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DEVELOPMENT OF *AGARICUS* SPECIES OTHER THAN *AGARICUS BISPORUS*

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REVIEW OF PROJECT ACHIEVEMENTS

1. To identify an *Agaricus* species (W4) (first thought to be *A. sylvaticus* 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*.

2. The identification of a strain of *Agaricus* W4 (IV) which is capable of producing 63.5 and 83% of the yield of commercial white hybrid and brown strains of *A. bisporus* under semi-commercial cropping conditions.

3. Significant differences in the cropping performance of different single spore isolates from the same original fruitbody were observed. This could be an important source of genetic variation for further improvements in yield potential.

4. Yields of over 200 kg/tonne compost at spawning have been achieved in crate experiments with *Agaricus* W4 strains. The best yield obtained in trays (45 kg compost) was 131 kg/tonne compost.

5. The substrate requirements of *Agaricus* W4 and *A. bisporus* appeared to be similar, with the best yields being obtained from commercial mushroom composts based on pre-wetted straw with horse and poultry manures.

6. A significant correlation was found between substrate pH and yield of *Agaricus* W4, with lower pH resulting in higher yield.

7. *Agaricus* W4 can be grown under similar conditions to *A. bisporus*, although covering of the compost and casing with paper to prevent desiccation and to maintain high CO_2 levels, the maintenance of high humidity levels (>90% r.h.), a shallower casing layer (35 mm) and avoidance of heavy watering are important cultural factors.

8. The use of growth rate data obtained from simple growth rate tubes is an approximate and rapid test for the productivity of different W4 strains, and could be used in the early stages of a screening programme where a large number of strains are involved.

9. Yields of over 170 kg/tonne compost were achieved with *Agaricus arvensis* strain Somycel R20.

RECOMMENDATIONS FOR FURTHER WORK

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

1. Single spore isolates of the most promising strain W4IV should be obtained and their yield potential assessed.

2. The use of spawned casing should be examined.

3. Other supplements (Champlus and Springboard) should be tested for use with W4 strains and *Agaricus arvensis*.

4. A casing material suited to the culture of these *Agaricus* species should be developed; different peat and chalk sources and proportions should be examined.

INTRODUCTION

While the cultivated mushroom, *Agaricus bisporus*, continues to dominate commercial production, there are over 40 naturally-occurring *Agaricus* relatives in the UK alone, offering a diversity in shape, texture, colour and flavour. Two in particular, the horse mushroom (*Agaricus arvensis*) and an as yet un-named woodland species (W4), have been identified as having commercial potential due to their strong flavours and attractive fruitbodies.

The objectives of the present project were:

1. To compare the yield potential of different strains of W4 obtained from different localities and different single spore isolates from the original fruitbodies

2. To establish nutritional and environmental optima for the growth and fruiting of W4

3. To produce the most promising strains on a semi-commercial scale

4. To examine the effect of protein-based supplementation (Betamyl 1000) on cropping

5. To examine the cropping performance of a commercial strain of *Agaricus arvensis* (Somycel R20) and a range of other strains held at HRI.

PART I - *AGARICUS* W4

MATERIALS AND METHODS

Agaricus strains

Four sources of *Agaricus* W4 found in different locations in the UK were used in these experiments (Table 1). Tissue cultures were obtained from fruitbody collections of W4II and W4IV. Cultures from single basidiospores were obtained from W4I fruitbodies using a micromanipulation technique. Single spore isolates were also obtained from the W4II and W4III isolates using a diluted suspension of basidiospores (strains W4I/A119 and W4II are shown in the Appendix, Photos 1 and 2). Two strains of *A. subfloccosus* were obtained from R. Stadelman, Gossau, Switzerland; a culture from the original tissue culture *A. subfloccosus* I and a single spore culture from this, 3V1. The strains were cultured on malt extract agar before spawn was prepared on rye grain. For comparison, two commercial strains of *A. bisporus*, white hybrid Somycel 609 and brown Somycel 856, were used.

