Project title:	Determination of the variation in the development and multiplication of mushroom pests when reared on various commercial mushroom spawns
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OBJECTIVES AND BACKGROUND

An important factor in the multiplication of pests within a mushroom crop could be the strain of mushroom used by the grower, as the way in which the two main mushroom pests - phorids (*Megaselia halterata*) and sciarids (*Lycoriella auripila*) - infest and develop within a mushroom crop is inextricably linked to growth and development of the mushroom itself.

Phorid female flies are attracted during spawn-running of compost and subsequently lay their eggs close to the growing mycelium. The eggs hatch into larvae which are obligate mycelial feeders *i.e.* they require mycelium as a food source in order to develop. Therefore the biological properties of the mycelium may have an important bearing on the ability of the adults to detect it and the larvae to use it as food.

Sciarids differ from phorids. The adults are probably not attracted to the smell of mushroom mycelium but are mostly attracted before spawn is added to the compost. Well-colonized compost actually inhibits oviposition by sciarid females. Sciarid larvae are not obligate mycelial feeders but react to mushroom mycelium either positively or negatively, depending on whether there is a little or a lot of it.

Variations in response (generation time, fecundity, increase rates) to commercial 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 - analogous to the 'virus-breaker' strains - and use them, with chemicals if necessary, in the management of any long-term pest problem.

The aim of this project was to determine, in small-scale pot tests, whether natural resistance to phorids and sciarids existed in various commercial mushroom spawns. This was assessed in terms of variation in population development, after the pests had been exposed to the various spawns growing through mushroom compost.

SUMMARY OF RESULTS

Spawn strains tested

Ten strains were chosen to represent the general 'types' of mushroom grown and these are shown in Table 1. These represent as much genetic diversity as possible within the constraints of commercial strain availability. Somycel 11 and Darlington 649 were included as representing the typical parent strains of the first hybrids.

Phorids

Populations of egg-laying phorids were exposed to the spawns in a number of multi-comparison trials. The number of phorids subsequently emerging from the various treatments were used then to determine which of the spawns was most resistant. Therefore, the results were a combination of both adult (attraction/repellance) and larval (mortality/antifeedant) effects. Statistical analysis of these trials was carried out to allow for both 'neighbour effect' - whereby a highly attractive spawn may have been placed next to a less attractive one; and the density of the initial egg-laying phorid population.

Table 1. Commercial spawn strains used in M 25

Spawn name	Spawn code	Spawn type
Amycel A2800	A2800	Hybrid
Hauser A12	A12	Hybrid
International Spawn 501	I501	Hybrid
Sylvan 130	S130	Hybrid
Royale Champignon 101	RC101	Brown
Le Lion C9	C9	Brown
Darlington 649	D649	Smooth white
Sinden A6	A6	Smooth white
Le Lion B62	B62	Rough white
Somycel 11	S11	Rough white

There was a wide variation in the number of adults emerging from the different strains and this is shown in Figure 1(a). Populations produced by one of the brown strains (RC101) were significantly higher than those from the other strains. There was an approximate spawn-type grouping apparent, whereby the brown and smooth-white strains were more susceptible than the rough-whites and the hybrids. One of the hybrid strains (I501) was the most resistant strain of those tested, producing only about a third of the number of flies that were produced by the most susceptible strain (RC101). Across all strains, there was little variation in the generation time of the phorids.

Sciarids

The sciarid tests were on a smaller and simpler scale to those with phorids, with no tests being done on the comparative attractivity/repellance of the various spawns. Instead, known numbers of sciarid larvae were added to the pots of spawned compost. Therefore, the results only give information on any larval (mortality/antifeedant) effects.

There was a less widespread variation than had occurred with phorids and this can be seen from Figure 1(b). One of the smooth white strains (A6) was most resistant, producing less than 50% of the number of flies produced by the most susceptible strain (C9), while one of the brown strains (RC101) was also fairly resistant. The result with RC101 was more or less the direct opposite to that seen with phorids. There did not appear to be any spawn-type grouping as occurred with phorids and, as with phorids, there was little variation in the generation time.

