Title of project:	Determination of the effect of commercially grown strains of cultivated mushrooms on the development and multiplication of mushroom pests.
Project number:	M 25a
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Date commenced:	April 1 1999
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Key words:	Mushroom, Strain, Insect pest, Sciarid, Phorid

CONTENTS

PRACTICAL SECTION FOR GROWERS

BACKGROUND

In MAFF funded work (HHH1735SMU and HHH1730SMU) and in HDC project M 25, it was found that mushroom pests reacted differently in terms of generation time, fecundity and increase rates when reared on different cultivable species of *Agaricus*.

This project, M 25a, tested twelve commercial strains of *Agaricus bisporus* to determine their susceptibility to sciarids and phorids. This was assessed in terms of variation in population development. The two strains demonstrating the most extreme effects on each pest were to be further tested in semi-commercial trials on the mushroom unit.

Variations in response to these spawns could be employed as a non-chemical aid to mushroom pest control on all types of mushroom farm, by limiting reproduction rates. It might also be possible to identify 'fly-breaker' strains - analogous to the 'virus-breaker' strains - and use them, with chemicals if necessary, in the management of any long-term pest problem. Knowledge of such natural resistance may also facilitate future breeding programmes.

SUMMARY OF RESULTS

LABORATORY TESTS

The twelve commercial strains of *Agaricus bisporus* tested are shown in Table 1.

Spawn company	Strain code
Amycel	2100
Amycel	2810
Hauser	A15
International Spawn	I501
International Spawn	I2001
Le Lion	C9
Le Lion	X22
Le Lion	X25
Somycel	S512
Sylvan	A12
Sylvan	S130
Sylvan	C3.8

Table 1. Spawn strains used in the laboratory tests.

These spawns were chosen to represent those spawns most used by the UK mushroom industry at the start of the project. Choice was also influenced by spawn variability as determined by existing DNA finger-printing data.

Commercial grain spawns of each strain were obtained to ensure that the results related to the normally available product. For the phorid tests, pots of compost were inoculated with a candidate spawn and the mycelium allowed to permeate it for a number of days before being exposed to known numbers of the pest. For the sciarid tests, pots of compost were inoculated with the spawn to give a range of spawn running periods (0, 1, 2 & 3 days) before being confined in small rearing chambers with known numbers of flies. In each case the pots were removed and placed in an incubator after four days. The number of flies subsequently emerging from the pots was used to determine the susceptibility of each spawn strain. Each test with each pest was repeated to validate the results.

Results

Phorids: The mean phorid emergence from both tests indicated that A15 was the most susceptible strain, closely followed by X25 (Fig. 1). Three strains appeared to constrain phorid numbers: 2810, I501 and C3.8. These were tested further and C3.8 proved to be the least susceptible strain. Thus A15 and C3.8 were chosen as being the most and least susceptible mushroom strains to phorids and were taken forward into the semi-commercial trials in the mushroom unit.

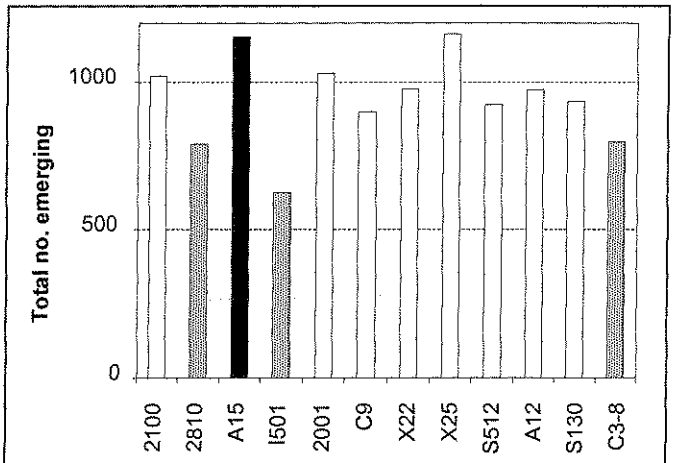

Figure 1. Phorid emergence from 12 strains of *Agaricus bisporus*

Sciarids: Strain C9 was the least susceptible to sciarid development in all the tests (Fig. 2). Sciarid development was strongly influenced by the amount of mycelial growth. Thus emergence from pots that had three days spawn growth before the introduction of flies produced the greatest effect. Five strains appeared to be the most consistently susceptible to sciarids: 2810, A15, S512, I510 and X25. These were re-tested and the results indicated that 2810 was the most susceptible strain. Thus 2810 and C9 were

