

## **FINAL REPORT AUGUST 1994**

Project Number : HDC M4a  
Date Project Commenced : February 1994  
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Project Co-ordinator : Mr. H.J. Linfield

### **THE CULTURE OF ALTERNATIVE *AGARICUS* SPECIES ON A COMMERCIAL AND SEMI-COMMERCIAL SCALE**

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## REVIEW OF PROJECT ACHIEVEMENTS

1. The identification of an *Agaricus* species (W4) which has an attractive appearance and distinct flavour, considered to be better than that of *A. bisporus*.
2. The identification of an *Agaricus* W4 isolate (IV) which is consistently able to produce 70-80% of the yield of a commercial brown strain of *A. bisporus* under semi-commercial cropping conditions.
3. Single spore isolates were obtained from W4IV which produced a higher yield than the parent culture in small-scale experiments.
4. A black peat casing was found to result in cleaner mushrooms than a brown peat casing. A casing material with a more uniform structure than the bulk material used in the present experiments would be preferable.
5. The use of the supplement Betamyl 1000 was found to increase the yield of some, but not all, *Agaricus* W4 isolates.
6. A UK source of *Agaricus arvensis* (93-7) which produced a higher yield in a small-scale experiment than a commercially available strain Somycel R20, was identified.
7. Commercial trials in trays, bags and deep troughs are currently being conducted.

## RECOMMENDATIONS FOR FURTHER WORK

It is likely that the full yield potential of both *Agaricus* W4 and *Agaricus arvensis* has not been realised. The following areas of work are suggested to develop this potential:

1. To compare the cropping performance of the new W4 single spore isolates with that of the parent isolate W4IV, on semi-commercial and commercial scales.
2. To compare the yield of a promising new isolate of *A. arvensis* (93-7) with that of a commercially available strain (Somycel R20) on a semi-commercial scale.
3. To assess the yield potential of the progeny of HRI *A. arvensis* isolates.
4. To investigate the effect of a wider range of cultural factors, including supplementation, on the yield of *A. arvensis*.

## INTRODUCTION

An *Agaricus* species (W4) (first thought to be *A. silvaticus* but later found to be more closely related to *A. subfloccosus*) with an attractive appearance and distinct flavour, considered to be better than that of *A. bisporus*, has been identified. Significant differences in the cropping performance of four separate sources of *Agaricus* W4 and different single spore isolates taken from these original cultures of *Agaricus* W4 were observed. These sources of variation were identified as methods for improving the yield of *Agaricus* W4. As a result of HDC Project M4, a strain was isolated (W4IV) which is capable of producing 63.5 and 83% of the yield of commercial white hybrid and brown strains of *A. bisporus*.

As part of Project M4, the cropping performance of a commercial strain of the horse mushroom, *Agaricus arvensis* (Somycel R20) was examined. Since this project, several other *A. arvensis* isolates have been collected from the wild in the UK, although their yield potential on composted substrates has yet to be determined.

The objectives of the present project were:

1. To compare the yield potential of single spore isolates taken from the best wild isolate of *Agaricus* W4, W4IV with that of the original *Agaricus* W4IV isolate.
2. To examine the use of supplements on the yield of *Agaricus* W4.
3. To examine the use of different casing materials for the culture of *Agaricus* W4.

4. To investigate the use of spawned casing, 'cassing' for *Agaricus* W4.
5. To compare the cropping performance of wild HRI isolates of *Agaricus arvensis* with that of the commercial strain, Somycel R20.

PART I - *AGARICUS* W4

## MATERIALS AND METHODS

Experiment 1 - Effect of compost supplementation at spawning on *Agaricus* species

## (a) Compost supplementation at spawning

- (i) Control (unsupplemented)
- (ii) Betamyl 1000 (1% w/w), based on soya-meal protein
- (iii) ADCO Springboard (1% w/w), based on fatty acids

(b) *Agaricus* strains/isolates

- |                        |       |   |
|------------------------|-------|---|
| <i>Agaricus</i> W4     | (i)   | A119  |
|                        | (ii)  | A130  |
|                        | (iii) | W4II  |
|                        | (iv)  | W4IV  |
| <i>A. bisporus</i>     | (v)   | Somycel 609, white hybrid                             |
|                        | (vi)  | Somycel 856, brown                                    |
| <i>A. subfloccosus</i> | (vii) | 3V1 (obtained from R. Stadelman, Hauser, Switzerland) |

Rye grain spawn was produced for the experiment by a commercial spawn producer. A commercial Phase II compost was spawned at 2% w/w and filled into wooden trays, each filled with 45 kg of substrate. The analysis of the substrate is shown in the Appendix.

