Project Title: Diagnosis & Detection of Mushroom Viruses

Project Leader: Dr S A Hill

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Project Title:	Diagnosis & Detection of Mushroom Viruses
Project Leader:	Dr S A Hill
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RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application: The aim of the project was to produce an antiserum for use in diagnostic tests for mushroom viruses, especially MV4. Two rabbits were injected with purified MV4 and MV1 and bleeds were taken at intervals. One of the antisera produced proved to be useful for the diagnosis of MV1 and MV4 and will be used in the diagnostic service provided by CSL.

Summary

Objective of project

The objective of the project was to produce a new antiserum, with activity against mushroom viruses, which could be used in the immunosorbent electron microscopy (IEM) test.

Results

An antiserum was produced which can be used to trap both MV1 and MV4 in the IEM test. The new antiserum was compared with the Barton antiserum currently used in diagnostic tests at CSL (Harpenden) and found to be at least as good, if not better, at trapping viruses.

Opportunity for Application

All mushroom virus testing in the UK is carried out at CSL so the new antiserum can be directly applied to the testing scheme on site.

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 25 nm (MV1) and 35 nm (MV4) spherical particles and 19 x 50 nm bacilliform particles. 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. Prior to 1991 this test was based on antisera produced originally by Barton & Atkey (1984), 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. The objective of this project was therefore to produce purified preparations of MV4 and MV1 and inject these into rabbits in order to produce a new antiserum for use in the IEM diagnostic test.

Materials and Methods

Virus Purification

Virus was purified from infected mushroom sporophores obtained from commercial crops as part of the testing scheme run at CSL or from artificially infected crops produced at CSL. The method used was similar to that of Schmidt, Proll, Richter & Zahn (1985). Mushrooms were stored at 4°C until used. For each preparation 200g of mushrooms were ground to a fine powder using liquid nitrogen in a mortar and pestle. This material was then homogenised in 400ml of 0.05M phosphate buffer pH7 containing 0.1% ß-mercaptoethanol and 0.1% thioglycollic acid. This

mixture was then centrifuged at 3,000 rpm for 30 min in an MSE 8x50 ml rotor at 5°C. The supernatant was removed, filtered through muslin and stored at -20°C.

The sample was then thawed and centrifuged at 3,000 rpm for 30 min in an MSE 8x50 ml rotor at 5°C. The supernatant obtained was then centrifuged at 26,000 rpm in a Kontron TFT 45.94 rotor for 90 min at 5°C. The resultant pellet was resuspended in a few ml of 0.02M phosphate buffer pH7. This was then centrifuged for 15 min at 10,000 rpm in an MSE 8x14 ml rotor at 5°C. The supernatant was kept and the pellet was re-extracted with phosphate buffer, re-centrifuged and the supernatants pooled and then frozen at -20°C. The preparation was thawed the following day and centrifuged for 15 min at 10,000 rpm in an MSE 8x14 ml rotor at 5°C. The supernatant was then centrifuged at 30,200 rpm for 2h in a Kontron TFT 55.38 rotor at 5°C. The pellets obtained were resuspended in a small amount of phosphate buffer and dialysed overnight against the same buffer at 4°C.

Immunisation of animals

Two Lop cross rabbits were immunised using purified virus prepared as described in the previous section. Injections were usually of 300-500 µl of the purified preparation. For the first injection this was mixed 1:1 (v:v) in Freund's Complete Adjuvant and given intramuscularly. Further injections were mixed 1:1 (v:v) with Freund's Incomplete Adjuvant and were again intramuscularly given. Test bleeds were taken about 2 weeks after each injection starting with the second injection.

Immunoelectron microscopy (IEM) method used for screening antisera

Antisera produced from test bleeds 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 was placed on a piece of Parafilm and a carbon collodion coated grid was placed on the drop for 15 min at room temperature. 20 µl drops of a known virus-infected mushroom exudate were placed on a second piece of Parafilm. The grids were washed with 20 µl drops of phosphate-buffered saline, drained and placed on the mushroom exudate drops for 1h 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 obtained were compared with those for negative controls (buffer or non-specific tobacco mosaic virus antiserum) and the positive control (Barton antiserum).