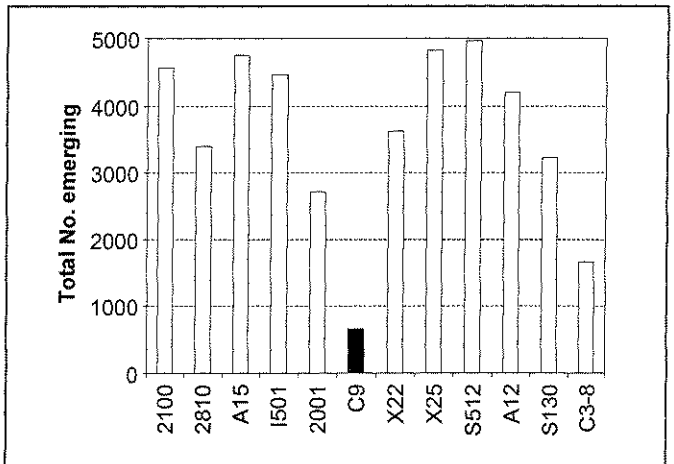

Figure 2. Sciarid emergence from 12 strains of *Agaricus bisporus*

chosen as being the most and least susceptible mushroom strains to sciarids and were taken forward into the semi-commercial trials in the mushroom unit.

Discussion

The different strains used in these tests showed varying effects on mushroom fly pest development.

The results from the two phorid tests were more variable than expected. This may have been due to the phorid infestation method - which required anaesthetisation and microscopic sexing of the adult flies - and a propensity for the flies to escape from the infestation chamber! However, strain A15 produced the largest mean number of phorids and, after re-testing three strains that produced low numbers of flies in both tests, strain C3-8 was found to be the least susceptible.

The results from the two sciarid tests were more consistent. Most strains showed a decrease in emergence as the amount of mycelium increased, the exceptions being strains I501, A15, S512, X25 and 2810 - the five strains re-tested to check which was most susceptible to sciarids. In both tests, C9 produced greater numbers of flies where the infestation coincided with spawning and where, therefore, there was no actively growing mycelium present.

MUSHROOM UNIT TRIAL

Method

General protocol

Two mushroom crops, prepared from the same compost, were grown under conditions typical of the UK mushroom industry. Trays (0.56 m^2) of peak-heated compost (50 kg/tray at spawning) were spawned with one of the selected spawn strains (spawning day = Day 0). The crops were spawn-run in four separate cropping houses for about 17 days before being cased with a pre-mixed commercial casing. The first mushrooms appeared about 2-3 weeks later and were produced in a sequence of flushes, each being at about 7-10 day intervals. The crops were harvested as would a commercial crop, with yield data (weight) being recorded on an individual tray basis.

MUSHROOM UNIT TRIAL

Four batches of HRI F3 compost, 24 trays per batch, were spawned with the chosen strains from the laboratory tests. Immediately after spawning, the trays were placed in the mushroom chambers, one for each strain. The crops were spawn-run, cased, watered and harvested according to HRI standard practice. There were three flushes of mushrooms. Five days after spawning two chambers with either 2810 or C9, 240 gravid female sciarids were released into each chamber at the rate of 10 per tray. Seven days after spawning two chambers with either A15 or C3.8, eight 'seed' pots of phorids were placed in each

chamber. In all four chambers, inverted sticky traps were placed on the surface of each tray immediately after casing to obtain an estimate of the number of flies developing. The number of flies caught was recorded daily where possible.

Results

Pest population development

Phorids crops: Phorids started to emerge 20 days after infestation in the A15 crop and two days later in the C3.8 crop. Fewer flies emerged form the C3.8 crop than the A15 crop throughout the cropping period. An overall reduction of 44% was evident by the end of the three-flush cropping period.

Sciarid crops: In both crops, sciarids started to emerge 21 days after infestation. Initially, a 64% reduction in flies was observed in the C9 crop, compared to the 2810 crop. As the crop progressed, the difference between the two crops became less pronounced such that, by the end of the three-flush cropping period, the overall reduction in the C9 crop was 31.5%.

Yield

It is not possible to compare the yield from the brown strain crops to that of the white strain crops as the former normally produce a lower yield of mushrooms. However, the weight of damaged mushrooms was reduced from 1.73% in the 2810 crop to 0.79% in the C9 crop.