Spawned compost was hydraulically pressed into the trays which were then covered with paper and stacked in a spawn-running room where the compost temperature was maintained at 25°C. After full mycelial colonization of the substrate, 20 days after spawning, the compost was cased with a moist mixture of peat (Bord na Mona medium grade) and chalk (9:1 v/v) to a depth of 35 mm. The trays were transferred to a cropping shed where the relative humidity of the air was maintained at 95% and the compost temperature kept at 25°C. The casing was covered with paper until mycelium became visible at the surface, 14 days after the casing was applied. Fresh air was then introduced in the cropping shed and the environmental conditions were altered to encourage pinhead initiation and fruitbody development. The air temperature, humidity and CO<sub>2</sub> concentration were reduced to levels of 16-17°C, 90-92% and 0.06-0.07% v/v respectively. The casing was kept moist by regular light watering after the first fruitbodies had developed to 10 mm diam. About 15% more water was applied to the *A. bisporus* treatments than to the *Agaricus* W4 treatments since previous trials had shown that over-watering inhibited pinhead formation of *Agaricus* W4. Fruitbodies, diam 35-45 mm, were harvested at the 'stretched veil' stage over a 45-day period. The stipes were trimmed (about 13% of the sporophore weight was removed) and the weight of fruitbodies harvested from each tray was recorded. A randomized block design was used, with six replicate trays of each strain/supplement treatment.

#### Experiment 2 - Effect of casing materials and spawned casing, 'cassing', on *Agaricus* species

##### (a) Casing treatments

- (i) Control, Bord na Mona medium grade peat + fine grade chalk (Needhams

SF16)

- (ii) Harte casing (Irish wet bog peat + fine grade chalk)
- (iii) Control casing + 'caccing' (spawn-run compost), caccing material was added at 250 g/m<sup>3</sup> casing.

(b) *Agaricus* strains/isolates

- Agaricus* W4 (i) A119
- (ii) A130
- (iii) W4II
- (iv) W4VI
- (v) Somycel 609, white hybrid
- (vi) Somycel 856, brown

Cultural conditions were similar to those in Experiment 1 with the exception of 'caced' casing treatments of *A. bisporus* Somycel 609 and 856. These trays were transferred from the case-running room after 6 days to a growing chamber where fresh air was introduced. The trays were then transferred back into the main cropping shed when this was aired, 14 days after casing. The spawned casing did not result in faster colonization of the casing for the *Agaricus* W4 treatments; these trays therefore remained in the main cropping shed throughout.

A randomized block design was used, with six replicate trays of each strain/casing treatment.



### Experiment 3 - Strain selection

Single spore isolates were obtained from a fruitbody of W4IV. The isolates were cultured on agar before rye grain spawn was prepared. Tests for mating reactions between single spore cultures were made but there was no evidence of any compatibility (fluffy mycelium at the juncture zone between cultures). Cultures were taken from the zone between two single spore isolate cultures to test if the cultures were the same as either of the single spore isolates. Six isolates of *Agaricus subfloccosus* from Canada were obtained from R. Kerrigan, Sylvan, USA.

Spawn was produced on rye grain for the different single spore isolates and mating test cultures.

#### Cultural procedure

The experiment was conducted in a controlled environment chamber. The substrate was filled into plastic crates each holding 5 kg substrate, and spawned at 2% w/w with grain spawn. The same substrate as in Expts 1 and 2 was used. Cultural conditions were similar to those described in Expt 1, except a shallower layer of casing (20 mm) was used.

Three replicate crates of each strain were 'blocked' in three tiers in the cropping chamber.

## RESULTS

### Experiment 1

The yields of *Agaricus* W4, *A. bisporus* and *A. subfloccosus* in supplemented and unsupplemented compost are shown in Table 1. The *A. bisporus* strains produced a significantly higher yield than the wild *Agaricus* isolates. W4IV resulted in a significantly higher yield than the other *Agaricus* W4 isolates. Overall, supplementation of compost with Betamyl increased yield although no effect was recorded on isolates I/A130, IV or *A. subfloccosus* 3V1. The supplement Springboard had a negative effect on the yield of *Agaricus* W4 isolates and these plots had large numbers of *Coprinus* fruitbodies.

### Experiment 2

Differences between casing treatments were not consistent for different isolates or strains. For *A. bisporus* Somycel 609, the black (Harte) peat resulted in a higher yield than the brown peat but for the other isolates and strains, there were no significant differences in yield between the black and brown peat casings. The black peat casing was supplied in bulk and had a lumpy texture resulting in uneven colonization of the casing. However, mushrooms from the black peat casing were significantly cleaner than those from the brown peat casing. The black peat casing had a higher moisture holding capacity than the brown peat casing resulting in a higher moisture content at application and during cropping (Fig. 1). The black peat casing moisture content of the *A. bisporus* treatments was slightly higher than that of the *Agaricus* W4 treatments due to the higher water application. The first picks shown in

Fig. 1 are for casing with 'cassing'. The casing treatment did not advance the cropping or affect the yield of *Agaricus* W4 but the cropping of *A. bisporus* was advanced by 5 days.

### Experiment 3

The yield and number of fruitbodies from the *A. bisporus* strains Somycel 609 and Somycel 856 were significantly higher than those of any of the *Agaricus* W4 or *A. subfloccosus* isolates (Table 3). All of the single spore isolates which survived through to spawn production resulted in fruitbody formation. There was a wide range in yields from single spore isolates of W4IV. Isolates 5 and 16 produced significantly lower yields and isolates 2 and 15 produced significantly higher yields than the original culture W4IV. Cultures taken from the zone between single spore isolate cultures resulted in yields similar to one or other of the constituent isolates.