Composted substrates

Wheat straw was the main carbon source in all of the substrates used (Table 2). With the exception of substrate i, various animal manures were used as the main source of nitrogen (N) and the quantities were adjusted to obtain a substrate N content of *c.* 2.5-3.0% of D.M. after processing. Substrates a, b and c were obtained from commercial mushroom compost producers. These were based on mixtures of pre-wetted wheat straw and deep litter poultry manure (a shredded mixture of poultry droppings, feathers and wood shavings), with or without horse or pig mature (a mixture of straw bedding and droppings). Following a mixing period of 3-4 d, the materials were stacked into windrows for 7 d, and turned at 2-d intervals (Phase I composting). Compost activator (molassed fibrous meal containing 26% sugar and 7.4% N, w/w D.W.) was added at 10 kg/tonne substrate to substrates a and b.

Formula 2 mushroom compost (Randle, 1974) was prepared by mixing wheat straw, poultry manure and compost activator in a stack, which was turned and wetted during a 13-d (Phase I) period (substrate d). Substrates f, g and h were prepared with chopped straw to assist

water absorption, and poultry manure. The materials were stacked, wetted and turned for either a 3 or 13-d Phase I period.

Substrate i was prepared with chopped straw and compost activator (10 kg/tonne substrate). A Phase I stacking period of 130 d was required to achieve a level of substrate degradation, based on ash content, similar to that obtained with substrates prepared with animal manures. Following an initial wetting and blending of materials, the stack was turned at 5-7 d intervals. Gypsum was added to all the substrates at the start of Phase I at 25 kg/tonne substrate to reduce the pH and release of ammonia.

All the Phase I substrates were filled into controlled aeration bulk pasteurization chambers or 'tunnels' and pasteurized at 58-60°C for 6 h and 'conditioned' at 46-49°C (Phase II composting) until ammonia could no longer be detected in the substrate with Draeger gas sampling tubes (CH20501), usually 6-7 d after filling. To examine the effect of the duration of Phase II beyond the clearance of ammonia, samples of substrate were conditioned at 46-49°C for a further period of 7 d (substrates e and g). All substrates were analysed for moisture, N, ammonium (NH_4^+) and ash contents and pH.

Cultural procedure

Small scale experiment (Expt 1). The experiments were conducted in controlled environment chambers (Flegg and Smith, 1977). The substrates were filled into plastic crates (490 x 290 x 230 (deep) mm) each holding 4 kg substrate, and spawned at 2% w/w with grain spawn.

After spawning, the surface of the substrate in the crates was covered with paper to prevent desiccation. The substrate temperature was maintained at 25°C and the relative humidity of the air in the chamber was maintained at 95%. After full mycelial colonization of the substrate, *c.* 21 d after spawning, the compost was covered (cased) with 2.2 kg of a moist mixture of peat and chalk (9:1 v/v) to a depth of 20 mm. The casing was covered with paper until mycelium became visible at the surface, *c.* 14 d after the casing was applied. Fresh air was then introduced in the chamber and the environmental conditions were altered to encourage pinhead initiation and fruitbody development. The air temperature, humidity

and CO_2 concentration were reduced to levels of 16-17°C, 90-92% r.h. and 0.06-0.07% v/v respectively. The casing was kept moist (70% moisture content) by regular light watering after the first fruitbodies had developed to 10 mm diam.

Sporophores, diam. 35-45 mm, were harvested at the 'stretched veil' stage over a 45 d period. The stipes were trimmed (about 13% of the sporophore weight was removed) and the number and weight of sporophores harvested from each container was recorded.

A split plot design was used with substrates a to h (Table 2) forming main plots and four *Agaricus* W4 strains (Table 4) and *Agaricus bisporus* S609 forming sub-plots. Three replicate crates of each strain were 'blocked' in three tiers in the cropping chamber. Three replicates of substrates a to h and one replicate of substrate i were prepared. For the third replicate of substrate a to h, 12 additional *Agaricus* W4 strains were introduced (Table 5), with three replicate crates of each strain arranged in blocked tiers.