CONCLUSION

In the laboratory tests and mushroom unit trials the brown strains C3.8 and C9 appear to be less susceptible than the white strains A15 and 2810 to phorid and sciarid development, respectively. Where economically feasible therefore, these brown strains could be used to help reduce fly populations on a mushroom farm. Strain C3.8 was the second least attractive strain to sciarids in the laboratory experiments so it might, therefore, be possible to grow this strain to reduce the populations of both fly pests by about 30 to 40%.

In the laboratory tests, there was also some variation in the ability of the white strains to support fly development. It would, however, be imprudent to extrapolate directly from these laboratory results straight to a recommendation suitable for growers to act upon. Candidate spawn strains would need to be field tested on the mushroom unit to determine whether there is sufficient variability between such strains for practical use before such recommendations could be proposed.

SCIENCE SECTION

INTRODUCTION

BACKGROUND

Pests are a constant threat to the successful commercial production of mushrooms and about £3.5M/annum is being spent on control measures. Although this mitigates the problem, losses in production of approximately £7M/annum are still being incurred due to direct larval action and associated disease spread by adult flies. Less quantifiable but often just as important, are 'fly' factors such as nuisance value to mushroom staff (especially pickers), presence in mushroom pre-packs and incursion into private dwellings. Uncontrolled populations cause far greater losses and can even cause complete crop failures.

Larval control relies on single insecticides for each pest species due to product withdrawal, crop intolerance and insecticide resistance. Up until very recently, the organophosphorous insecticide, diazinon, was available (and remained viable) for control of mushroom phorid larvae (*Megaselia halterata*). However, production of diazinon has now stopped and the limited supplies that remain available to growers will soon run out. Phorid control in the very near future, therefore, will be extremely problematic. Chemical control of sciarid fly larvae relies on just two, similarly-acting, insect growth regulators - diflubenzuron and methoprene – and even here, the latter insecticide is likely to be withdrawn in the very near future. There is a similar dearth of approved adulticides. Reliance on so few active compounds leaves pest control extremely vulnerable to further resistance or product-withdrawal problems. Pest control in the future will be increasingly difficult yet, without adequate protection, the mushroom crop is extremely susceptible to pest attack.

An important factor in the multiplication of pests within a mushroom crop is the strain of mushroom used by the grower, as the way in which the three main mushroom pests - sciarids (*Lycoriella auripila*), phorids (*Megaselia halterata*) and cecids (*Heteropeza pygmaea* and *Mycophila* spp) - infest and develop within a mushroom crop is inextricably linked to growth and development of the mushroom itself. The way in which a pest attacks a crop is determined by the various properties of the developing mycelium, for example the production (or otherwise) of attractant or repellent volatiles. Conversely, the degree of mycelial development and subsequent cropping will depend on the habits and abundance of the pest.

For example, it is well established that phorid female flies are attracted to the spawn-running phase of mushroom production, subsequently laying their eggs close to the growing mycelium. A number of volatiles, produced by the mycelium as it grows through mushroom compost, have been isolated and tested for their potential to attract phorids. It is also known that phorid larvae are obligate mycelial feeders *i.e.* they require mycelium as a food source in order to develop.

The sciarid differs from the phorid in a number of ways. The adults are not attracted to the smell of mushroom mycelium to the same degree as phorids, but are mostly attracted

before mushroom spawn is added to the compost. It has even been shown that a compost, well colonised by mushroom mycelium, actually inhibits oviposition by sciarid females. Sciarid larvae, although not obligate mycelial feeders, do react to mushroom mycelium in a number of ways. In a newly spawned compost, larvae feed quite happily on the edge of the growing mycelial front. Conversely, large amounts of mycelium inhibit their development - probably due to the accumulation of a metabolic by-product of mycelial growth.

From each grower's point of view, it would be extremely advantageous to know whether the strain of mushroom that he was growing encouraged or discouraged mushroom pests. Mushroom farms can often be described as 'phorid' or 'sciarid' farms, being mostly infested by one or other of the major pests. Strains of mushrooms, resistant to pests, could be employed to good effect to minimize or eradicate mushroom pests. Such strains are likely to be of use wherever in the world they are grown, as the pests afflicting mushrooms are similar, world-wide. Environmentally and from the consumer's point of view, such strains would have a beneficial effect as the use of pesticides, harmful to the operator, the crop and the environment, could be minimized or even eliminated. Such strains could be used by all types of mushroom farm, irrespective of the system used for growing mushrooms

It is not known how mushroom pests react, in terms of generation time, fecundity, increase rates etc, to the current commercial spawns. Variations in response to these spawns could be employed as a non-chemical aid to mushroom pest control, by limiting reproduction rates. It might also be possible to identify 'fly-breaker' strains. These would be analogous to the 'virus-breaker' strains in as much as they would reduce the general pest problem on a farm yet allowing economically viable crops to be grown. They could be used, therefore, with chemicals if necessary, in the management of any long-term pest problem.