Two of the six Canadian *A. subfloccosus* isolates produced a spawn-run but failed to produce fruitbodies (K1542 and K1565). The isolate K1552 produced a higher yield than the other Canadian (K) and Swiss (3V1) isolates (Table 3). However, the quality of fruitbodies was poor since the caps were often misshapen.

### CONCLUSIONS

These experiments have confirmed that W4IV is the most productive parent isolate of *Agaricus* W4, compared with the other sources. On a semi-commercial scale, a yield of just under 150 kg/tonne compost at spawning was achieved. Two single spore isolates obtained

from W4IV produced a slightly higher yield than W4IV in crates, although this difference needs to be examined on a larger scale. The *A. subfloccosus* isolate K1552 produced a yield of 160 kg/tonne in a crate experiment although the quality of fruitbodies was poor.

The supplement Betamyl 1000 resulted in a significant yield increase in both *A. bisporus* and *Agaricus* W4 although not all the W4 isolates produced a significant response. No positive effects of the supplement Springboard were found although in an earlier trial, a yield increase comparable to that produced by Betamyl 1000 was found using the strain Somycel 609. The use of spawn-run compost (cacking) did not result in a faster colonization of the casing. A higher rate of cacking than that used in the present experiment (250 g/m<sup>3</sup>) may be necessary to achieve a positive effect.

The black peat casing resulted in cleaner mushrooms but there was no overall yield advantage for the *Agaricus* W4 isolates. A black peat casing with a less lumpy structure resulting in a more uniform colonization of the casing would be preferable.

Table 1 Yield of *Agaricus* W4 isolates, *A. bisporus* and *A. subfloccosus* on unsupplemented compost and compost supplemented with Betamyl 1000 or Springboard, Kg tonne<sup>-1</sup> compost at spawning, Expt 1. Each value is the mean of 6 replicate trays

Supplement	<i>Agaricus</i> W4 isolates				<i>A. bisporus</i>		<i>A. subfloccosus</i>
	I/A119	I/A130	II	IV	S609	S856	3V1
Control	92	127	108	146	201	175	130
Betamyl	117	124	122	148	223	202	121
Springboard	59	105	89	118	202	181	113
Mean	89	119	106	137	209	186	121

LSD (5%) for strain means = 16.6; for strain x supplement = 28.9

Table 2 Yield of *Agaricus* W4 isolates and *A. bisporus* strains using different casing treatments, Expt 2. Each value is the mean of 6 replicate trays

Casing treatment	<i>Agaricus</i> W4 isolates				<i>A. bisporus</i>	
	I/A119	I/A130	II	IV	S609	S856
Brown peat	64	134	107	133	204	192
Black peat	63	110	102	147	249	192
Brown peat + 'cassing'	78	138	95	134	212	202
Mean	68	127	101	138	222	195