INTERPRETATION OF RESULTS

This project represents an important and vital initial step in determining natural resistance by commercial mushroom strains to insect pests. The results gained so far are extremely encouraging as they indicate that important and potentially useful differences may occur.

However, it cannot be emphasized too strongly that these are preliminary results and that, at present, pest management practices should not be changed in light of them

These were small, pot-based, laboratory tests and would need to be repeated on a larger scale before specific action points could be reported. The findings achieved with the phorid tests, especially, are the result of *multi-comparison* trials - equivalent to a grower growing at least four spawn strains in one house at the same time. There was no scope within this project to determine whether the results achieved with any particular spawn strain would have been repeated if that strain had been tested in isolation.

PRACTICAL AND FINANCIAL ANTICIPATED BENEFITS

From each grower's point of view, of course, 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. The use of pesticides, harmful to the operator, the crop and the environment, could be minimized or even eliminated. Knowledge of such natural resistance will aid insect control strategies on all types of mushroom farm and will even facilitate future breeding programmes.

SCIENCE SECTION

INTRODUCTION

Background

An important factor in the multiplication of pests within a mushroom crop is likely to be 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 repellant 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.

The larval stages of phorids and cecids are both obligate mycelial feeders *i.e.* they require mycelium as a food source in order to develop. It has been shown in the past that cecid larvae reproduce at quite different rates on various (now obsolete) strains of the cultivated mushroom *Agaricus bisporus*. For example on one strain of mushroom, *M.speyeri* produced 14 young in 6.5 days, while on another strain it produced 25 young in 5.4 days. After a period of just three weeks, such differences would produce populations of 3,800 larvae with the first strain and 294,000 larvae with the second - a 77-fold difference.

The sciarid differs from the previous two pests in a number of ways. The adults are not attracted to the smell of mushroom mycelium, as with 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.

Target

It is not known how mushroom pests react, in terms of generation time, fecundity, increase rates etc, to the spawns in current use. 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 - analogous to the 'virus-breaker' strains - and use them, with chemicals if necessary, in the management of any long-term pest problem. The aim of this project was to determine whether natural resistance to sciarids and phorids exists in various spawns. This was assessed in terms of variation in population development. Knowledge of such natural resistance will aid insect control strategies on all types of mushroom farm and will facilitate future breeding programmes.

Related work

This project is linked with a similar project funded by MAFF, whereby eight cultivable fungi are being screened for the presence of natural resistance to mushroom phorids. With the exception of one commercial strain, which is common to both projects, the fungi chosen for the MAFF project are not commercial *Agaricus bisporus* strains but represent as much genetic diversity as possible within the compost colonising members of the genus *Agaricus*. In addition, as part of a MAFF-funded ROAME, the interaction of paedogenetic mushroom cecid larvae (*H.pygmaea* and *M.speyeri*) with a range cultivable fungi, including commercial strains of *A.bisporus*, is also being investigated to identify whether any natural resistance is present.

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.

MATERIALS AND METHODS

Spawn strains tested

Ten strains were chosen to represent the general 'types' of mushroom grown. There were four hybrid (H), two brown (B), two smooth-white (SW) and two rough-white (RW) strains and these are shown in Table 1. They represent as much genetic diversity as possible within the constraints of commercial strain availability. Somycel 11 and Darlington 649 were included as representing the typical parent strains of the first hybrids.

Table 1. Commercial spawn strains used in M 25

Spawn name	Spawn code	Spawn type
Amycel A2800	A2800	Hybrid
Hauser A12	A12	Hybrid
International Spawn 501	I501	Hybrid
Sylvan 130	S130	Hybrid
Royale Champignon 101	RC101	Brown
Le Lion C9	C9	Brown
Darlington 649	D649	Smooth white
Sinden A6	A6	Smooth white
Le Lion B62	B62	Rough white
Somycel 11	S11	Rough white

Grain spawns of each strain were made, where necessary and, to ensure appropriate comparisons in subsequent experiments, the comparative growth rates of these strains were determined in compost 'race' tubes.