Results

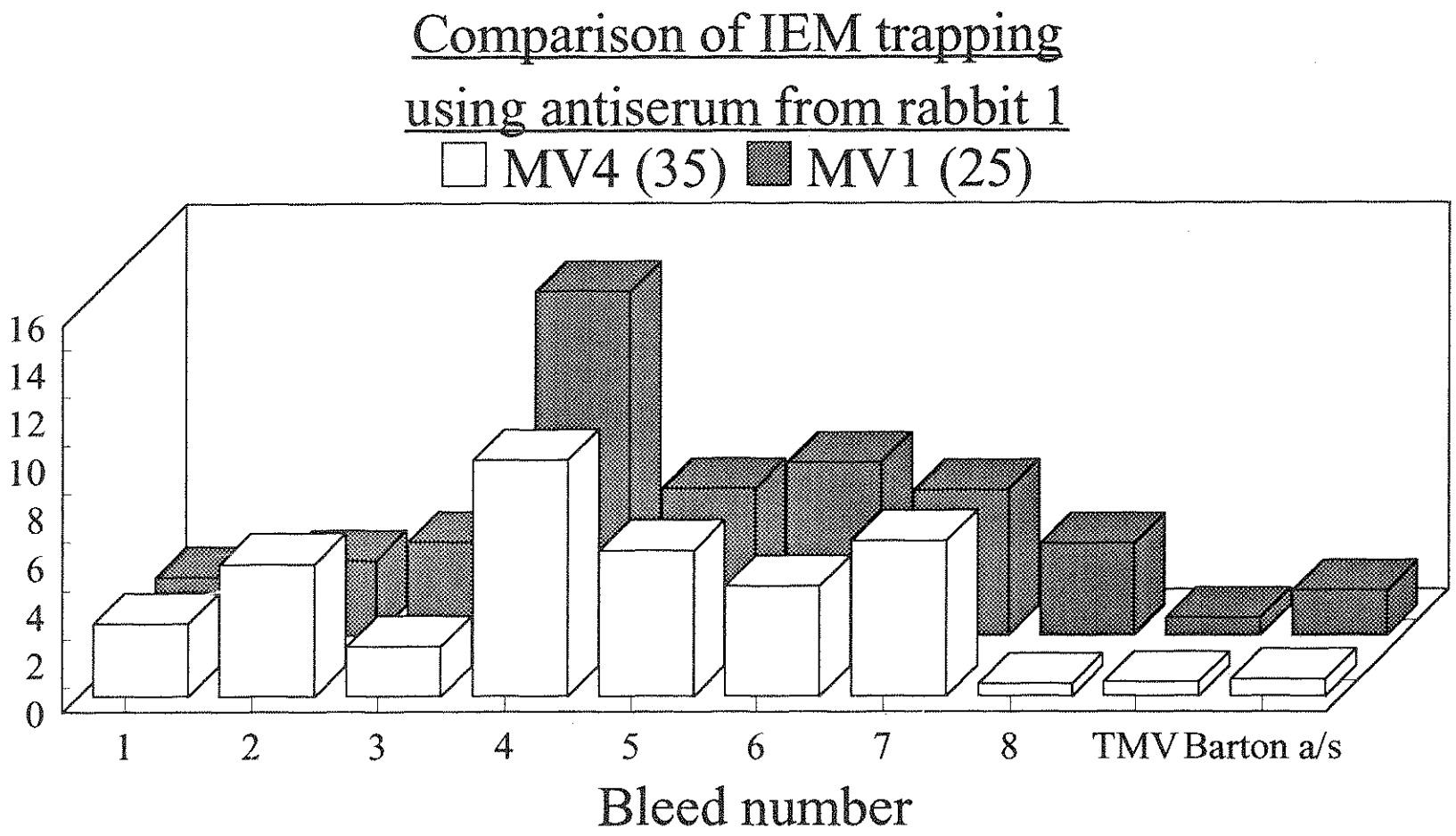
Antisera from test bleeds from the two rabbits were compared for their ability to trap mushroom viruses in IEM. Of the two rabbits used, rabbit 1 gave the best results. A summary of the results from rabbit 1 is presented in the figure (page 5). From this it can be seen that almost all of the test bleeds from rabbit 1 gave antisera which trapped MV1 and MV4 better than either TMV (tobacco mosaic virus) antiserum or the Barton antiserum. A comparison of the test bleeds shows some variation but bleeds from the middle of the immunisation programme were generally best, as would be expected. Bleed 4 from rabbit 1 was chosen as the best for IEM tests and was tested several times. It gave consistently good results but its trapping ability did vary from experiment to experiment. This is probably because IEM is an inherently subjective technique.

Tests on the ability of the new antiserum to trap MV3 have shown that it has no activity against this virus. This is what would be expected as the purified virus preparation injected contained very few, if any, MV3 particles.

The new antiserum was compared with the Barton antiserum using about 30 mushroom samples from growers. Preliminary results suggested that the new antiserum traps MV1 to a similar extent but that trapping of MV4 was inconsistent. This suggests that the interpretation of results obtained with the new antiserum will need to be modified compared to interpretation of results obtained with the Barton antiserum. Further investigation is required.

Conclusions

A new antiserum was made and was found to be able to trap MV1 and MV4 in IEM tests. This antiserum does not, however, trap MV3. The new antiserum will provide an alternative to the Barton antiserum currently used in IEM tests for the detection of virus diseases in mushroom samples at CSL. The Barton antiserum will not last for ever and it is vital to have an alternative to this when stocks eventually run out.


