Project Title: Calibration of the new virus antiserum for use in immunosorbent electron microscopy (IEM)

Project Leader: Christine Henry

Final Report:	29/1/96
Project Number:	M7a
Project Leader:	Christine Henry
Location of Project:	Central Science Laboratory MAFF Hatching Green Harpenden Herts AL5 2BD
Project Coordinator:	Richard Gaze
Date Project Commenced:	August 1994
Date Project Completed:	December 1995
Key Words:	- Mushroom - La France disease

CONTENTS

RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application: The aim of the project was to calibrate a new antiserum for use in diagnostic tests for MV1 and MV4 based an immunosorbent electron microscopy (IEM).

Summary:

(1) Objective of the project

The objective of the project was to calibrate the new antiserum against the Barton antiserum for use in immunosorbent electron microscopy (IEM).

(2) Results

The ability of the new antiserum to trap virus particles of MV1 and MV4 was compared with that of the Barton antiserum. Although results from both antisera were broadly comparable the new antiserum was found to be slightly less effective in trapping both viruses.

(3) Opportunity for Application

All mushroom virus testing using IEM is carried out at CSL so information on the calibration of this antiserum with the Barton antiserum can be immediately used in decision making.

EXPERIMENTAL SECTION

Introduction

La France disease of mushrooms was first described in the USA. Its symptoms include a delay in appearance of sporophores, reduced yield, misshapen sporophores and accelerated post-harvest deterioration. Hollings (1962) and Hollings & Stone (1971) found that the disease is associated with the presence of virus-like particles, especially mixtures of 25nm (MV1) and 35nm (MV4) spherical particles and 19 x 50 nm bacilliform particles (MV3). The diagnosis and detection of these viruses was originally carried out by electron microscopy (EM), looking for the characteristic virus particles associated with the disease. Barton & Atkey (1984) developed antisera against purified mushroom virus preparations. These antisera have been used in immunosorbent electron microscopy tests (IEM) to diagnose virus problems in crops. IEM tests have been shown to be more sensitive than EM in the early diagnosis of virus problems. Both EM and IEM are currently used at CSL for the diagnosis of viruses in samples of mushrooms sent in by growers.

The diagnostic test provided by CSL is used by many mushroom growers with up to 2,000 samples being tested each year. Of these tests most are done by IEM, which is a popular test with growers who wish to monitor virus levels in their crops in order to get an early warning of increased virus levels before they cause severe yield loss.

At present CSL is the only UK-based laboratory offering this testing service. The IEM test is dependent on the availability of a good quality antiserum for trapping mushroom viruses onto EM grids. This test is based on antisera produced originally by Barton & Atkey (1994), however, with supplies of these antisera beginning to run out it seemed sensible to produce a new antiserum against these viruses, particularly against MV4 which appears to be the virus most frequently associated with yield loss. Under project M7 an antiserum was produced for use in the IEM test. The objective of this project was to assess the performance of this antiserum when compared to the Barton antiserum in trapping of MV1 and MV4 in the IEM test.

MATERIALS AND METHODS

Immunoelectron microscopy (IEM) method

Antisera were tested for their ability to trap viruses in IEM. The antisera were diluted 1:500 in carbonate buffer pH 9.6 and used as a trapping antibody in IEM as described below.

20 µl drops of each test serum dilution were placed on a piece of Parafilm and a carbon collodion coated grid was placed on each drop for 30 minutes at room temperature. A piece of mushroom was taken from each of 10 sporophores and placed on a muslin square. Mushroom sap was extracted by squeezing the sporophores through the muslin in a garlic press into an eppendorf tube and this exudate was centrifuged for one minute at 10,000 rpm and finally diluted 1:10 in PBS 20 µl drops of this were placed on Parafilm. The grids were washed with 20 µl drops of phosphate-buffered saline, drained and placed on the diluted mushroom exudate drops for 1 hour at room temperature. The grids were then washed with 30 drops of distilled water, followed by 5-6 drops of 2% uranyl acetate, drained and examined under the electron microscope.

The number of particles of MV1 and MV4 trapped were counted for 20 fields of view at 100,000 x magnification.

RESULTS

The results for trapping using the Barton and HDC antisera are shown in Table 1. Overall, the results are generally comparable for the two antisera. No trapping of MV3 using the HDC antiserum would be expected (see previous report on project M7). Levels of trapping of MV1 using the HDC antiserum were generally lower than using the Barton antiserum, being $\underline{c}$ 80-90% of the level obtained using the Barton antiserum. The relatively low level of trapping of MV4 by the HDC antiserum was surprising since previous comparisons (see project M7) had suggested that the HDC antiserum performed much better than the Barton antiserum. Previous tests were performed using higher levels of virus than the tests reported here and it is possible that the HDC antiserum performs well in trapping high numbers of MV4 particles but less well with low levels of virus. There also remains the possibility that there are serologically distinct types of MV4 which infect mushrooms.