Large scale experiment (Expt 2). The experiments were conducted in controlled environment growing rooms using wooden cropping trays (internal dimensions 0.91 x 0.61 x 0.18 (deep) m), each filled with 45 kg of substrate. G.C.R.I. Formula 2 mushroom compost (substrate d) was used and grain spawn was added at a rate of 2% w/w. Four W4 strains, two *A. bisporus* strains (S609, white hybrid, and S856, brown) and two *A. subfloccosus* strains were used (Table 8). The compost was supplemented with the soya meal-based 'Betamyl 1000', containing formaldehyde denatured protein at rates of 0 (control), 0.5 and 1% of compost fresh weight. The effect of compost supplementation was not examined on W4IV or *A. subfloccosus*.

Spawned compost was hydraulically pressed with a pressure of 1.03×10^7 Pa into the trays which were stacked in a spawn-running room for 21 d. The compost was then cased to a depth of 35 mm using the same casing mixture described in Expt 1. Environmental conditions in the growing rooms and procedures for covering the compost and casing with paper and harvesting of sporophores were the same as in Expt 1. About 30% less water was applied to the casing of W4 and *A. subfloccosus* treatments than the *A. bisporus* since preliminary tests had shown that heavy watering tended to inhibit pinhead initiation and

development of these species, while the less vigorous growth in the casing layer also had a lower water requirement.

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

Growth rate of Agaricus strains in composted substrates

A method for measuring mycelial growth in culture tubes was adapted from Straatsma *et al.* (1989). Samples of substrate (30 g) were filled into the tubes (internal dimensions 195 x 30 mm) to a level of 110 mm from the closed end, which contained seven rye grains of *Agaricus* spawn. The open ends of the tubes were plugged with cotton wool. The tubes were kept in an incubator at 25°C, 95% r.h., and the position of the mycelial front was recorded at 2-d intervals until the substrate was fully colonized.

In Expt 1, one sample of substrates a to h was used, inoculated with the *Agaricus* strains shown in Table 5. In Expt 2, substrate d was used, inoculated with the *Agaricus* strains shown in Table 7. Three replicate growth tubes of each strain were allotted to three 'blocks' in the incubator.

RESULTS

Analysis of substrates, Experiment 1

Differences in pH and dry weight, N and NH_4^+ content between substrate types a to h were not significant at $P = 0.05$, although there was some indication that substrate g had relatively high N and NH_4^+ contents whereas substrates a and e had relatively low N and NH_4^+ contents.

Substrate types a and b had significantly higher ash contents than substrate types e, d, f and g. Substrate type i, which was not replicated, had relatively low dry weight, N and NH_4^+ contents compared with the other substrates.

Experiment 1

Cropping in plastic crates. The yields of four W4 strains and *A. bisporus* S609 from nine substrate types are shown in Table 4. The yield from strain I/A129 was significantly lower than that from the other W4 strains. Substrates b and c resulted in significantly higher yields than substrates e and i. The overall mean yield of the best three W4 strains was 43% of the overall mean yield of *A. bisporus*, although in the best substrates b and c, this increased to 50%. A cropping crate is shown in Photo 3.

