the pot. The traps were placed in the pots as soon as the first emerged adults were observed and kept in place for 14 days.

Results and Discussion

Results are given in Table 3. All the fungi reduced the sciarid fly populations although the overall level of control was low (c. 25% - 40% for L2 – L3) and the concentration of fungal spores applied is one that we consider to be relatively high. Still, the finding is a useful one because ascomycete entomopathogenic fungi are not usually associated with causing infections in larval diptera. As a general rule, the level of control increased with the age of the larvae, i.e. control was better when the fungus was applied to L2-L3 larvae than when it was applied prior to egg hatch. There are a number of possible explanations: (i) the fungal spores may not have persisted well in the substrate; (ii) the fungus has contact action, and larvae that moult before the spores have grown into the haemocoel are able to rid themselves of infection. Hence applications to early stage larvae present more opportunities for larvae to escape infection; (iii) later instar larvae are larger and therefore may pick up

larger numbers of spores from the substrate; (iv) we know from previous studies that spores applied to substrate tend to be concentrated in a band in the top few cm of the substrate. It may be that the later stage larvae are migrating more often to the top of the substrate surface to feed on the soya flour and hence exposing themselves to more fungal inoculum.

Table 3: Percentage emergence of adult sciarid flies from pots treated with different insect pathogenic fungi

Treatment	**Timing of application**		
	Pre egg hatch	**L1-L2**	**L2 –L3**
Water	91%	88%	85%
Metarhizium Met52	78% (14.3% infected)	74% (18.2% infected)	53% (18.1% infected)
Lecanicillium from adult sciarid	82% (20.3% infected)	76% (31.6% infected)	59% (16.9% infected)
Beauveria from Naturalis product	62% (15.2% infected)	56% (16.1% infected)	48% (20.8% infected)

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

04/3 Attractiveness of growing media as egg-laying sites for sciarid and shore flies (ADAS)

In Year 1, a pilot research glasshouse experiment had indicated that a Klassman organic substrate was more attractive to shore flies for egg laying and a glasshouse experiment on a commercial herb nursery showed that 40% composted green waste substrate was more favourable for the development of sciarid fly populations than peat-based or wood-fibre substrates. In the Year 2 work reported here, a glasshouse experiment was done on a commercial nursery to quantify shore fly population development in peat-based substrate compared with those containing composted green waste or wood fibre. In addition, laboratory olfactometer studies were done to determine whether sciarid and shore flies are attracted to the scent of volatile compounds released from the substrates.

Nursery experiment with shore flies

Materials and Methods

The experiment was done at Delfland Nursery, Dodington, Cambs (glasshouse vegetable propagator) and in a research glasshouse at ADAS Boxworth. All substrates were supplied by Bulrush Horticulture Ltd. and were made up in the following proportions by volume:

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

Twenty replicate plastic plant pots (6x6 cm) were filled to 2 cm below the rim with each of the five treatment substrates. The pots of substrate were watered to full water-holding capacity one day before transferring the pots to the nursery on 26 January 2010. The pots were placed beside a gutter where algae was present and beside some tomato plants grown in rockwool blocks with algae visible on the rockwool surface. Shore flies were observed on the algae on the rock wool blocks and on the vertical polythene sheet hanging beside the tomatoes. The pots were placed in 20 replicate blocks with one pot of each treatment placed randomly within each block. In order to maintain all substrates at full water-holding capacity, the grower was asked water the pots as necessary. The pots were left on the nursery for 23 days to allow the algae to develop on the substrate surface as a food source for the shore flies and for the females to lay eggs on the substrate. The pots were removed to ADAS Boxworth on 18 February 2010.

The % volume moisture of the five treatment substrates was taken in five replicate pots of each substrate on sequential dates. Four readings were taken per pot to obtain a mean reading per pot. The readings were taken at the set up of the experiment on 26 January and on 4 and 18 February just before the pots were removed from the nursery. Two further readings were taken on 24 February and 3 March when the pots of substrates were in a research glasshouse at ADAS Boxworth.