RELATED WORK

This project follows up the earlier HDC-funded project (M 25), in which variation in phorid and sciarid development, when reared on a number mushroom strains, was shown to exist. A six month MAFF-funded ROAME (HH1730SMU (Determination of the variation in the development and multiplication of mushroom phorids (*Megaselia halterata*) when reared on various cultivable fungi - completed March 1996) screened various cultivable fungi for the presence of natural resistance to mushroom phorids. In an extant MAFF-funded ROAME (HH1735SMU - Determination of the variation in the development and multiplication of mushroom sciarids when reared on various cultivable fungi) the fungi were screened for the presence of natural resistance to mushroom sciarids. With the exception of one commercial strain, which is common to all projects, the fungi chosen for the MAFF projects are not commercial strains but represent as much genetic diversity as possible within the compost colonising members of the genus *Agaricus*.

With these exceptions and as far as is known, no similar work on mushroom pests is being carried out, although analogous work is being done on diseases.

TARGET

The overall objective of the project was to determine which, if any, of the commercial strains of *Agaricus bisporus* tested possessed natural resistance to sciarids and phorids. This was assessed in terms of variation in population development. Specific objectives that link in to the Milestones and Programme of Work were:

1. Obtain twelve commercial mushroom strains from commercial sources and determine their rate of growth in a standard mushroom compost.
2. Determine the effect of the mushroom strains on sciarid development using a pot-based testing system in the laboratory.
3. Determine the effect of the mushroom strains on phorid development using a pot-based testing system in the laboratory.
4. Identify the four mushroom strains that, in laboratory tests, are most and least susceptible to phorid and sciarid population development.
5. Carry out crop validation trial of the identified strains against the mushroom phorid.
6. Carry out crop validation trial of the identified strains against the mushroom sciarid.
7. Prepare report of research findings.

These objectives were broadly interdependent and sequential, progression to any particular objective being dependent on the successful completion of the previous one.

LABORATORY INVESTIGATIONS

Method

Fungi:

The twelve commercial strains of *Agaricus bisporus* tested are shown in Table 1.

Spawn company	Strain code
Amycel	2100
Amycel	2810
Hauser	A15
International Spawn	I501
International Spawn	I2001
Le Lion	C9
Le Lion	X22
Le Lion	X25
Somycel	S512
Sylvan	A12
Sylvan	S130
Sylvan	C3.8

Table 1. Spawn strains used in the laboratory tests.

These spawns were chosen to represent those spawns most used by the UK mushroom industry at the start of the project. Choice was also influenced by spawn variability as determined by existing DNA finger-printing data.

Stage 1: Laboratory tests on spawn vigour

Commercial grain spawns of each strain were obtained to ensure that the results related to the normally available product. To ensure appropriate comparisons in subsequent experiments, the comparative growth rates of these strains was determined. For each strain, six grains of spawn were placed in the bottom of 28 mm diameter test tubes (3 tubes per strain). 30g of freshly pasteurised compost was then placed into the tube and compressed to a 100 mm depth thus ensuring intimate contact with the spawn grains. The tubes were then placed into an incubator at 25 C. The extent of mycelial growth through the compost was assessed on a daily basis to determine the time taken for the mycelium of each strain to grow 10 mm from the inoculum point.

Stage 2: Laboratory tests - phorids

The performance of each spawn strain was tested against phorids separately to assess individual effects. For each strain, eight pots of compost - inoculated according to the results from Stage 1 to give 10 mm diameter colonies for all the strains - were confined in a small rearing chamber. 40 each of female and male phorids were introduced into each chamber. The pots were kept in the infestation chamber for four days before removing them to an incubator at 25 C. Subsequent emergence of adults was used to determine the effects of the spawn strains on pest development. To ensure that the results were consistent, the test was repeated.