Pots of compost were inoculated with a candidate spawn and the mycelium allowed to permeate it for various numbers of days before being exposed to either phorids or sciarids.

Phorid protocol

A number of multi-comparison trials were done, whereby the strains were kept in their natural 'type' groupings (the hybrids were put into two groups H1 & H2). Each was performed as follows. Two strains from two of the above 'types' were tested together in an infestation chamber (800 x 470 x 530 mm). For each strain, five pots of compost were inoculated with grain spawn. The pots were then incubated for a pre-determined number of days (ascertained by the 'race' tube tests) to ensure that all four strains tested had the same amount of mycelial growth at the time of phorid infestation. The five pots from each of the four strains in the test were placed in one of the quadrants of the infestation chamber (Figure 2). Four further pots of compost, containing emerging populations of phorids, were placed down one centre-line of the chamber. The chamber was rotated through 180° every two hours, during an eight-hour illuminated 'day' to prevent light-induced effects. 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 phorid attraction and larval mortality. Each test took 4-5 weeks to complete.

To enable sufficient comparisons to be made between the strains, each 'type' grouping (H1, H2, B, SW & RW) were in separate tests with a minimum of two of the other 'types' (*e.g.* H1/B, H1/SW; B/SW, B/RW; etc).

Sciarid protocol

Pots of compost were inoculated with the candidate strains in a similar manner to the phorid tests *i.e.* ensuring equality of mycelial spawn-run at the time of sciarid infestation. The pots were then inoculated with 20 fertile sciarid eggs and put in an incubator at 25°C. Subsequent emergence of adults was used to determine the effects of the spawn strains on larval mortality.

RESULTS

Phorids

The number of phorids emerging from the compost spawned with the various strains were used to determine which of the spawns was most resistant. Therefore, the results were a combination of both adult (attraction/repellance) and larval (mortality/antifeedant) effects. Statistical analysis of the trials was carried out to allow for both 'neighbour effect' - whereby a highly attractive spawn may have been placed next to a less attractive one; and the density of the initial egg-laying phorid population.

There was a wide variation in the number of adults emerging from the different strains and this is shown in Figure 1(a). The statistical analysis of the trials is shown in Figure 3(a).

There was an approximate spawn-type grouping apparent, whereby the brown and smooth-white strains were more susceptible than the rough-whites and the hybrids. The population produced by the brown strain RC101 was, by far, the largest producing almost 40% more flies than the next highest producers, the smooth-white strains. RC101 was significantly higher than all other strains at $P<0.05$ or better. The hybrid strain I501 was the most resistant strain of those tested, yielding only about a third of the number of flies that were produced by the most susceptible strain RC101. At the 5% level, the number of flies produced by I501 was significantly lower than the brown and smooth-white strains. Within the hybrid strains, the 36% difference between I501 and A2800 was the biggest, although this was only significant at the 8.6% level

Across all strains, there was little variation in the generation time of the phorids (Figure 4(a)). The biggest difference was only 2.4 days and this occurred between two of the hybrid strains (I501 and A2800).

Sciarids

The sciarid tests were on a smaller and simpler scale to those with phorids, with no tests being done on the comparative attractivity/repellance of the various spawns. Instead, known numbers of sciarid larvae were added to the pots of spawned compost. Therefore, the results only give information on any larval (mortality/antifeedant) effects.

There was a less widespread variation than had occurred with phorids and this can be seen from Figure 1(b). The statistical analysis of these tests is shown in Figure 3(b).