After returning the pots of substrate to a research glasshouse at ADAS Boxworth, each pot was placed in a larger ‘emergence’ pot with a small yellow sticky trap secured onto the inside of the emergence pot with a paper clip. Each emergence pot was covered with a ‘lid’ of insect-proof mesh to prevent emerging shore flies from escaping. The pots were laid out in a randomised block design and kept for five weeks in the glasshouse to allow all shore fly adults to emerge and to be caught on the sticky traps. The substrate in each pot was kept damp by using a piece of wet capillary matting in the base of each ‘emergence’ pot and by

daily overhead watering using a watering can with a fine rose. Numbers of shore fly adults per sticky trap were recorded. The data was analysed using analysis of variance (ANOVA).

Results and Discussion

Mean numbers of shore flies emerging per pot are shown in Figure 1. Significantly more shore fly adults emerged from the 10% composted green waste substrate than from the other substrates ($P<0.05$). The results indicate that either shore fly females were more attracted to the 10% composted green waste substrate than the other substrates for egg laying, and/or this substrate was more suitable for shore fly larval development.

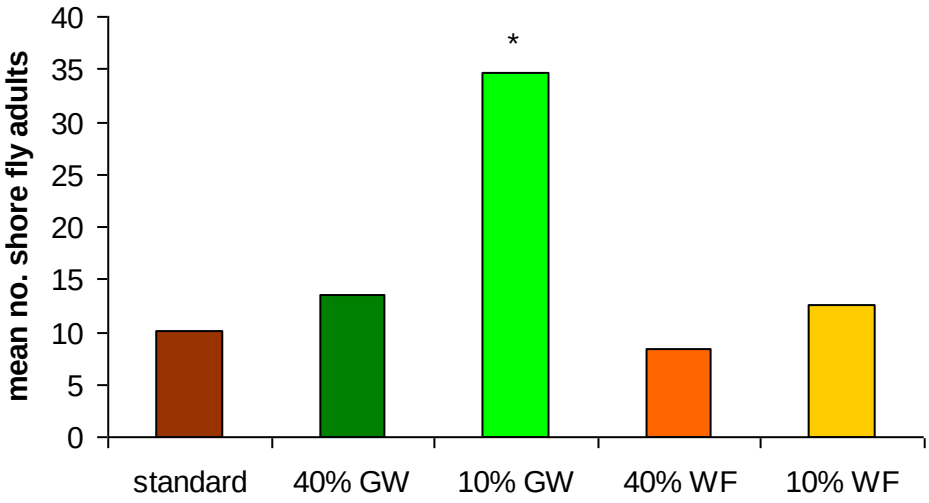

Figure 1. Mean number of shore fly adults emerging per pot. GW = composted green waste, WF = wood fibre. * Significantly more in composted green waste substrate than other substrates, *P*<0.05.

The mean % volume moisture readings are shown in Table 4. The composted green waste substrates had consistently higher moisture readings than the other substrates and this difference was significant for both green waste mixes on 18 and 24 February and on 3 March. The 40% composted green waste substrate had significantly higher moisture readings than the 10% composted green waste substrate on 4, 18 and 24 February. The two wood fibre substrates and the standard substrate have similar readings throughout. Although these results indicated that the composted green waste substrates had higher % volume water than the other substrates, there were no visible differences in the amount of algae growing on each substrate type. However, this was difficult to quantify as the algae grew on the strands of wood fibre whereas it covered the whole surface of the other substrates.

Table 4. Mean % volume moisture readings in the substrate treatments. GW = green waste, WF = wood fibre.

Date	26 Jan 2010	4 Feb	18 Feb	24 Feb	3 March
standard	29.6	42.5	40.9	48.4	39.6
10% GW	25.9	46.0	48.3	54.4	50.8
40% GW	29.5	50.8	50.2	58.2	53.5
10% WF	25.6	43.3	41.2	49.7	45.1
40% WF	22.1	38.6	41.8	50.4	44.1

Olfactometer studies

Olfactometer studies were done with both sciarid and shore fly adults to determine any attraction to the scent of the substrates used in the experiments on commercial nurseries in both Years 1 and 2. The static air olfactometer was supplied by Warwick HRI and has previously been used for work with mushroom sciarid flies (Tibbles *et al*. 2005). The olfactometer consists of a 20 x 12 x 8cm perspex release arena with 2 cm diameter ventilation holes in the lid, covered with fine mesh (Figure 2). The fly release device consists of a specimen tube that is secured in the base of the arena with Blue-tack ®. Flies are pooted into the tube using an aspirator and the tube is placed in the arena for release. The test pots (clear plastic pots with white polythene lids) are positioned underneath the release arena and connected to the arena by glass tubes (6cm x 0.6cm, four to each test pot). Holes were burnt into the plastic base of the release arena and glass tubes positioned flush to the arena floor and held in place by all-purpose glue, creating a pit-fall trap from which the flies cannot easily escape. The lids of the test pots had corresponding holes burnt in them and the lids were pushed into the glass tubes, which protrude from the base of the arena. The pots were prepared with a 30 gram layer of plaster of Paris (3:4 ratio of water to plaster). The plaster of Paris is moistened with water to maintain the humidity of the test substrates placed in the test pots.