CONCLUSIONS

The HDC antiserum performs in a fairly similar way to the Barton antiserum in trapping MV1. A best-fit line can be drawn using the data on numbers of particles trapped onto grids (see Figure 1). This could be used to translate results from one antiserum to the other for IEM on MV1. Results for MV4 were less satisfactory with the only conclusion being that the HDC antiserum traps MV4 less well than the Barton antiserum at low virus levels. In order to produce an adequate comparison of the two antisera these experiments should be repeated using a range of mushrooms known to be infected with MV4 at higher levels.

In general, it appears that although the HDC antiserum could be used for IEM on MV1 it is not adequate for use with MV4. The difficulties is encountered in production of antibodies to these non-immunogenic proteins are well known (Schmidt *et al*, 1995). It remains highly desirable that a good antiserum to both these viruses should be produced. Work is being carried out at CSL under MAFF funding to look at the possibilities of using gene expressions systems in bacteria to produce large quantities of the coat proteins of these viruses for production of monoclonal antibodies. This work may eventually lead to the production of better quality antibodies for use in IEM or enzyme linked immunoassay (ELISA) tests.

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GLOSSARY

MV1 = Mushroom Virus 1, a spherical 25nm diameter virus particle.

MV3 = Mushroom Virus 3, a bacilliform 19 x 50nm virus particle.

MV4 = Mushroom Virus 4, a spherical 35nm diameter virus particle.

REFERENCES

Barton, R.J. & Atkey, P.T. (1984). Detection of viruses in *Agaricus bisporus* (Lange) Imbach by immunosorbent electron microscopy. *Annual Report of the Glasshouse Crops Institute for 1982*, p.115-116.

Hollings, M. (1962). Viruses associated with a die-back disease of cultivated mushroom. *Nature 196*, p.962-965.

Hollings, M. & Stone, O. (1971). Viruses that infect fungi. *Annual Review of Phytopathology* 9, p.93-118.

Schmidt, H.B., Proll, E., Richter, J. & Zahn, R. (1985). Investigations of purification of mushroom viruses. *Achives für Phytopathologie und Pflanzenschutz 21*, p.121-130.

Table 1: Results of a Comparison of Trapping of Virus Particles of MV1, MV3 and MV4 in IEM using the Barton and HDC Antisera