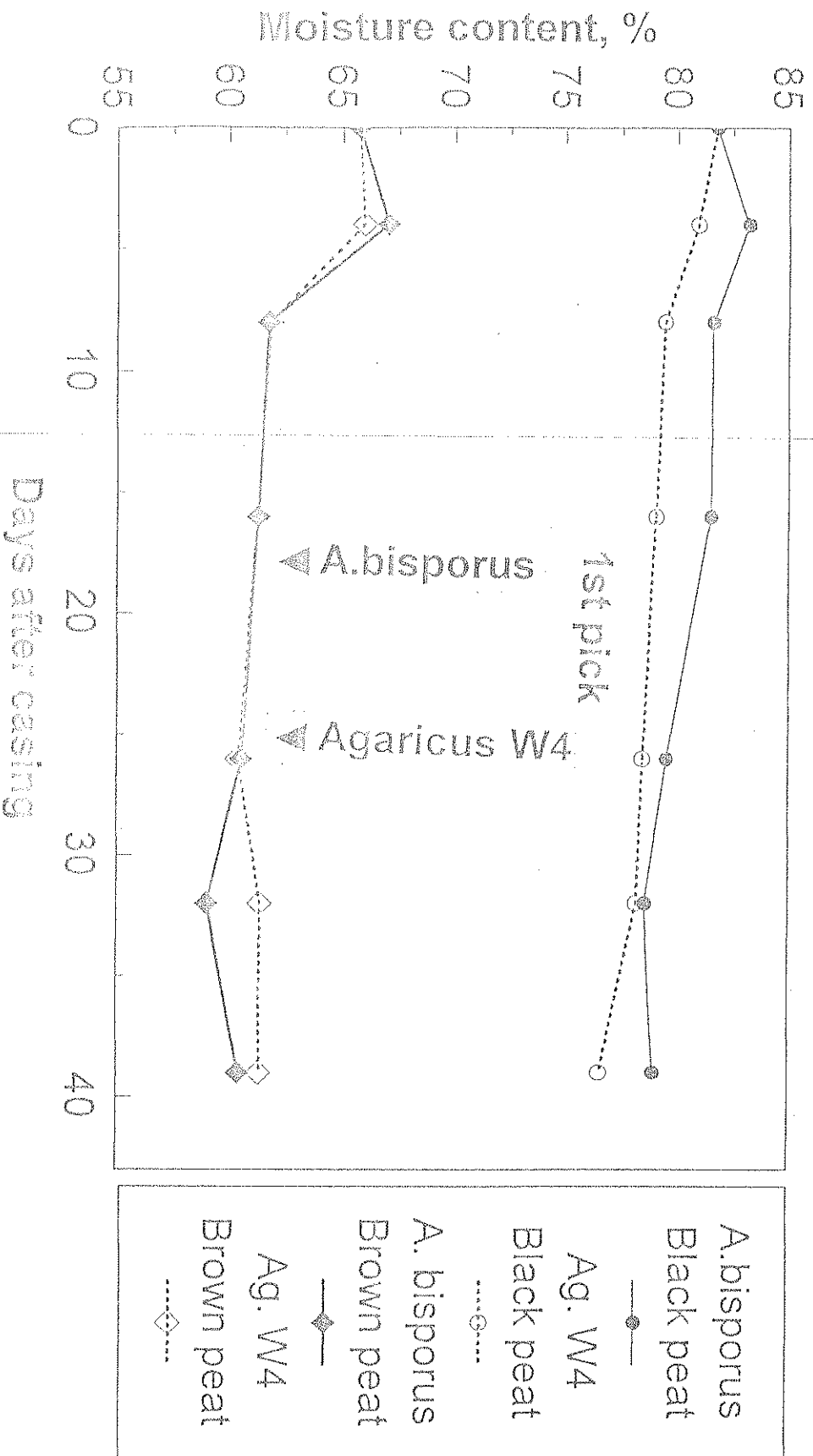
LSD (5%) fir strain means = 17.7; for strain x casing treatments = 30.7

Table 3 Yield and number of fruitbodies from single spore isolates of W4IV and cultures taken from the zone between two single spore isolate cultures, Expt 3. Each value is the mean of 3 replicate crates

Species	Isolate or Strain	Yield, g/kg substrate	No. fruitbodies/crate	
<i>Agaricus</i> W4 Parent cultures	I/A130	132	31	
	II	109	25	
	IV	138	33	
Single spore isolates	IV/ssi1	131	33	
	IV/ssi2	154	30	
	IV/ssi3	127	34	
	IV/ssi5	81	28	
	IV/ssi8	147	31	
	IV/ssi13	136	32	
	IV/ssi14	146	46	
	IV/ssi15	152	34	
	IV/ssi16	88	23	
	IVssi1/ssi2	156	39	
	IVssi1/ssi3	126	28	
	IV/ssi13/ssi14	113	28	
	IV/ssi14/ssi16	155	36	
	IV/ssi15/ssi16	91	24	
	<i>A. subfloccosus</i>	K1543	49	17
		K1552	160	41
K1553		67	15	
K1570		30	10	
3V1		110	26	
<i>A. bisporus</i>	Somycel 609	251	53	
	Somycel 856	239	61	

L.S.D. ( $P < 0.05$ ) = 14 (*Agaricus* W4 isolates)

Fig. 1 Casing moisture content, Expt 2





PART II - AGARICUS ARVENSIS

MATERIALS AND METHODS

*Agaricus arvensis* isolates and strains

- (i) 93-7
- (ii) 93-9
- (iii) 93-10
- (iv) Somycel R20

The sources of the three new HRI isolates are shown in Table 4.

Cultural procedure

The cultural procedure was the same as for *Agaricus* W4 described in Expt 3 except lighting (150 lux) was used for 12 h/d after fresh air was introduced into the chamber. The same compost was used (see Appendix for analysis). Sporophores were harvested closed with a cap diameter of 45 mm. The base of the stem was trimmed (average trimmed weight of fruitbodies was 35 g). Three replicate crates of each isolate/strain were prepared.

RESULTS

All the isolates produced a first flush about 25 days after casing and had flushing intervals

of about 12 days. Four to five flushes were picked during a cropping period of 45 days.

Yields from the different isolates are shown in Table 5. The isolate 93-7 produced a higher yield than the strain Somycel R20 although the difference was not quite significant at  $P = 0.05$ . The appearance and quality of fruitbodies from the different sources of *A. arvensis* was similar. The HRI isolates were slightly paler than R20. However, fruitbodies of R20 became paler as the flushes progressed.

Table 4 Source of *Agaricus arvensis* isolates

Isolate	Collected by:	Location	Date	Habitat
93-7	H. Grogan	Littlehampton, West Sussex	26 Sept. 1993	Coniferous leaf litter
93-9	H. Grogan	Littlehampton, West Sussex	5 Oct. 1993	Cupressus hedge
93-10	R.H. Gaze	Bedham, West Sussex	11 Oct. 1993	Composting grass clippings

Table 5 Yield and number of fruitbodies of *Agaricus arvensis* isolates and strains. Each value is the mean of 3 replicate crates.