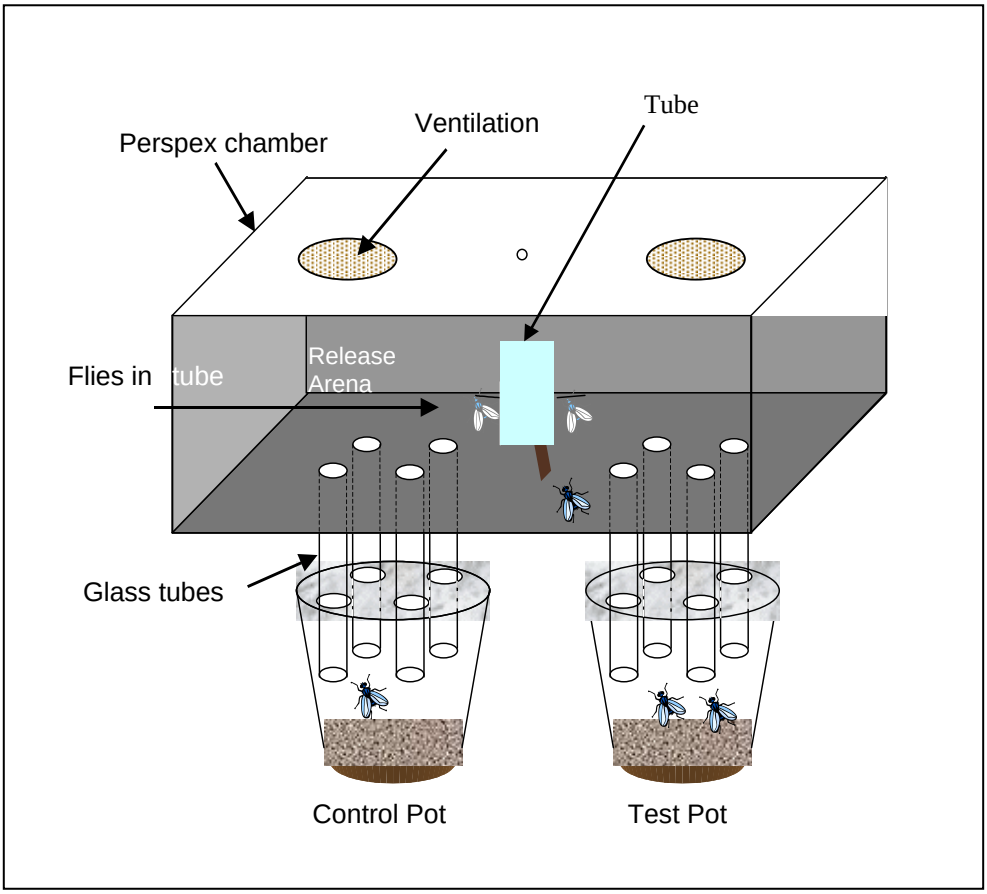

Figure 2. Static air olfactometer, adapted from Tibbles *et al*. 2005.

The olfactometer was used to test the preference for sciarid or shore flies for the following pairs of treatments:

1. Blank control (damp cotton wool) vs. standard peat-based substrate
2. Blank control vs. 40% composted green waste, 60% standard peat based substrate
3. Blank control vs. 40% wood fibre, 60% standard peat based substrate
4. Standard peat based substrate vs. 40% composted green waste, 60% standard peat based substrate
5. Standard peat based substrates. 40% wood fibre, 60% standard peat based substrate
6. 40% composted green waste vs. 40% wood fibre substrate

Between four and eight replicate bioassays for each paired treatment were done. The bioassays for treatment pairs 3 (sciarid flies), 4 (shore flies) and 5 and 6 (both flies) are still to be completed and will be reported in the final report.