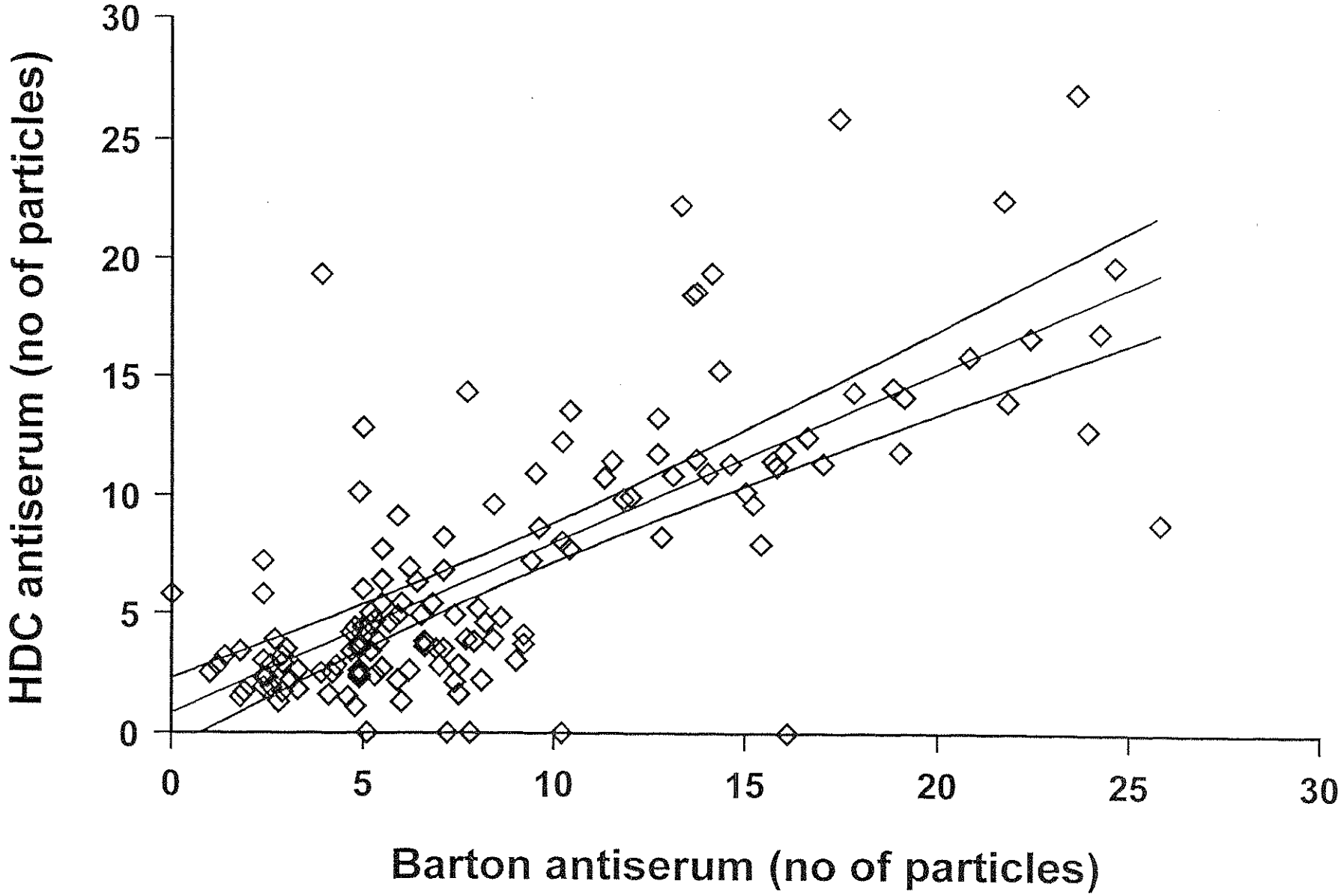
Mean Number of Particles Trapped

Sample No	MV1		MV4		MV3		
	Barton	HDC	Barton	HDC	Barton	HDC	
1	8.4	3.9	0.8	0.8	0	0	
2	5.9	2.2	0	0	0	0	
3	7.7	14.3	0	0	0	0	
4	2.9	2.9	0	0	0	0	
5	3.9	19.3	0	0	0	0	
6	7.5	-	0.6	-	0	-	no grids
7	4.9	10.1	0	0	0	0	
8	6.2	2.6	0	0	0	0	
9	13.7	18.5	0	0	0.3	0	
10	5.9	9.1	0	0	0	0	
11	2.4	2.3	0	0	0	0	
12	22.4	16.6	0	0	0	0	
13	5.5	5.4	1.1	0	0	0	
14	9.2	3.7	0.8	0	0	0	
15	1.4	3.2	0	0	0	0	
16	7.0	2.8	0	0	0	0	
17	6.6	3.8	0	0	0	0	
18	2.8	1.3	0	0	0	0	
19	4.2	2.5	0	0	0	0	
20	2.6	2.8	0	0	0	0	
21	7.4	4.9	0.6	0	0	0	
22	4.3	2.8	0	0	0	0	
23	15.8	11.2	0	0	0	0	
24	16.0	11.8	0	0	0	0	
25	17.4	25.8	0	0	0	0	
26	21.8	13.9	0	0	0	0	
27	13.6	18.4	0	0	0	0	
28	6.0	1.3	0	0	0	0	
29	16.6	12.4	0	0	0	0	
30	19.0	11.8	0	0	0	0	
31	17.0	11.3	0	0	0	0	
32	24.2	16.8	0	0	0	0	
33	17.8	14.3	0	0	0	0	
34	6.6	3.6	1	0	0	0	
35	2.4	7.2	0	0	0	0	
36	5.3	4.7	0	0	0	0	
37	5.3	2.4	0.9	0	0.1	0	
38	6.0	5.4	0	0	0	0	
39	1.9	1.7	0	0	0	0	
40	1.8	3.4	0.9	0	0	0	
41	7.7	3.9	0	0	0	0	
42	4.6	1.5	0	0	0	0	
43	2.9	3.2	0.7	0	0	0	
44	2.4	5.8	0	0	0	0	
45	4.1	1.6	0	0	0	0	