Isolate or strain	Yield g/kg substrate	No. fruitbodies/crate
93-7	133	21
93-9	67	14
93-10	62	10
Somycel R20	120	17
L.S.D. ( $P < 0.05$ )	15	

APPENDIX

Analysis of compost at spawning

N	NH <sub>4</sub> <sup>+</sup>	Ash	Moisture	pH
Percentage of Dry Weight				
2.51	0.21	15.3	75.8	7.7

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3. Single spore isolates were obtained from W4IV which produced a higher yield than the parent culture in small-scale experiments.
4. A black peat casing was found to result in cleaner mushrooms than a brown peat casing. A casing material with a more uniform structure than the bulk material used in the present experiments would be preferable.
5. The use of the supplement Betamyl 1000 was found to increase the yield of some, but not all, *Agaricus* W4 isolates.
6. A UK source of *Agaricus arvensis* (93-7) which produced a higher yield in a small-scale experiment than a commercially available strain Somycel R20, was identified.
7. Commercial trials in trays, bags and deep troughs are currently being conducted.

## RECOMMENDATIONS FOR FURTHER WORK

It is likely that the full yield potential of both *Agaricus* W4 and *Agaricus arvensis* has not been realised. The following areas of work are suggested to develop this potential:

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2. To compare the yield of a promising new isolate of *A. arvensis* (93-7) with that of a commercially available strain (Somycel R20) on a semi-commercial scale.
3. To assess the yield potential of the progeny of HRI *A. arvensis* isolates.
4. To investigate the effect of a wider range of cultural factors, including supplementation, on the yield of *A. arvensis*.



## INTRODUCTION

An *Agaricus* species (W4) (first thought to be *A. silvaticus* but later found to be more closely related to *A. subfloccosus*) with an attractive appearance and distinct flavour, considered to be better than that of *A. bisporus*, has been identified. Significant differences in the cropping performance of four separate sources of *Agaricus* W4 and different single spore isolates taken from these original cultures of *Agaricus* W4 were observed. These sources of variation were identified as methods for improving the yield of *Agaricus* W4. As a result of HDC Project M4, a strain was isolated (W4IV) which is capable of producing 63.5 and 83% of the yield of commercial white hybrid and brown strains of *A. bisporus*.

As part of Project M4, the cropping performance of a commercial strain of the horse mushroom, *Agaricus arvensis* (Somycel R20) was examined. Since this project, several other *A. arvensis* isolates have been collected from the wild in the UK, although their yield potential on composted substrates has yet to be determined.

The objectives of the present project were:

1. To compare the yield potential of single spore isolates taken from the best wild isolate of *Agaricus* W4, W4IV with that of the original *Agaricus* W4IV isolate.
2. To examine the use of supplements on the yield of *Agaricus* W4.
3. To examine the use of different casing materials for the culture of *Agaricus* W4.

4. To investigate the use of spawned casing, 'cassing' for *Agaricus* W4.
5. To compare the cropping performance of wild HRI isolates of *Agaricus arvensis* with that of the commercial strain, Somycel R20.

PART I - *AGARICUS* W4

## MATERIALS AND METHODS

Experiment 1 - Effect of compost supplementation at spawning on *Agaricus* species

- (a) Compost supplementation at spawning
- (i) Control (unsupplemented)
  - (ii) Betamyl 1000 (1% w/w), based on soya-meal protein
  - (iii) ADCO Springboard (1% w/w), based on fatty acids
- (b) *Agaricus* strains/isolates
- |                        |       |   |
|------------------------|-------|---|
| <i>Agaricus</i> W4     | (i)   | A119  |
|                        | (ii)  | A130  |
|                        | (iii) | W4II  |
|                        | (iv)  | W4IV  |
| <i>A. bisporus</i>     | (v)   | Somycel 609, white hybrid                             |
|                        | (vi)  | Somycel 856, brown                                    |
| <i>A. subfloccosus</i> | (vii) | 3V1 (obtained from R. Stadelman, Hauser, Switzerland) |

Rye grain spawn was produced for the experiment by a commercial spawn producer. A commercial Phase II compost was spawned at 2% w/w and filled into wooden trays, each filled with 45 kg of substrate. The analysis of the substrate is shown in the Appendix.

Spawned compost was hydraulically pressed into the trays which were then covered with paper and stacked in a spawn-running room where the compost temperature was maintained at 25°C. After full mycelial colonization of the substrate, 20 days after spawning, the compost was cased with a moist mixture of peat (Bord na Mona medium grade) and chalk (9:1 v/v) to a depth of 35 mm. The trays were transferred to a cropping shed where the relative humidity of the air was maintained at 95% and the compost temperature kept at 25°C. The casing was covered with paper until mycelium became visible at the surface, 14 days after the casing was applied. Fresh air was then introduced in the cropping shed and the environmental conditions were altered to encourage pinhead initiation and fruitbody development. The air temperature, humidity and CO<sub>2</sub> concentration were reduced to levels of 16-17°C, 90-92% and 0.06-0.07% v/v respectively. The casing was kept moist by regular light watering after the first fruitbodies had developed to 10 mm diam. About 15% more water was applied to the *A. bisporus* treatments than to the *Agaricus* W4 treatments since previous trials had shown that over-watering inhibited pinhead formation of *Agaricus* W4. Fruitbodies, diam 35-45 mm, were harvested at the 'stretched veil' stage over a 45-day period. The stipes were trimmed (about 13% of the sporophore weight was removed) and the weight of fruitbodies harvested from each tray was recorded. A randomized block design was used, with six replicate trays of each strain/supplement treatment.