For the blank control pot, 5g of cotton wool was placed in the pot on top of the plaster of Paris and moistened with 37.5 g of water to ensure the two pots have the same relative humidity. Each test substrate was wetted up with water (approx. 100 ml to one litre of substrate). The % volume moisture was checked using a Delta T SM200 moisture meter. Five replicate readings were taken to provide a mean % volume moisture. More water was added if required to give 35-45% volume moisture. For each of the test substrates 100ml of substrate was placed on top of the plaster of Paris in the test pot and pushed down slightly to ensure there was space between the substrate and the glass tubes to allow the flies to enter the pot.

Approximately 100 adult sciarid flies or 200 shore flies were collected from laboratory or glasshouse cultures respectively and pooted into the release tube using an aspirator. The tube was then placed within the release arena. The flies were released and the lid placed on the release arena as quickly as possible to prevent escape of flies. For paired treatments 4-6 the control blank pot was replaced by the other test compost. The pots were labelled with the treatment and replicate number. Once the flies had been added the olfactometer was placed in an incubator for 24 hours at 25°C with no lighting, to reduce any visual stimuli from the test pots. It was then placed in a refrigerator for a few minutes to reduce fly activity and potential escape when the test pot lids were removed. The lids with the glass tubes were then removed from each test pot and a piece of yellow sticky trap placed over the top of each pot. The sticky traps completely covered the top of the pots to prevent fly escape. Once the sticky traps were in position the pots were placed in an incubator at 25°C with lights on a 16 hour light:8 hour dark cycle. The pots were left in the incubator for a minimum of 24 hours to allow all flies within the pot to be caught on the sticky trap. Any flies remaining in the olfactometer arena were pooted into a tube to which 70% alcohol was added.. The numbers of male and female flies in the tube were recorded after examination under a low power microscope. The sticky traps from the test pots were examined under a microscope and the numbers of males and females recorded. The data was analysed using Regression analysis.

Results and Discussion

Sciarid flies: The results are shown in Table 5. There were no significant differences between the proportion of sciarid fly adults in the control pots with damp cotton wool and in the test pots containing standard peat-based compost. This result indicates that sciarid flies respond to the higher humidity in the control and test pots than in the release arena. A significantly higher proportion of sciarid fly adults (67.1%) were recorded in the pots with 40% composted green waste substrate than in the control pots (23.7%, $P<0.05$). This result indicates that sciarid flies respond to the smell of the 40% composted green waste substrate.

When given the choice of peat-based or 40% composted green waste substrates, there was no significant difference in the proportion of sciarid flies in either test pot, but a significantly higher proportion chose to move to the pot with the 40% composted green waste (47%) rather than remaining in the release arena (18.1%).

Table 5. Percentage of sciarid fly adults in the blank control pot, the test substrate pot and the release arena 24 hours after releasing adults to the olfactometer arena. GW = green waste. * Significantly more in 40% composted GW substrate than in control. $^{+}$ Significantly more in 40% composted GW substrate in release arena ($P<0.05$).

% flies in blank control	% flies in standard peat-based substrate	% flies in 40% composted GW	% flies remaining in release arena
43.5	51.8	N/A	4.7
23.7	N/A	67.1*	9.2
N/A	34.9	47.0^{+}	18.1

Shore flies: The results are shown in Table 6. A significantly higher proportion of shore fly adults (52%) were recorded in the pots with the standard peat-based compost than in control pots with damp cotton wool (32.2%, $P<0.05$). There were no significant differences between the proportion of shore flies in the control pots and in the pots with 40% composted green waste substrate. A significantly higher proportion of shore fly adults (48.9%) were recorded in control pots with damp cotton wool than in the pots with 30% wood fibre substrate (35.8%, $P<0.05$). These results should be interpreted with caution as fresh substrates were used in these initial olfactometer bioassays with shore flies. The same substrates will be re-tested using substrates on which algae have been allowed to grow in a glasshouse, as shore flies are likely to be attracted to their food source (algae) and algae may grow more readily on some of the test substrates. As more shore fly adults emerged from 10% composted green waste substrate on which algae had been allowed to grow in the nursery experiment reported above, this substrate with algae will also be used in further olfactometer bioassays with shore flies.