Sample No	MV1		MV4		MV3	
	Barton	HDC	Barton	HDC	Barton	HDC
46	1.2	2.8	0	0	0	0
47	4.8	1.1	0	0	0	0
48	2.4	1.9	0	0	0	0
49	3.3	1.8	0	0	0	0
50	3.3	2.6	0	0	0	0
51	2.5	2.3	0	0	0	0
52	5.0	4.4	0	0	0	0
53	-	-	-	-	-	-
54	4.7	3.4	0	0	0	0
55	2.4	3	0	0	0	0
56	0	5.8	0	0	0	0
57	4.9	2.4	0	0.3	0	0
58	5.5	6.4	0	0	0	0
59	6.8	5.4	0	0	0	0
60	14	10.9	0	0	0	0
61	9.6	8.6	0	0	0	0
62	7.1	3.5	0	0	0	0
63	15.7	11.4	0	0	0	0
64	13.7	11.5	0	0	0	0
65	10.2	12.2	0	0	0	0
66	13.3	22.2	0	0	0	0
67	24.6	19.6	0	0	0	0
68	12	9.9	0	0	0	0
69	2.7	3.9	0	0	0	0
70	1.8	1.5	0	0	0	0
71	1	2.5	0	0	0	0
72	12.7	11.7	0	0	0	0
73	8.6	4.8	0	0	0	0
74	20.8	15.8	0	0	0	0
75	15.4	7.9	5.6	1	0	0
76	7.4	2.1	0.7	0	0	0
77	7.2	0	1.5	0	0	0
78	14.1	19.3	0	0	0	0
79	5.2	4.5	0.6	0	0	0
80	4.7	4.2	0.3	0	0	0
81	9.4	7.2	1.4	0.6	0	0
82	7.1	8.2	0.7	0	0	0
83	16.1	0	19.2	0	0	0
84	10.2	0	9	0	0	0
85	6.2	6.9	0	1.3	0	0
86	5	12.8	0.6	0	0	0
87	5.1	0	2.9	0	0	0
88	7.8	0	1.3	0	0	0
89	7.9	3.8	6.7	0	0	0
90	10.4	13.5	0.7	0	0	0
91	9.5	10.9	0	0	0.2	0
92	23.6	26.8	0	0	0	0
93	8.4	9.6	0	0	0	0
94	23.9	12.7	0	0	0	0
95	10.2	8	0	0	0	0
96	25.8	8.8	0	0	0	0
97	2.6	1.9	0	0	0	0

Sample No	MV1		MV4		MV3	
	Barton	HDC	Barton	HDC	Barton	HDC
98	4.9	2.3	0	0	0.5	0
99	4.9	3.5	0	0	0	0
100	3.9	2.5	0	0	0	0
101	4.9	2.6	0	0	0	0
102	9	3.0	0	0	0	0
103	4.8	4.4	0	0	0	0
104	4.9	3.7	0	0	0	0
105	3.1	2.2	0.1	0	0	0
106	5.5	2.7	0	0	0	0
107	12.8	8.2	0.9	0	0	0
108	19.1	14.1	2.2	1.5	0.5	0
109	5.4	3.8	0	0	0	0
110	8.1	2.2	0.7	0	0	0
111	7.5	2.8	0	0	0	0
112	9.2	4.1	0	0	0	0
113	11.5	11.4	0	0	0	0
114	15	10.1	0	0	0	0
115	18.8	14.5	0	0	0	0
116	5.2	5	0	0	0	0
117	3	3.5	0	0	0	0
118	5.2	3.4	0	0	0	0
119	14.3	15.2	0	0	0	0
120	12.7	13.2	0	0	0	0
121	5.5	7.7	0	0	0	0
122	7.1	6.8	0	0	0	0
123	5.7	4.6	0	0	0	0
124	6.4	6.3	0	0	0	0
125	6.5	4.9	0	0	0	0
126	15.2	9.6	0	0	0	0
127	2.9	1.7	0	0	0	0
128	5	6	0	0	0	0
129	11.8	9.8	0	0	0	0
130	21.7	22.4	0	0	0	0
131	11.3	10.7	0	0	0	0
132	10.4	7.7	0	0	0	0
133	13.1	10.8	0	0	0	0
134	14.6	11.3	0	0	0	0
135	7.5	1.6	0	0	0.3	0
136	6.9	3.5	1.1	0.4	0	0
137	8.2	4.6	0.7	0	0	0
138	nd	nd	nd	nd	nd	nd
139	5.9	4.9	0.2	0.6	0	0
140	8	5.2	1	0	0	0

Figure 1 Trapping of MV1 using the Barton and HDC antisera



Contract between CSL (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

1. TITLE OF PROJECT Contract No: M7a

CALIBRATION OF THE NEW MUSHROOM VIRUS ANTISERUM FOR USE IN IMMUNOSORBENT ELECTRON MICROSCOPY (IEM)

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Virus disease of mushrooms or La France disease was first described in the USA in the 1950's but it is now endemic in UK crops. The most obvious effect of the disease is to cause bare patches in mushroom beds and therefore loss of yield. It also causes discoloured and deformed mushrooms and an accelerated post-harvest deterioration leading to loss of quality.