Stage 3: Laboratory tests - sciarids

The performance of each mushroom strain against sciarids was tested separately to assess individual effects. Pots of compost were inoculated with each strain to give a range of spawn running periods (0, 1, 2 & 3 days) before being confined in small rearing chambers. For each spawn-running period, eight replicate pots of each strain were tested. Twenty four sciarid females and six males were then introduced into each chamber. The pots were kept in the infestation chambers for four days before removing them to an incubator at 25°C. Subsequent emergence of adults was used to determine the effects of the spawn strains on pest development. To ensure that the results were consistent, the test was repeated.

Stage 4: Analysis of laboratory tests results

From the results, the putative susceptibility of each strain to phorids and sciarids was determined. For each pest, the most and the least susceptible strains were tested further on the HRI Mushroom Unit in crop validation trials.

Results

Race tube tests

Most strains reached 10 mm diameter after six days but strains A2100 and C3.8 took seven days, I501, 2001 and X22 took five and A15 only four days.

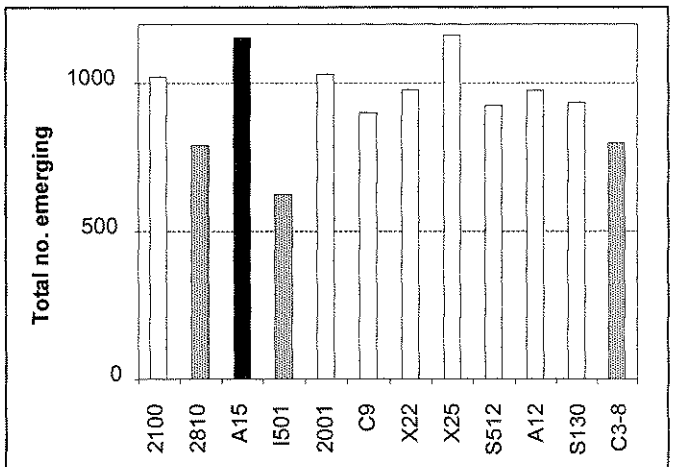

Figure 1. Phorid emergence from 12 strains of *Agaricus bisporus*

Phorids

Phorids emerged from the pots of compost after 22 days. There were no obvious strain effects on emergence time. There were variations in response of the phorids to the strains between the two tests but A15 was consistently attractive and I501, C3.8 and 2810 were both in the lower end of the 'attractiveness' in the two tests. The mean number of phorids from both tests is shown in Figure 1.

Three of the least susceptible strains were re-tested to determine which should go forward to the semi-commercial trials. The results from this re-test showed that strain C3.8 produced 34% and 62% fewer phorids than strains 2810 and I501 respectively.

Sciarids

Flies started to emerge from the pots of compost after about 24 days but with strains C9, X22 and X25 emergence was delayed by one or two days from the pots spawn-run for three days prior to infestation. The mean numbers of sciarids that emerged from both tests are shown on Figure 2.

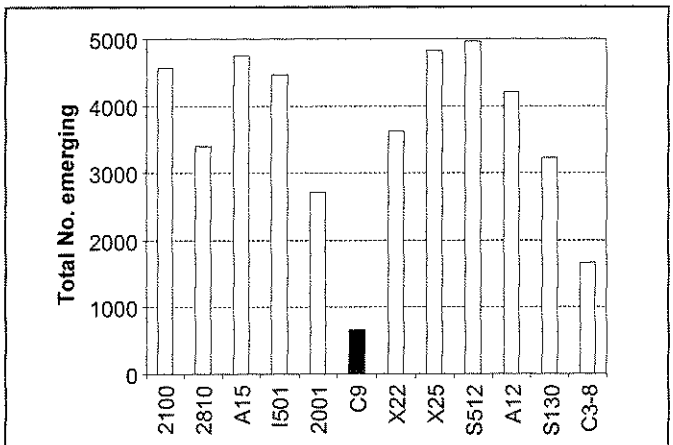

Figure 2. Sciarid emergence from 12 strains of *Agaricus bisporus*

The five most susceptible strains were re-tested to determine which should go forward to the semi-commercial trials (Fig. 3). Strain 2810 produced 1%, 5%, 24% and 39% more sciarids than strains I501, A15, S512 and X25, respectively.

Discussion

The different strains used in these tests showed varying effects on mushroom fly pest development.