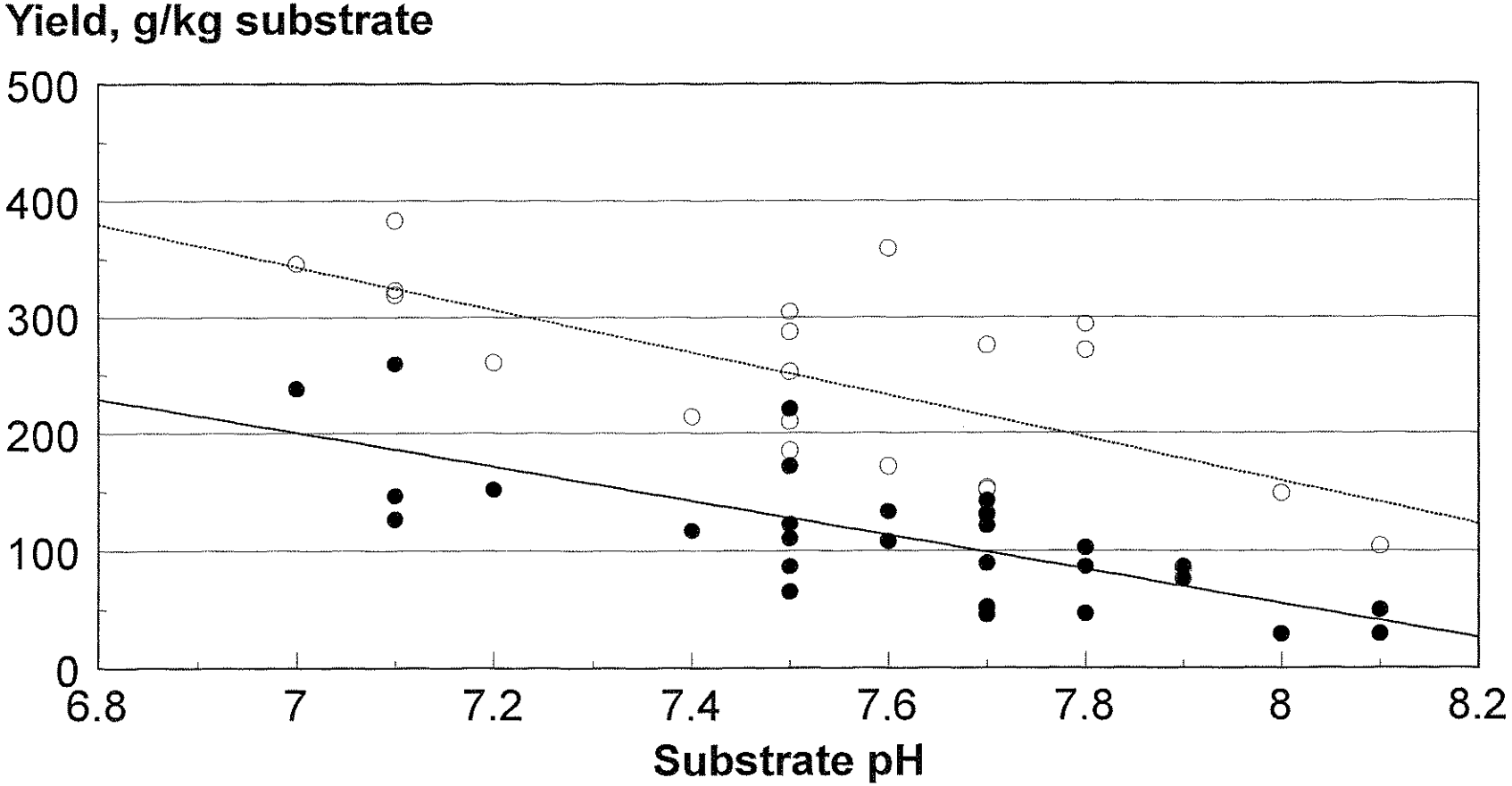
The relationship between the mean yield of strains W4I/A119 and A130 and W4II and different compost analysis factors was determined. There were no significant relationships between yield and compost N, NH_4^+, ash or dry weight contents but there was a significant negative correlation between yield and substrate pH (Fig. 1). There were also significant negative correlations between the yield of *A. bisporus* and substrate pH and moisture content. The yields of W4 strains and *A. bisporus* in the different substrates were also significantly correlated.

The yield from a wider range of W4 strains is shown in Table 5. The yields of strains W4I/A119 and A130, W4II, W4II/A1, W4III/A9 and W4IV were not significantly different. There were significant differences in the yields of single spore isolates from W4I; isolates A119 and A130 produced significantly higher yields than A118, A125 and A129. Yields of single spore isolates from W4III were not significantly different, except A9 which produced a significantly higher yield.