Control of the disease is largely by disposal of infected crops and increased hygiene precautions on farms. This routine management of crops to contain the spread of virus disease is dependent on our ability to monitor crops for the presence of virus reliably.

Three viruses are found in UK crops, MV1 (a spherical 25nm virus), MV3 (a bacilliform, 19 x 50nm virus) and MV4 (a spherical 35 nm virus). These viruses can be diagnosed by the use of electron microscopy (EM) but the use of immunosorbent electron microscopy (IEM) increases the sensitivity of the test and provides an early warning of the development of a virus problem in crops.

CSL provides a diagnostic service, including EM and IEM tests, to mushroom growers. The current IEM test is based on an antiserum developed by Barton & Atkey in 1984. Stocks of this antiserum are running out and a replacement will be needed soon if this testing service is to continue.

An antiserum reacting against MV1 and MV4 was produced under a project funded by the Horticultural Development Council (HDC contract No. M7). This antiserum can be used instead of the Barton antiserum to detect viruses using the IEM test, however it requires calibration alongside the Barton test to enable ADAS and CSL to provide useful advice based on the new test.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

Mushrooms are the most valuable horticultural crop in the UK with an annual value of £350 million in 1992. The majority of mushrooms grown are of the white mushroom, Agaricus bisporus, type which is generally susceptible to virus disease. In the past, changes in cropping practices have improved virus control considerably, however crop losses due to virus diseases still occur sporadically. ADAS surveys carried out in 1976 showed that 27% of

mushroom crops had low levels of virus and 6% of crops had medium to high levels. High levels of virus have been linked to yield loss but the effect of low levels of virus is not known. Even a loss of 6% of the UK crop would result in a decrease of £21 million in revenue. Thus the potential saving from forecasting and good diagnosis of virus problems in the UK crop is substantial.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

Interpretation of the results of EM and IEM tests in a cropping context is always difficult. The advice given to growers as a result of these tests is based on the extensive experience of CSL and ADAS with the type of results produced by the Barton antiserum. A new antiserum will have different characteristics making interpretation of the results from IEM tests based on the new antiserum difficult.

The aim of this work would be to compare the results obtained using the new antiserum with those obtained using the Barton antiserum on a range of routine samples tested by the diagnostic team. This would allow CSL diagnostic staff and the National Mushroom Adviser, Richard Gaze, to interpret these results and provide meaningful advice to growers.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

Diagnostic tests using EM and IEM have been carried out by CSL staff for the last 7 years.

Currently, there is a MAFF-funded project at CSL on comparisons of diagnostic test methods for mushroom virus diagnosis.

This work would provide a follow-up to work funded by HDC under contract No. M7 to develop the routine use of the antiserum produced in this project.

6. DESCRIPTION OF THE WORK

The new antiserum would be compared with the Barton antiserum in routine IEM tests for viruses. All samples being processed by the virology routine IEM testing scheme would be tested in parallel using the new antiserum. Results of the tests would be analysed and compared with any reported symptoms seen in the crops. This would provide the background experience necessary to give advice to growers.

Based on past experience it is likely that approximately 250 samples would need to be tested in this project to give a reasonable picture of the performance of the new antiserum.

Constraints:

The work is dependent on the number and type of samples sent in by growers. It is therefore difficult to predict what range of results we would expect to get from the tests. It is also difficult to guarantee that 250 samples will be received within the time specified.

7. **COMMENCEMENT DATE AND DURATION**

Start date: 01.07.94; duration 1 year. The experimental work will be completed by the end of March and the final report will be produced by the end of July, 1995.

8. **STAFF RESPONSIBILITIES**

Project Leader:	Ms C M Henry
Staff Involved:	Miss D Wright
	Miss E Hobbs
	Miss A Thompson
Others:	Mr R Gaze (HRI: Nat. Mushroom Adviser)

9. **LOCATION**

CSL, Harpenden

10. **COSTS**

Contract No: M7a
Date: 11.8.94

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature.....

Position Research Director

Date 31. 8. 94.

Signed for the Contractor(s)

Signature.....

Position.....

Date.....

Signed for the Council

Signature.....

Position CHIEF EXECUTIVE

Date 17. 8. 94.