Table 6. Percentage of shore fly adults in the blank control pot, the test substrate pot and the release arena 24 hours after releasing adults to the olfactometer arena. GW = composted green waste substrate. WF= wood fibre. * Significantly more than in paired test pot (P<0.05).

% flies in blank control	% flies in standard peat-based substrate	% flies in 40% composted GW	% flies in 40% WF substrate	% flies remaining in release arena
32.2	52.0*	N/A	N/A	15.8
51.1	N/A	44.9	N/A	4.0
48.9*	N/A	N/A	35.8	15.3

A mixture of male and female sciarid and shore fly adults were used in these olfactometer bioassays. This was partly due to the difficulties in determining the sex of the flies when alive and active, and also so as not to interfere with the natural fly behaviour in a mixed sex population. Further analyses of the data will be done to determine any preferences for female sciarid and shore flies to selected substrates for egg-laying.

O4/4 Effect of temperature on the development of sciarid fly (Bradysia difformis)

Materials and Methods

The aim of this work package was to measure the rate of development of *B. difformis* from egg to adulthood in peat-based substrate at different temperatures. The effect of temperature on the development of the shore fly *S. tenuicosta* has already been determined (Vanninen, 2001). The effect of temperature on the rate of development of *B. difformis* has not been measured before although some information is available for other *Bradysia* species (Harris *et a*l., 1996).

Cultures of *B. difformis* originated from a grower's holding and were reared on a mixture of 25 g soya flour and 500 g Irish moss peat (35% moisture content) (Vitax, Coalville, Leicestershire, UK) within a plant propagator (25x18x20 cm, Stewart Plastics, Croydon, UK) at 25°C in darkness. The lid of each propagator was vented with two 2 cm diameter holes plugged with cotton wool. Cultures were initiated by introducing 50 gravid adult female *B. difformis* into the propagator. The first filial generation of adult *B. difformis* emerged after approximately 21 days.

For egg collection, an adaptation of the method for obtaining different larval instars of *Lycoriella ingenua* (Binns, 1973) was used. Gravid adult female *B. difformis* (180–220) were

incubated for 3 days at 25°□C within an oviposition chamber, which consisted of a cylindrical plastic frame (5 cm high x 6 cm diameter) covered in fine mesh nylon gauze, placed on the base of a Petri dish (7.5 cm diameter), which contained 25 g Irish moss peat. The oviposition chamber was placed on moistened tissue paper in a plant propagator (23 cm high x □9 cm diameter, Stewart Plastics) to maintain a humid atmosphere. Eggs were extracted from the surface of the peat by flotation on water, then pipetted onto a mixture of 5% wt/wt soya flour in Irish moss peat within Petri dishes and incubated at 5 temperatures 10, 15, 20, 25 and 30°C in darkness. Dishes were examined daily and because there are no simple morphological changes during the development of sciarid larvae (Binns, 1981a,b), instars were identified from the size of the head capsule using light microscopy (White & Smith, 2000).

Results and Discussion

Results are given in Table 6. Total development time was inversely proportional to temperature, with the optimal development temperature (i.e. the shortest development time) being > 30°C. At the lowest temperature studied, 10°C, eggs hatched slowly and did not develop to complete the larval stage. There was no emergence of adults at 10°C even after 70 days of incubation, however when the pots were transferred to 20°C a small number of adults emerged after 7 days (5 – 10%). At all the temperatures used, larvae were seen moving close to the soil surface prior to pupation. At lower temperatures (≤ 20°C) larvae were observed on the soil surface for greater periods than at the higher temperatures. This may indicate a mismatch between the optimum temperature for sciarid development and the best conditions for the transmission of *Furia* infections: the fungus is likely to be transmitted more effectively at lower temperatures because transmission occurs on the substrate surface. Infected larvae move up to the surface to die, then *Furia* ballistospores are formed and actively discharged into the air. Spores that are acquired by a sciarid larva will germinate and penetrate the insect, whereas spores that do not contact a larva will germinate to form secondary ballistospores that are actively discharged into the air again. Thus, the longer a larva remains at the surface, the more likely it is to contact *Furia* ballistospores. We do not yet know the optimum temperature for the development of *Furia* infections, but we can predict that transmission of the disease is likely to be higher at or below 20°C.