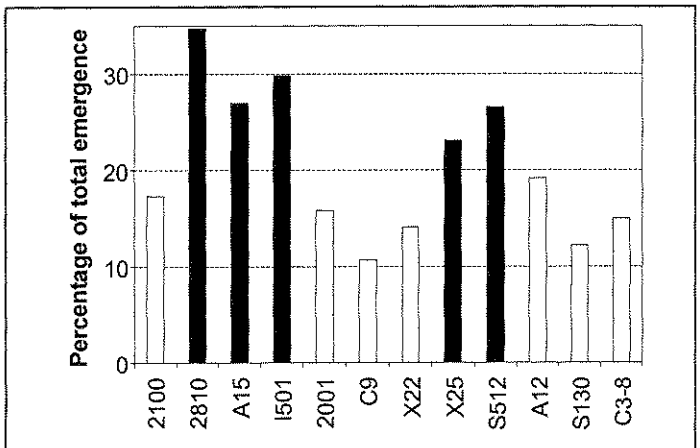

Figure 3. Sciarid emergence from 12 strains of *Agaricus bisporus* on day 3

The results from the two phorid tests were more variable than expected. This may have been due to the phorid infestation method - which required anaesthetisation and microscopic sexing of the adult flies - and a propensity for the flies to escape from the infestation chamber! However, strain A15 produced the largest mean number of phorids and, after re-

testing three strains that produced low numbers of flies in both tests, strain C3-8 was found to be the least susceptible.

The results from the two sciarid tests were more consistent. Most strains showed a decrease in emergence as the amount of mycelium increased, the exceptions being strains I501, A15, S512, X25 and 2810 - the five strains re-tested to check which was most susceptible to sciarids. In both tests, C9 produced greater numbers of flies where the infestation coincided with spawning and where, therefore, there was no actively growing mycelium present.

MUSHROOM UNIT TRIAL

Method

General protocol

Two mushroom crops, prepared from the same compost, were grown under conditions typical of the UK mushroom industry. Trays (0.56 m^2) of peak-heated compost (50 kg/tray at spawning) were spawned with one of the selected spawn strains (spawning day = Day 0). The crops were spawn-run in four separate cropping houses for about 17 days before being cased with a pre-mixed commercial casing. The first mushrooms appeared about 2-3 weeks later and were produced in a sequence of flushes, each being at about 7-10 day intervals. The crops were harvested as would a commercial crop, with yield data (weight) being recorded on an individual tray basis.

Phorids

Specific protocol: Each crop (A15 and C3.8) consisted of 24 trays arranged in 2 rows each of three stacks of four trays. In addition to any natural infestation that may have occurred, each crop was artificially infested with eight 'seed' pots of phorids on Day 7. To determine the subsequent population development in the crop, an emergence trap, consisting of an inverted 'U'-shaped sticky trap (sticky side down), was placed on the surface of each tray after the casing was applied. These were counted daily, where possible, and replaced at suitable intervals for the duration of the crop.

Sciarids

The *General* and *Specific protocols* for the sciarid tests were the same as outlined for phorids, except that the crops (2810 and C9) were artificially infested with 240 gravid female sciarids three days after spawning.

Results

Phorid population development

In the A15 crop, phorids started to emerge 20 days after infestation (Fig. 4). Flies from the C3.8 crop started to emerge two days later. Fewer flies emerged form the C3.8 crop than the A15 crop throughout the cropping period. An overall reduction of 44% was evident by the end of the three-flush cropping period.

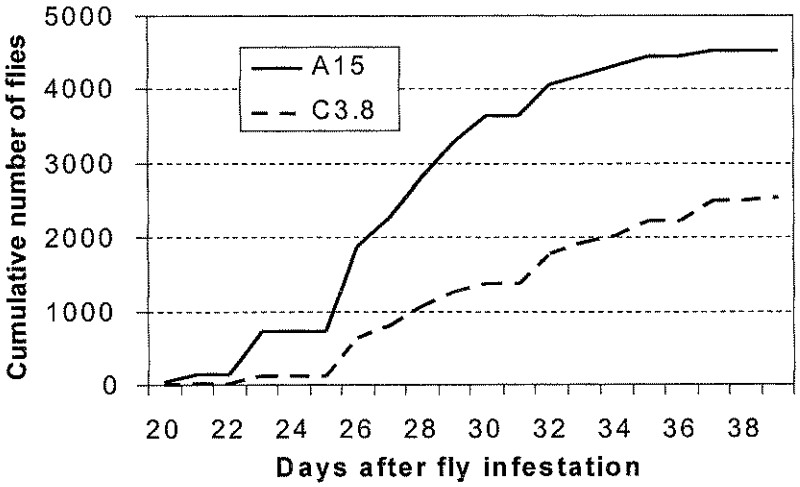

Figure 4. Phorid emergence from two mushroom crops

Sciarid population development

Sciarids started to emerge 21 days after infestation with 230 flies counted on the traps from strain 2810 and only 82 flies counted from strain C9 – a 64% reduction. As the crop progressed, the difference between the two crops became less pronounced such that, by the end of the three-flush cropping period, the overall reduction in the C9 crop was 31.5% (Fig. 5).