Growth rate in composted substrates

The growth rate of W4 strains and *A. bisporus* S609 is shown in Table 6. The W4I single spore isolates resulted in slower growth rates than W4III single spore isolates and the strain W4IV. However, within single spore isolates there were also significant differences in growth rate. The strains with the highest growth rates were W4III/A7 and W4IV. However, the mean growth rate of these strains was 47% of that of *A. bisporus* S609.

Fig. 1 Relationship between substrate pH and yield



Experiment 2

Cropping in trays. The yield of W4 strains, *A. bisporus* and *A. subfloccosus* is shown in Table 7. The strain W4IV produced a significantly higher yield than the strain W4II. The yield of strains W4I/A119 and A130 was not significantly different and was between those of W4II and W4IV. The single spore isolate of *A. subfloccosus* 3V1 produced a significantly higher yield than strain I. The strain W4IV produced 83% of the yield of *A. bisporus* (S865, brown) and *A. subfloccosus* (3V1) and 63.5% of the yield of *A. bisporus* (S609, white hybrid). The use of the supplement Betamyl 1000 significantly increased the yield of *A. bisporus* S609 but there was no significant effect on the yield of *A. bisporus* S856 or on the three W4 strains tested. The effect of Betamyl 1000 on W4IV and *A. subfloccosus* was not examined. Cropping of W4 strains in trays is shown in Photo 4.

Growth rate of W4, A. bisporus and A. subfloccosus

The growth rate of the strains used in Expt 2 in the same substrate is shown in Table 8. The growth rate of *A. bisporus* was significantly higher than that of *A. subfloccosus*, which was higher than that of the W4 strains. Within species there were also significant differences in growth rate. The single spore isolate of *A. subfloccosus* (3V1) resulted in a faster growth rate than the original strain (I). Within W4, strain W4/A1 and W4IV resulted in the fastest growth rates.

CONCLUSIONS

The results of these experiments have shown that yields of 63.5 and 83% of commercial white hybrid and brown strains of *A. bisporus* can be achieved with W4IV on a semi-commercial scale. Yields of over 200 kg/tonne compost at spawning were obtained in crates containing 4 kg of substrate, although the best yield obtained in trays containing 45 kg substrate was 131 kg/tonne substrate.

Yields of mushrooms from W4 and *A. bisporus* in different substrates were significantly correlated. Similar substrate factors are therefore related to high productivity in both

species. Substrates with a lower pH resulted in a higher yield in both species, although substrate dry weight content was only correlated with the yield of *A. bisporus*. The two best substrates were commercial mushroom composts based on pre-wet straw, with horse and poultry manures.

The use of the supplement Betamyl 1000 had no significant effect on the yield of W4 or brown *A. bisporus* (S856), although the yield of white hybrid *A. bisporus* (S609) was significantly increased.

The use of growth rate data is an approximate guide to the productivity of different strains and could be used in the early stages of a screening programme where many strains are involved. However, differences between the relative growth rate and the relative productivity of different strains were found. Differences were also found in the relative performance of different strains in small-scale crates and larger-scale tray experiments. The final selection of strains can therefore only be based on the results of larger scale experiments.

Table 1 Source of W4 and *Agaricus subfloccosus* isolate

Isolate	Collected by:	Location	Date/Year	Habitat
W4I	P. Grimbly	Littlehampton, West Sussex	1975	Leaf litter beneath frondose trees
W4II	R.H. Gaze	Epsom, Surrey	28 Feb, 1977	Leaf litter beneath a *Cupressus* tree
W4III	R. Watling	Dunkeld, Tayside	1979	Roadside verge
W4IV	J.T. Fletcher	Olantigh, Kent	7 May, 1992	Leaf litter beneath a *Cupressus* hedge
A. subfloccosus				
I	R. Stadelman	Bäretswil-Zürich, Switzerland	22 Oct, 1990	Soil bank, formerly planted with *Picea abies*

Table 2 Animal manure and compost activator ingredients, and straw pre-treatments used in preparing the substrates, and durations of Phases I and II of composting