Comparison of IEM trapping
using antiserum from rabbit 1
MV4 (35)
MV1 (25)
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
TMV Barton a/s
Bleed number

Preliminary results from comparisons of both antisera using grower (commercial) mushroom samples suggest that the interpretation of results using the new antiserum will be difficult at first as it has different properties from the Barton antiserum. This problem is common to all antisera as each one is unique. Further comparisons between the two will therefore be necessary before the new antiserum could replace the Barton antiserum in routine testing.

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

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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.

Contract (excluding cost structure)

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

PROPOSAL

1. TITLE OF PROJECT **Contract No: M7**

MUSHROOMS - DIAGNOSIS AND DETECTION OF MUSHROOM VIRUSES.

2. BACKGROUND AND COMMERCIAL OBJECTIVE

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) found that the disease was associated with the presence of virus-like particles, especially mixtures of 25nm. (MV1) and 35nm. (MV4) spherical particles and 19 x 50nm. bacilliform (MV3) particles. The diagnosis and detection of these viruses has been difficult because of lack of good serological tests, however, 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 direct electron microscopy (EM) in the early diagnosis of virus problems. Both IEM and EM tests are currently used at the Central Science Laboratory Harpenden for the diagnosis of mushroom virus in samples sent in by growers.

The diagnostic service provided by Harpenden Laboratory is used by many mushroom growers with 1169 samples being tested this year (1990). Of these, 1021 samples were tested by IEM, which is a popular test with mushroom 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, Harpenden Laboratory is the only UK-based laboratory offering this type of service. Unfortunately, this testing service is unlikely to continue beyond next year if further

suitable supplies of antisera are not made available soon. The aim of this project would be to produce new and better antisera for use in the IEM diagnostic tests carried out at Harpenden Laboratory so that this service can continue.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

Mushrooms are the single most valuable horticultural crop in the UK. In 1987 the total value of the UK crop was £223 million. 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 yield effect of low levels is not known. Even a loss of 6% of the UK crop would be a revenue loss of £13 million. 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

The antisera available at present have a low activity against MV4 which is considered to be the most important of the three viruses involved in crop loss. The aim of the work would be to produce a new rabbit polyclonal antiserum which has a better affinity for MV4 than the antisera available at present. This would improve the sensitivity of the IEM test for the detection of MV4 and thus allow better and earlier warnings of problems in mushroom crops to be given. An improved antiserum might also make the interpretation of results more effective allowing better advice to be given on the management of crops.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

Barton (1984) purified mushroom viruses and produced an antiserum against these viruses. This antiserum has been used in diagnostic tests at Harpenden Laboratory for the last 5 years.

Work has been going on at Harpenden Laboratory on the development of antisera and monoclonal antibodies against plant viruses (eg barley yellow dwarf virus, beet necrotic yellow vein virus, etc.) for many years and a great deal of expertise has been accumulated. This expertise would be used in the development of a mushroom virus antiserum.

Currently, there is a MAFF-funded project at Harpenden Laboratory on the epidemiology of mushroom viruses and comparisons of diagnostic test methods (EM, IEM and PAGE). The two projects would complement each other.

6. DESCRIPTION OF THE WORK

The production of a polyclonal antiserum against mushroom viruses would be achieved as follows:

Mushroom viruses would be purified from infected sporophores at Harpenden. They would be purified using an extraction procedure followed by differential centrifugation and purification using sucrose gradients as described by Schmidt, Proll, Richter & Zahn (1985). It is anticipated that yields would be in the order of 100 μg/100g of infected mushrooms. purity of the preparations would be assessed by spectrophotometry and electron microscopy.

The purified preparations would be injected into 2 rabbits and test bleeds would be taken at intervals after injections. These test bleeds (3/rabbit) would be tested for their ability to trap virus in IEM tests.

When test bleeds show good results, a large scale bleed will be taken from the rabbit and tested. The ability of this new antiserum to trap virus particles in IEM tests will be compared with that of the Barton antiserum. If suitable the new antiserum will then be used in routine virus tests at the Harpenden Laboratory.

Constraints: It will be necessary to use at least 2 rabbits because of the low immunogenicity of mushroom viruses.

7. COMMENCEMENT DATE AND DURATION

The suggested start date would be 1 May 1991 with a finishing date of 30 April 1992.

8. STAFF RESPONSIBILITIES

Project Leader: Dr S A Hill
Staff Involved: Ms C M Henry
Mrs V Harju
Dr I Barker (Home Office Licence)

9. LOCATION

MAFF Central Science Laboratory
Hatching Green
Harpenden
Hertfordshire AL5 2BD