Table 6. Time (days) between different developmental stages of *B. difformis* reared at different temperatures

	Developmental stage				
Temperature	**Egg**	**Larval**	**Pupal**	**Adult**	**Total**
10°C	14		-	-	-
15°C	7	16	7	4	34
20°C	6	8	4	3	21
25°C	6	5	3	3	17
30°C	5	5	3	3	15

O4/5 Multifactorial experiment: interaction of substrate type, moisture and temperature on sciarid development

The original aim of this experiment was to test a hypothesis that although fly development rate from egg to adult is mainly a function of temperature, survival of the flies is affected by substrate composition and substrate moisture. The experiment as outlined in the grant application would quantify the development time from egg to adult and survival rate of flies in selected growing media with contrasting properties at constant and fluctuating temperatures in a range of controlled substrate moisture levels. The experimental design was finalized in consultation with our industry partners and has been modified from the original plan, partly because of new information obtained from the analysis of the physico-chemical properties of different substrates done in Year 1. This showed that standard (peat based) bedding substrate, composted green waste substrate and wood fibre substrate had very large differences in conductivity. There was no correlation between substrate composition and microbial activity but there was a correlation between water content of the medium and microbial biomass (this is important to our project because sciarid larvae feed on microorganisms within the substrate). The industry partners advised that, given this new information, the original experiment had too many interacting variables and this was likely to make interpretation of the results difficult. Because the different substrates have different water retaining capacities and bulk densities, the mathematical relationship between moisture content and temperature is likely to vary between different substrates, and this is likely to have knock on effects on microbial biomass accumulation which in turn will affect fly development. Therefore, a new experiment was designed with a reduced number of variables. This is ongoing at the time of writing. The protocol is as follows: Adult *Bradysia difformis* originate from a growers holding as described above and reared on a mixture of soya flour and moss peat at 25°C in darkness. Three substrates will be compared: standard peat based bedding substrate, composted green waste substrate and wood fibre substrate, obtained from Bulrush Horticulture. The composted green waste and wood fibre substrates are mixed by hand with peat to a 40% total volume and total wet weight adjusted to that of the standard bedding peat substrate. Batches of 100 sciarid fly eggs are placed on the substrate within experimental pots (10 x 8 cm polystyrene, vented lids) containing 100g of

substrate including 5g soya flour, pressed by hand to a depth of 75mm . Pots are incubated at 25°C, 16:8 L:D, and after 7 days each pot removed and covered with a sticky trap. The traps are used to catch the adult flies emerging from the substrate. The pots are maintained at 25 °C, 16:8 L:D, in a controlled environment room for 17 days, after which the sticky traps are inspected daily for 14 days and the number of adult *B. difformis* caught on each trap per day is recorded.

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 substrate 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.
- *Furia* infection can occur within two weeks of young sciarid larvae hatching from eggs laid on freshly potted plants.
- Healthy sciarid larvae can eat infected larvae, during which they could pick up infection by coming into contact with the infective spores on the body of the dead larvae.
- *Furia* infection can reduce sciarid fly populations to such low levels that the fungus can die out quickly on nurseries due to lack of host insects.
- The optimum temperature for the development of sciarid flies from egg to adult is greater than 30°C. Larvae will not develop at 10°C, and development is very slow at 15°C, taking 30 days from egg hatch to adult emergence. Transmission of the *Furia* infection is probably more effective at lower temperatures (20°C and below) when sciarid larvae appear to spend more time on the substrate surface and hence are more likely to pick up spores.
- Populations of sciarid larvae may be partially controlled using commercially available fungal biopesticides.
- In an experiment on a commercial nursery, more shore flies emerged from substrate with 10% composted green waste than from peat-based substrate or with 40% composted green waste or with 10% or 40% wood fibre. This indicates that shore fly females are more attracted to 10% composted green waste, and/or this substrate is more suitable for shore fly larval development.

- Olfactometer studies indicated that sciarid fly adults are attracted to the smell of 40% composted green waste substrate. This result is consistent with those in glasshouse experiments in Year 1 and with grower observations that composted green waste substrates lead to greater problems with sciarid flies.

Knowledge and Technology Transfer

- STING (IOBC newsletter) October 2009
- HDC news November 2009
- HDC/BPOA Technical seminar February 2010
- ADAS Bedding and Pot plant notes article June 2010
- BOPP AGM and Seminar `A place to take root - optimising growing media use and plant nutrition – July 2010
- Consortia meetings on 11 February 2010 (full consortium) and 11 March 2010 (science partners).

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