Yield

It is not possible to compare the yield from the brown strain crops to that of the white strain crops as the former normally produce a lower yield of mushrooms. However, the weight of damaged mushrooms was reduced from 1.73% in the 2810 crop to 0.79% in the C9 crop. Table 2 shows the total yield from each crop and the percentage of buttons, open, closed and damaged mushrooms.

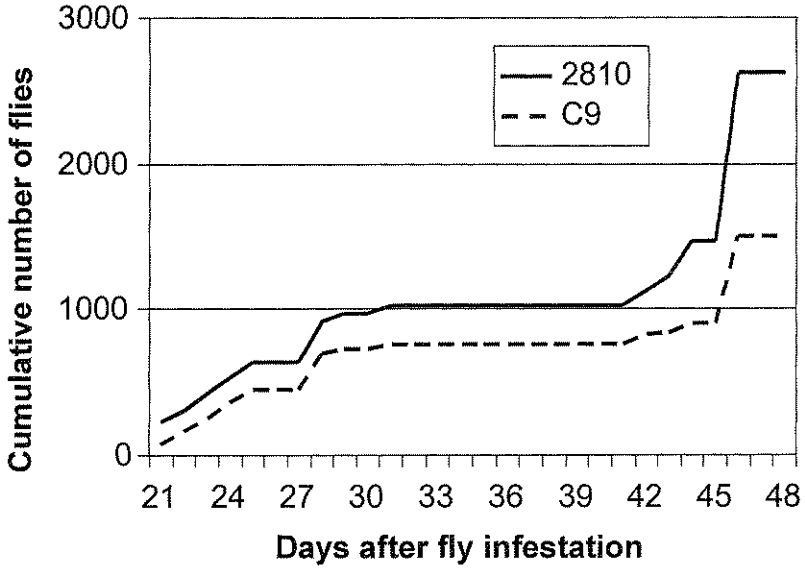

Figure 5. Sciarid emergence from two mushroom crops

Strain	% Buttons	% Closed	% Open	% Damaged	Total yield (kg)
A15	12.26	62.30	24.84	0.60	297.3
C3.8	5.73	53.81	40.28	0.17	274.5
2810	13.28	54.69	30.30	1.73	297.3
C9	17.08	47.97	34.16	0.79	246.2

Table 2. Cropping profile of the four strains tested in semi-commercial trial.

Discussion and Conclusions

These trials have demonstrated that the strain of *A. bisporus* used has an effect on mushroom fly pest development. They show that the brown strains are less susceptible to both phorids and sciarids - C3.8 reducing the phorid population by 44.2% and C9 reducing the sciarid population by 31.5%. The brown strains C3.8 and C9 appear to be less susceptible than the white strains A15 and 2810 to phorid and sciarid development, respectively. Where economically feasible therefore, these brown strains could be used to help reduce fly populations on a mushroom farm. Strain C3.8 was the second least attractive strain to sciarids in the laboratory experiments so it might, therefore, be possible to grow this strain to reduce the populations of both fly pests by about 30 to 40%.

In the laboratory tests, there was also some variation in the ability of the white strains to support fly development. It would, however, be imprudent to extrapolate directly from these laboratory results straight to a recommendation suitable for growers to act upon. Candidate spawn strains would need to be field tested on the mushroom unit to determine whether there is sufficient variability between such strains for practical use before such recommendations could be proposed.

REFERENCES

MAFF HHH1730SMU: Determination of the variation in the development and multiplication of mushroom pests when reared on various cultivable fungi. Project Leader P F White.

MAFF HH1735SMU: Determination of the variation in the development and multiplication of mushroom sciarids when reared on various cultivable fungi. Project Leader P F White, HRI Wellesbourne.

HDC M 25: Non-chemical control of mushroom sciarids using compost or peat-based substrates as bait traps. Project Leader P F White.