There did not appear to be any spawn-type grouping as had occurred with phorids. The smooth white strain A6 was most resistant, with a brown strain (RC101) being also fairly resistant. These two strains were clearly more resistant than the other strains producing populations that were significantly different from 7 or 4 of the other strains, respectively ($P<0.05$, Fig.3(b)). A6 produced less than 50% of the number of flies produced by the most susceptible, the brown strain C9. The two brown strains were significantly different from each other, as were the two smooth white strains. The result with RC101 was more or less the direct opposite to the effect achieved with phorids.

Across all strains, there was little variation in the generation time of the sciarids (Figure 4(b)) although there did appear to be a spawn-type grouping, with the smooth and rough whites taking a shorter time than the hybrids to develop through to flies. However, the biggest difference was only 2.1 days and this occurred between the smooth white strain A6 and the hybrid A12.

CONCLUSIONS

These results indicate that important and potentially useful differences occur between the strains tested and they represent an important and vital initial step to determine whether fungi possess natural resistance to mushroom pest species. However, it should be emphasized that these are results from preliminary experiments carried out on a small scale. The findings achieved with the phorid tests, especially, are the result of *multi-comparison* trials - equivalent to a grower growing at least four spawn strains in one house at the same time. There was no scope within this project to determine whether the results achieved with any particular spawn strain would have been repeated if that strain had been tested in isolation.

With this caveat in mind, however, the overall conclusions which can be inferred from this project are:

Phorids (combined adult/larval effects)

- There was a wide variation in the number of adults emerging from the different strains
- Significantly higher populations developed on a brown strain (RC101)
- Brown and smooth-white strains were more susceptible
- The hybrid strain (I501) appeared most resistant
- There was little variation in generation time

Sciarids (larval effects only)

- There was less widespread variation
- The smooth-white strain (A6) was the most resistant
- The brown strain (RC101) also appeared to be fairly resistant (*cf* phorids)
- There was little variation in generation time

THE FUTURE

Current MAFF-funded research is concentrating on the natural resistance of species of *Agaricus* other than *A. bisporus*. Without further funding from HDC, it is unlikely that this extremely interesting and potentially important area of work against commercial strains of *A. bisporus* will be continued.

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. The use of pesticides, harmful to the operator, the crop and the environment, could be minimized or even eliminated. Knowledge of such natural resistance will aid insect control strategies on all types of mushroom farm.

Further research needs to be done both to extend the host range (spawn types) and to substantiate the results from these initial experiments. Knowledge from such research has the potential to be used in future breeding programmes for cultivated mushrooms and IPR would need to be protected.

Figure 1(a) Phorids: Effect of ten strains of *Agaricus bisporus* on phorid development. Data adjusted for neighbour effect and initial fly number.