Substrate	Manure		Activator added[2]	Straw treatment	Duration (days)	
	Type	Quantity[1]			Phase I	Phase II
a	Poultry	28	Yes	Pre-wet	7	7
b	Horse & Poultry	46 5	Yes	Pre-wet	7	7
c	Horse, Pig & Poultry	16, 16 8	No	Pre-wet	7	7
d & e	Poultry	15	Yes	None	13	7 or 14
f & g	Poultry	29	No	Chopped	3	7 or 14
h	Poultry	38	No	Chopped	13	7
i	None	0	Yes	Chopped	130	7

[1]% of substrate fresh weight, excluding water added to the substrate or straw
[2]1% of substrate fresh weight, including water added

Table 3 Analysis of the substrates used in the experiments. Each value is the mean of three replicates (one replicate for substrate i (Expt 1) and d (Expt 2))

	% of D.W.				
Substrate	N	NH_4^+-N	Ash	% D.W.	pH
a (Expt 1)	2.12	0.12	24.7	31.8	7.40
b	2.57	0.16	23.2	29.9	7.37
c	2.66	0.22	21.5	28.2	7.33
d	2.43	0.09	16.3	24.0	7.60
e	2.29	0.02	18.3	27.7	7.73
f	2.57	0.17	17.8	29.4	7.43
g	3.12	0.29	19.5	24.3	7.77
h	2.49	0.21	21.0	27.1	7.67
i	1.43	0.08	12.1	19.0	8.10
d (Expt 2)	2.56	0.03	21.2	25.5	7.80
S.E. of the difference between means a-h (Expt 1)	0.335	0.077	1.63	2.58	0.179

Table 4 Yield sporophores of W4 strains and *A. bisporus* grown on eight composted substrate types, g kg^{-1} substrate at spawning, Expt 1. Each value is the mean of 3 substrate replicates x 3 replicate containers

	W4 Strain				*A. bisporus*
Substrate	II	I/A119	I/A129	I/A130	S609
a	81.8	104.5	41.6	92.2	296.3
b	194.3	154.8	44.8	130.3	310.6
c	92.0	145.5	61.6	164.8	271.9
d	116.1	107.5	55.7	78.9	243.7
e	65.7	108.1	53.9	88.2	220.0
f	102.7	127.9	54.7	86.5	240.8
g	102.7	100.9	56.3	64.4	221.9
h	113.4	112.1	40.0	133.8	242.4
i*	54.5	47.9	6.7	46.6	103.2
Mean a-h	108.6	120.2	51.1	104.9	256.0

*Single substrate replicate, mean of 3 containers
S.E. of difference between means: strains = 9.43; substrates = 20.63; strains x substrates = 26.64

Table 5 Yield of sporophores of W4 strains grown on composted substrates, g kg^{-1} substrate at spawning, Expt 1. Each value is the mean of eight substrates x three replicate containers

Original fruitbody	W4I	W4II	W4III	W4IV
Tissue culture	-	**113.6**	-	**112.3**
Single spore isolates	A118	A1	A4	
	46.4	**88.7**	**54.7**	
	A119		A5	
	100.3		**59.0**	
	A125		A6	
	45.6		**52.2**	
	A129		A7	
	20.7		**44.5**	
	A130		A9	
	104.0		**90.1**	
			A10	
			39.7	
			A11	
			62.5	

Mean yield of *A. bisporus* (S609) = 233.1
S.E. of the difference between means = 10.91

Table 6 Growth rate of W4 strains in composted substrates, mm d^{-1}, Expt 1. Each value is the mean of eight substrates x three replicate growth tubes

Original fruitbody	W4I	W4II	W4III	W4IV
Tissue culture	-	**2.28**	-	**2.66**
Single spore isolates	A118	A1	A4	
	1.10	**2.16**	**2.31**	
	A119		A5	
	2.17		**2.22**	
	A125		A6	
	1.80		**2.47**	
	A129		A7	
	1.76		**2.67**	
	A130		A9	
	2.12		**2.42**	
			A10	
			2.02	
			A11	
			2.24	