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##### (a) Casing treatments

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SF16)

- (ii) Harte casing (Irish wet bog peat + fine grade chalk)
- (iii) Control casing + 'cacing' (spawn-run compost), cacing material was added at 250 g/m<sup>3</sup> casing.

(b) *Agaricus* strains/isolates

- Agaricus* W4 (i) A119
- (ii) A130
- (iii) W4II
- (iv) W4VI
- (v) Somycel 609, white hybrid
- (vi) Somycel 856, brown

Cultural conditions were similar to those in Experiment 1 with the exception of 'caced' casing treatments of *A. bisporus* Somycel 609 and 856. These trays were transferred from the case-running room after 6 days to a growing chamber where fresh air was introduced. The trays were then transferred back into the main cropping shed when this was aired, 14 days after casing. The spawned casing did not result in faster colonization of the casing for the *Agaricus* W4 treatments; these trays therefore remained in the main cropping shed throughout.

A randomized block design was used, with six replicate trays of each strain/casing treatment.

### Experiment 3 - Strain selection

Single spore isolates were obtained from a fruitbody of W4IV. The isolates were cultured on agar before rye grain spawn was prepared. Tests for mating reactions between single spore cultures were made but there was no evidence of any compatibility (fluffy mycelium at the juncture zone between cultures). Cultures were taken from the zone between two single spore isolate cultures to test if the cultures were the same as either of the single spore isolates. Six isolates of *Agaricus subfloccosus* from Canada were obtained from R. Kerrigan, Sylvan, USA.

Spawn was produced on rye grain for the different single spore isolates and mating test cultures.

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The experiment was conducted in a controlled environment chamber. The substrate was filled into plastic crates each holding 5 kg substrate, and spawned at 2% w/w with grain spawn. The same substrate as in Expts 1 and 2 was used. Cultural conditions were similar to those described in Expt 1, except a shallower layer of casing (20 mm) was used.

Three replicate crates of each strain were 'blocked' in three tiers in the cropping chamber.

## RESULTS

### Experiment 1

The yields of *Agaricus* W4, *A. bisporus* and *A. subfloccosus* in supplemented and unsupplemented compost are shown in Table 1. The *A. bisporus* strains produced a significantly higher yield than the wild *Agaricus* isolates. W4IV resulted in a significantly higher yield than the other *Agaricus* W4 isolates. Overall, supplementation of compost with Betamyl increased yield although no effect was recorded on isolates I/A130, IV or *A. subfloccosus* 3V1. The supplement Springboard had a negative effect on the yield of *Agaricus* W4 isolates and these plots had large numbers of *Coprinus* fruitbodies.

### Experiment 2

Differences between casing treatments were not consistent for different isolates or strains. For *A. bisporus* Somycel 609, the black (Harte) peat resulted in a higher yield than the brown peat but for the other isolates and strains, there were no significant differences in yield between the black and brown peat casings. The black peat casing was supplied in bulk and had a lumpy texture resulting in uneven colonization of the casing. However, mushrooms from the black peat casing were significantly cleaner than those from the brown peat casing. The black peat casing had a higher moisture holding capacity than the brown peat casing resulting in a higher moisture content at application and during cropping (Fig. 1). The black peat casing moisture content of the *A. bisporus* treatments was slightly higher than that of the *Agaricus* W4 treatments due to the higher water application. The first picks shown in

Fig. 1 are for casing with 'caccing'. The caccing treatment did not advance the cropping or affect the yield of *Agaricus* W4 but the cropping of *A. bisporus* was advanced by 5 days.

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The yield and number of fruitbodies from the *A. bisporus* strains Somycel 609 and Somycel 856 were significantly higher than those of any of the *Agaricus* W4 or *A. subfloccosus* isolates (Table 3). All of the single spore isolates which survived through to spawn production resulted in fruitbody formation. There was a wide range in yields from single spore isolates of W4IV. Isolates 5 and 16 produced significantly lower yields and isolates 2 and 15 produced significantly higher yields than the original culture W4IV. Cultures taken from the zone between single spore isolate cultures resulted in yields similar to one or other of the constituent isolates.

Two of the six Canadian *A. subfloccosus* isolates produced a spawn-run but failed to produce fruitbodies (K1542 and K1565). The isolate K1552 produced a higher yield than the other Canadian (K) and Swiss (3V1) isolates (Table 3). However, the quality of fruitbodies was poor since the caps were often misshapen.