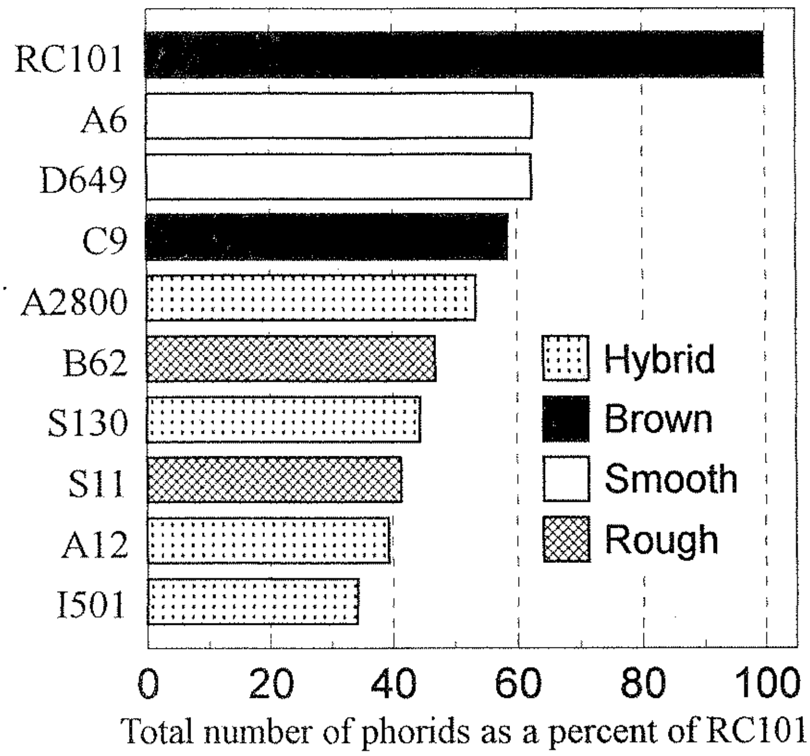

Figure 1(b) Sciarids: Effect of ten strains of *Agaricus bisporus* on larval development and emergence

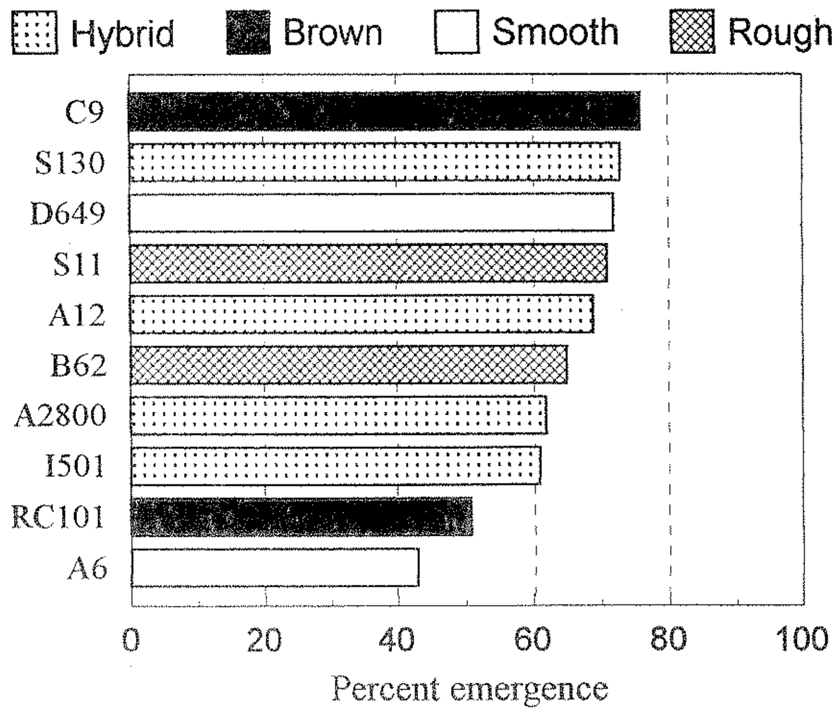

Figure 2. Phorid choice chamber. In this depiction, two hybrid strains are being compared to two rough white strains. The four phorid-infested pots of compost have not been shown.