Mean growth rate of *A. bisporus* (S609) = 5.84
S.E. of the difference between means = 0.064

Table 7 Yield of sporophores of W4 strains, *A. bisporus* and *A. subfloccosus* on unsupplemented compost and compost supplemented with Betamyl 1000, kg tonne^{-1} compost at spawning, Expt 2. Each value is the mean of 6 replicate trays

Betamyl 1000	W4 Strains				*A. bisporus*		*A. subfloccosus*	
% w/w	I/A119	I/A130	II	IV	S609	S856	I	3V1
0	95	108	82	131	206	156	105	159
0.5	112	105	73	-	239	159	-	-
1.0	103	103	71	-	252	165	-	-
Mean	104	105	75	-	232	160	-	-

S.E. of the difference between means: strain means = 8.9;
strain x Betamyl = 15.4

Table 8 Growth rate of W4 strains, *A. bisporus* and *A. subfloccosus* on compost, mm d^{-1}, Expt 2. Each value is the mean of three replicate growth tubes

Agaricus species	W4				*A. bisporus*		*A. subfloccosus*
Strain	I	II	III	IV	S609	S856	I
	-	2.40	-	2.72	8.44	7.66	3.73
Single spore isolates	A119	A1	A9				3V1
	2.02	2.87	1.31				5.20
	A130		A11				
	1.71		1.24				

S.E. of the difference between means = 0.952

PART II - AGARICUS ARVENSIS

MATERIALS AND METHODS

Substrates

The substrates used are shown in Table 9. These were G.C.R.I. Formula 2 compost, using chopped and unchopped straw (substrates 1 and 2) and two commercial mushroom composts (substrates 3 and 4). A single replicate of substrates 1 to 3 and five replicates of substrate 4 were produced. The analysis of substrates is shown in Table 10.

Cropping procedure

The experiment was conducted in controlled environment chambers fitted with fluorescent lighting (200 lux above the cropping surface). Substrate was filled into trays at 45 kg/tray and spawned at the rate of 1.5% of fresh weight with rye grain spawn. The strain Somycel R20 was used in all the crops and the strain W27C was used in two crops. Cultural conditions were similar to those described for *Agaricus* W4 except lighting was used for 12 h/d after fresh air was introduced into the chamber. Sporophores were harvested closed with a cap diameter of 45 mm. The base of the stem was trimmed (average trimmed weight of sporophores was 33 g).

RESULTS

In two crops grown on commercial mushroom compost, yields of over 170 kg/tonne were achieved with the strain R20. In crops where the strain W27C was grown, fruitbodies were obtained but the yields were low compared with R20. There was an insufficient number of composts to determine which compost factors were related to yield. Fruitbodies of strains R20 and W27C are shown in Photos.

Table 9 Animal manure and straw ingredients in the composts, and durations of Phase I and II of composting (*Agaricus arvensis*)

Crops	Compost	Manure	Straw treatment	Duration, d	
				Phase I	Phase II
1	1	Poultry	Chopped	13	7
2	2	Poultry	None	13	7
3	3	Horse/poultry	Pre-wet	7	7
4-8	4	Horse/Pig/Poultry	Pre-wet	7	7

Table 10 Analysis of composts at spawning and yield of *Agaricus arvensis*, R20 and W27C

Crop	Percentage of D.W.			Moisture %	pH	Yield, kg/tonne	
	N	NH_4^+	Ash			R20	W27C
1	2.74	0.23	22.4	70.2	7.6	113	*
2	2.99	0.35	21.2	76.7	7.8	142	26
3	2.36	0.22	23.2	73.2	7.1	174	*
4	3.05	0.38	22.2	73.7	7.3	119	*
5	2.38	0.11	22.7	72.7	7.8	177	*
6	2.37	0.13	19.7	71.6	7.5	91	*
7	2.12	0.23	22.1	72.8	7.3	141	*
8	2.04	0.23	17.6	71.6	7.5	123	53

*Not tested