### CONCLUSIONS

These experiments have confirmed that W4IV is the most productive parent isolate of *Agaricus* W4, compared with the other sources. On a semi-commercial scale, a yield of just under 150 kg/tonne compost at spawning was achieved. Two single spore isolates obtained



from W4IV produced a slightly higher yield than W4IV in crates, although this difference needs to be examined on a larger scale. The *A. subfloccosus* isolate K1552 produced a yield of 160 kg/tonne in a crate experiment although the quality of fruitbodies was poor.

The supplement Betamyl 1000 resulted in a significant yield increase in both *A. bisporus* and *Agaricus* W4 although not all the W4 isolates produced a significant response. No positive effects of the supplement Springboard were found although in an earlier trial, a yield increase comparable to that produced by Betamyl 1000 was found using the strain Somycel 609. The use of spawn-run compost (cacking) did not result in a faster colonization of the casing. A higher rate of cacking than that used in the present experiment (250 g/m<sup>3</sup>) may be necessary to achieve a positive effect.

The black peat casing resulted in cleaner mushrooms but there was no overall yield advantage for the *Agaricus* W4 isolates. A black peat casing with a less lumpy structure resulting in a more uniform colonization of the casing would be preferable.

Table 1 Yield of *Agaricus* W4 isolates, *A. bisporus* and *A. subfloccosus* on unsupplemented compost and compost supplemented with Betamyl 1000 or Springboard, Kg tonne<sup>-1</sup> compost at spawning, Expt 1. Each value is the mean of 6 replicate trays

Supplement	<i>Agaricus</i> W4 isolates				<i>A. bisporus</i>		<i>A. subfloccosus</i>
	I/A119	I/A130	II	IV	S609	S856	3V1
Control	92	127	108	146	201	175	130
Betamyl	117	124	122	148	223	202	121
Springboard	59	105	89	118	202	181	113
Mean	89	119	106	137	209	186	121

LSD (5%) for strain means = 16.6; for strain x supplement = 28.9

Table 2 Yield of *Agaricus* W4 isolates and *A. bisporus* strains using different casing treatments, Expt 2. Each value is the mean of 6 replicate trays

Casing treatment	<i>Agaricus</i> W4 isolates				<i>A. bisporus</i>	
	I/A119	I/A130	II	IV	S609	S856
Brown peat	64	134	107	133	204	192
Black peat	63	110	102	147	249	192
Brown peat + 'cassing'	78	138	95	134	212	202
Mean	68	127	101	138	222	195

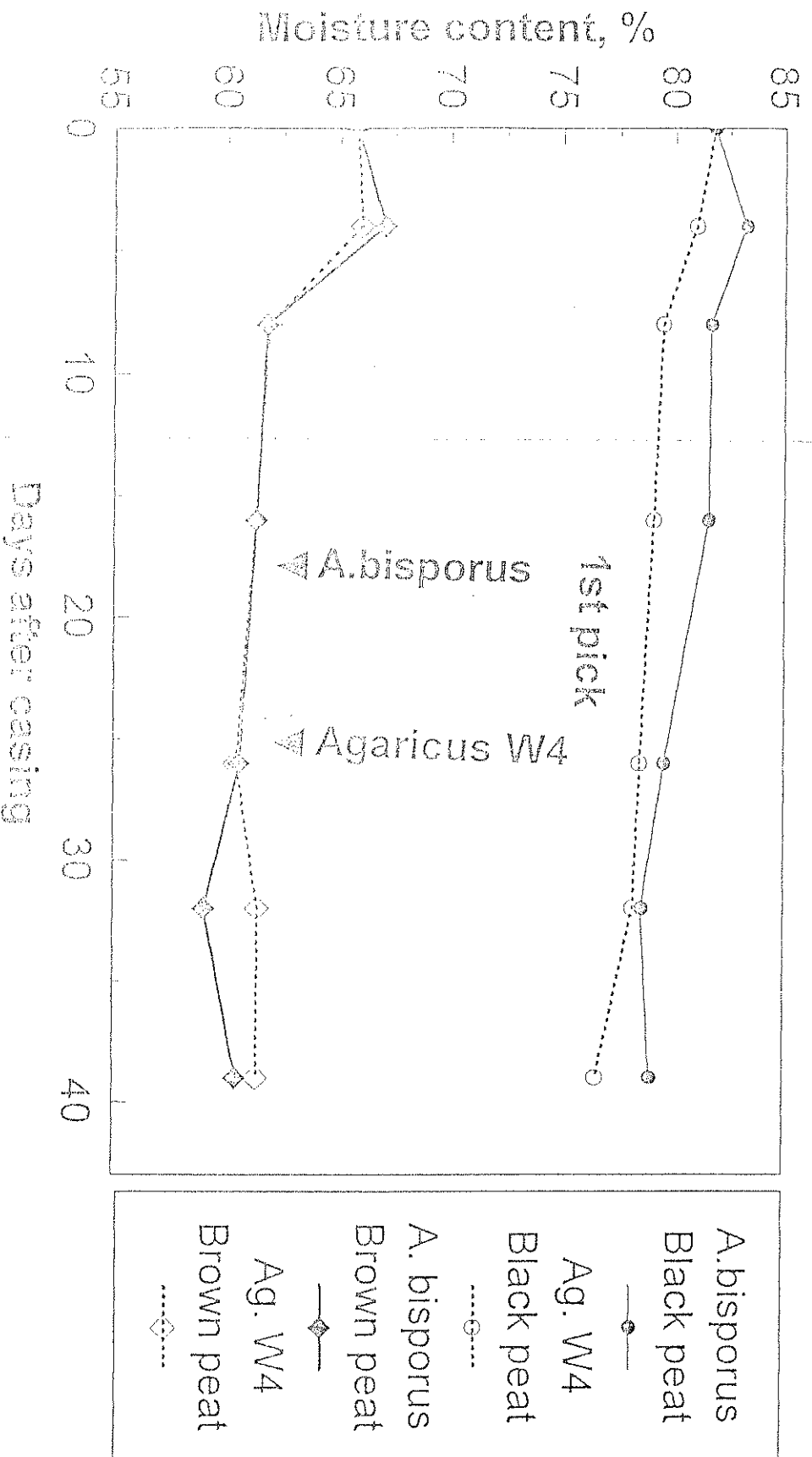
LSD (5%) fir strain means = 17.7; for strain x casing treatments = 30.7