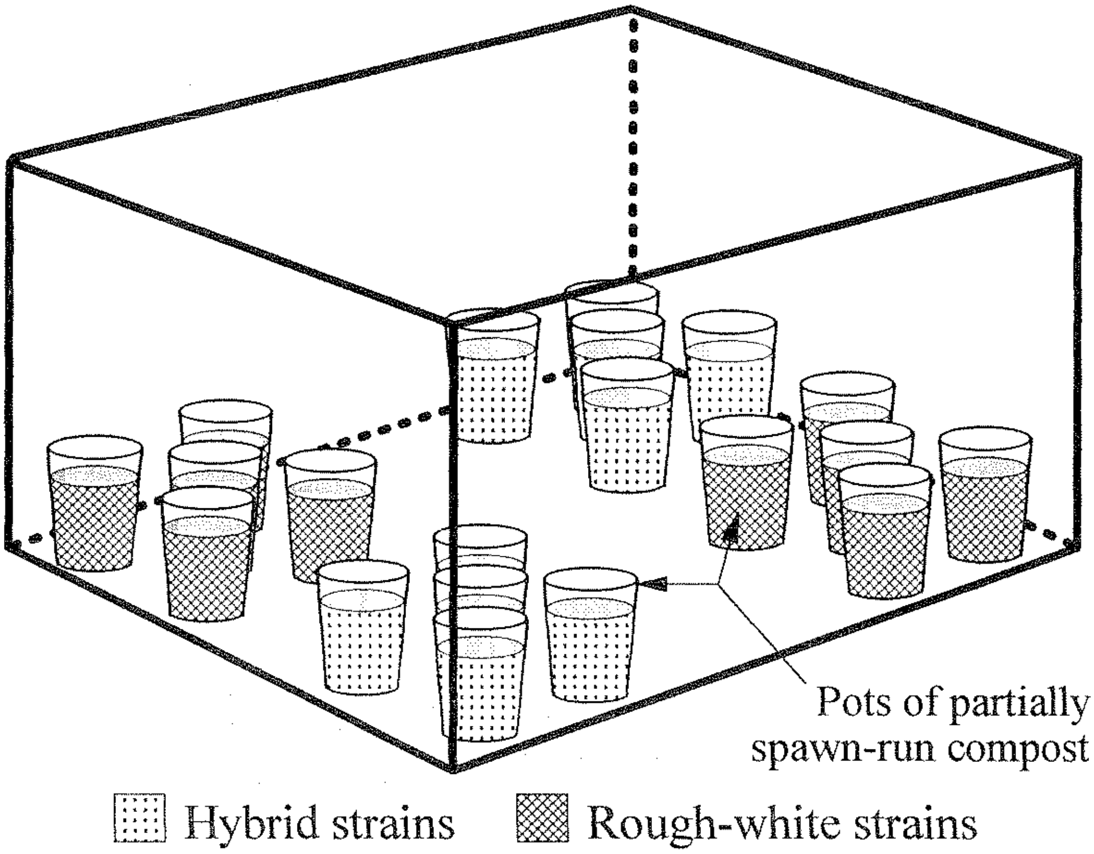

Figure 4(a) Phorids: Mean day of emergence

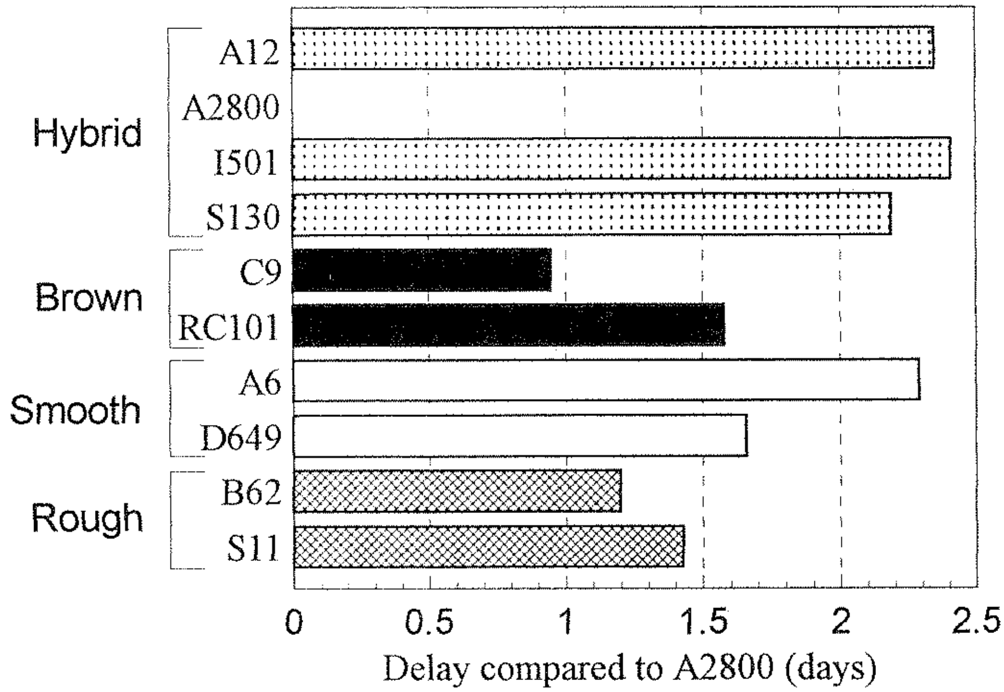

Figure 4(b) Sciarids: Mean day of emergence

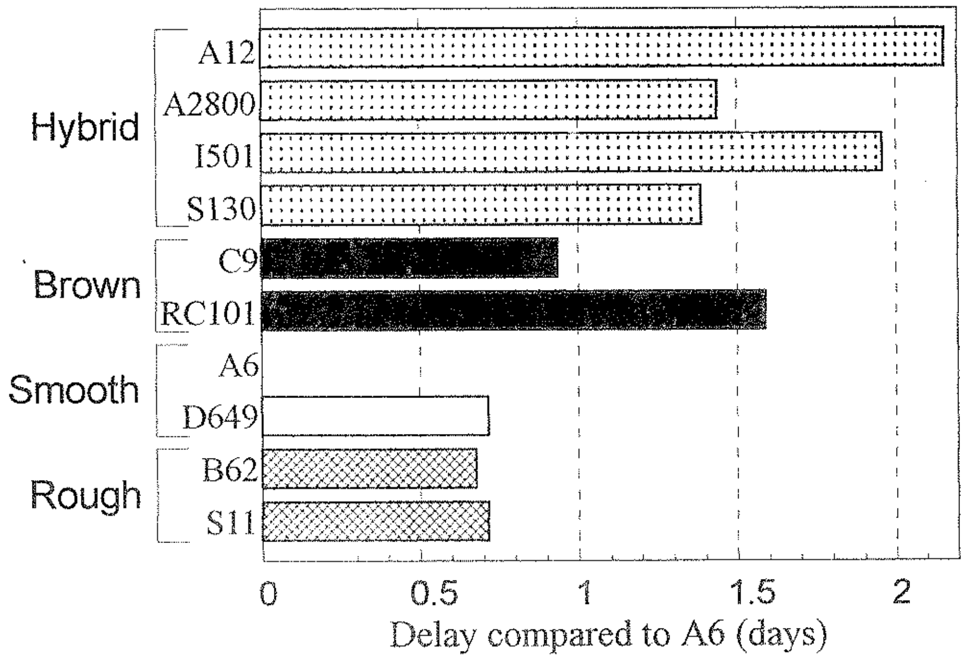

Figure 3(a) Phorids: Significance values (*P*) of comparisons between strains.

	A6	D649	C9	A2800	B62	S130	S11	A12	I510
RC101	4.2	4.1	2.9	3.2	1.0	0.7	0.6	0.6	0.3
A6		98.1	69.9	45.7	14.9	10.0	6.1	5.2	2.0
D649			71.5	46.7	15.2	10.3	6.3	5.4	2.0
C9				65.8	24.3	16.3	9.7	8.1	2.9
A2800					54.4	40.0	26.0	20.8	8.6
B62						75.7	48.2	36.8	12.7
S130							68.0	52.2	18.7
S11								79.4	31.6
A12									46.4

Figure 3(b) Sciarids: Significance values (*P*) of comparisons between strains

	S130	D649	S11	A12	B62	A2800	I510	RC101	A6
C9	74.3	66.2	58.6	44.6	23.4	13.2	10.7	0.9	0.1
S130		91.3	82.7	66.2	38.4	23.4	19.5	2.0	0.2
D649			91.3	74.3	44.6	27.8	23.4	2.6	0.3
S11				82.7	51.3	32.8	27.8	3.4	0.4
A12					66.2	44.6	38.4	5.5	0.7
B62						74.3	66.2	13.2	2.0
A2800							91.3	23.4	4.3
I510								27.8	5.5
RC101									38.4