Table 3 Yield and number of fruitbodies from single spore isolates of W4IV and cultures taken from the zone between two single spore isolate cultures, Expt 3. Each value is the mean of 3 replicate crates

Species	Isolate or Strain	Yield, g/kg substrate	No. fruitbodies/crate	
<i>Agaricus</i> W4	I/A130	132	31	
Parent cultures	II	109	25	
	IV	138	33	
Single spore isolates	IV/ssi1	131	33	
	IV/ssi2	154	30	
	IV/ssi3	127	34	
	IV/ssi5	81	28	
	IV/ssi8	147	31	
	IV/ssi13	136	32	
	IV/ssi14	146	46	
	IV/ssi15	152	34	
	IV/ssi16	88	23	
		IVssi1/ssi2	156	39
		IVssi1/ssi3	126	28
		IV/ssi13/ssi14	113	28
		IV/ssi14/ssi16	155	36
		IV/ssi15/ssi16	91	24
	<i>A. subfloccosus</i>	K1543	49	17
		K1552	160	41
K1553		67	15	
K1570		30	10	
3V1		110	26	
<i>A. bisporus</i>	Somycel 609	251	53	
	Somycel 856	239	61	

L.S.D. ( $P < 0.05$ ) = 14 (*Agaricus* W4 isolates)

Fig. 1 Casings moisture content, Expt 2



## PART II - AGARICUS ARVENSIS

### MATERIALS AND METHODS

*Agaricus arvensis* isolates and strains

- (i) 93-7
- (ii) 93-9
- (iii) 93-10
- (iv) Somycel R20

The sources of the three new HRI isolates are shown in Table 4.

#### Cultural procedure

The cultural procedure was the same as for *Agaricus* W4 described in Expt 3 except lighting (150 lux) was used for 12 h/d after fresh air was introduced into the chamber. The same compost was used (see Appendix for analysis). Sporophores were harvested closed with a cap diameter of 45 mm. The base of the stem was trimmed (average trimmed weight of fruitbodies was 35 g). Three replicate crates of each isolate/strain were prepared.

### RESULTS

All the isolates produced a first flush about 25 days after casing and had flushing intervals

of about 12 days. Four to five flushes were picked during a cropping period of 45 days.

Yields from the different isolates are shown in Table 5. The isolate 93-7 produced a higher yield than the strain Somycel R20 although the difference was not quite significant at  $P = 0.05$ . The appearance and quality of fruitbodies from the different sources of *A. arvensis* was similar. The HRI isolates were slightly paler than R20. However, fruitbodies of R20 became paler as the flushes progressed.

Table 4 Source of *Agaricus arvensis* isolates

Isolate	Collected by:	Location	Date	Habitat
93-7	H. Grogan	Littlehampton, West Sussex	26 Sept. 1993	Coniferous leaf litter
93-9	H. Grogan	Littlehampton, West Sussex	5 Oct. 1993	Cupressus hedge
93-10	R.H. Gaze	Bedham, West Sussex	11 Oct. 1993	Composting grass clippings



Table 5 Yield and number of fruitbodies of *Agaricus arvensis* isolates and strains. Each value is the mean of 3 replicate crates.

Isolate or strain	Yield g/kg substrate	No. fruitbodies/crate
93-7	133	21
93-9	67	14
93-10	62	10
Somycel R20	120	17
L.S.D. ( $P < 0.05$ )	15	

## APPENDIX

Analysis of compost at spawning

N	NH <sub>4</sub> <sup>+</sup>	Ash	Moisture	pH
Percentage of Dry Weight				
2.51	0.21	15.3	75.8	7.7