VIRUS TESTING OF PERENNIAL PROPAGATING STOCK

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INTRODUCTION

The presence of plant viruses can often be difficult to detect and, even if detected, the casual virus can be difficult to identify. This is in contrast to the obvious presence of most fungal disease and the ready identification of fungi. This difficulty creates a problem in perennials as the systemic nature of viruses in plant tissue and their persistence in plants means that all progeny obtained by vegetative propagation from infected perennial stock will also be infected.

The detection and identification of plant viruses is carried out by several means. Many virus diseases are self-indicating by their symptoms on leaves, flowers, or fruit. In cultivars that do not show clear symptoms infection can often be demonstrated by budding or grafting tissue into known sensitive "indicator" cultivars. Similarly herbaceous plants can be used as indicators if they show diagnostic symptoms when viruses are transmitted to them by insects or sap inoculation.

Other approaches to virus detection are electron microscopy and serology. Electron microscopy of sap preparations is effective when viruses are in high concentration and have particles of distinctive morphology—such as rod shapes. Serology relies on the use of an antiserum (which has been raised in an animal against a specific plant virus) producing a visible reaction when it reacts with this virus in plant sap

It is in serological tests, particularly the development of "enzyme linked immunosorbent assay" (ELISA), that there have been recent advances that benefit propagators. ELISA, described for plant viruses in 1977 (1), is a very sensitive test as it enhances otherwise undetectable virus—antiserum reactions by linking one component to an enzyme. The presence of the enzyme (and thus the virus) is revealed by adding a substrate that undergoes a visible colour change in the presence of the enzyme

DEVELOPMENT OF ELISA TESTING IN TASMANIA

1. **Antiserum availability.** ELISA is dependent upon the availability of an anti-serum against each virus to be tested. Thus when ELISA testing of hops *(Humulus lupulus)* was first commenced in our laboratory in 1980 it was dependent upon the

supply by the East Malling Research Station, East Malling, England, of antisera against six viruses known to infect hops. These antisera enabled the location or production of virus-free stock of major hop cultivars for propagation purposes (2). Subsequently, further supplies of antisera were produced against three of these viruses in our laboratory.

Antisera are now becoming commercially available. Although most antisera are produced in research establishments these establishments are now aware of the widespread demand for the antisera (for ELISA tests) so supplies are sold either directly or through commercial outlets. The cost of A $100 to 400 per milliliter reflects the 2 to 6 months work, expertise, and sophisticated laboratory equipment needed to produce an antiserum.

Testing is based, where possible, on the use of available commercial antisera: thus pollen-borne viruses of stone fruits are tested using two commercial antisera. For current tests on viruses of tulips and *Lilium* three commercial antisera are being used, plus two for which it had to be produced in our laboratory because of the lack of a commercial source.

2. **Rapid sap extraction.** The advantages of ELISA are its sensitivity plus its suitability for screening large numbers of specimens. For large scale testing of plant samples it is necessary to devise an automated method of sap extraction as use of a mortar and pestle is too slow. A motorised roller extractor is available commercially and similar equipment has been manufactured in our workshop for the following purposes:

i) To extract sap from leaves. A geared motor driving stainless steel rollers was constructed for approximately A$1,000.

ii) To extract sap from bulbs. A similar extractor (Figure 1) utilising matching helical rollers was constructed for approximately A$1,300. Similar equipment is used extensively for bulbs by the Dutch Bulb Inspection Service.

iii) To extract sap from woody tissue, such as dormant hop crowns, and also for bulk testing of numbers of samples. Tissue is placed in steel tubes and macerated with a rotary file driven by an electric drill. This is the technique developed by Washington State University's commercial ELISA laboratory at Prosser, Washington, USA, and used for all types of samples.

Figure 1. Extractor utilizing matching helical rollers.

3. **Developing reliability.** Under optimal conditions ELISA accurately detects virus infection. However, it is necessary to determine the optimal conditions and their limits for each virus-plant-antiserum system. Some of the variable conditions are: time of sampling of plants, the occurrence of virus strains, optimum buffer strength, and use of additives and degree to which samples are bulked.

Time of sampling is important as detectability may decrease with plant maturity. Thus tests for pollen-transmitted viruses in stone fruit are only carried out in spring (3); similarly we have noted a decreased accuracy of hop virus detection towards harvest.

The strains of tulip breaking virus are detected to differing degrees by two antisera (4) and in our tests there was a 5% difference in their ability to detect infection.

Bulb tissue may require cellulase to assist virus extraction and use of high molarity buffers to stabilise pH (5).

Bulking of several plants in one test decreases the cost per plant and is feasible if a low percentage of positives is expected and if these can still be detected when mixed with healthy samples (Figure 2).

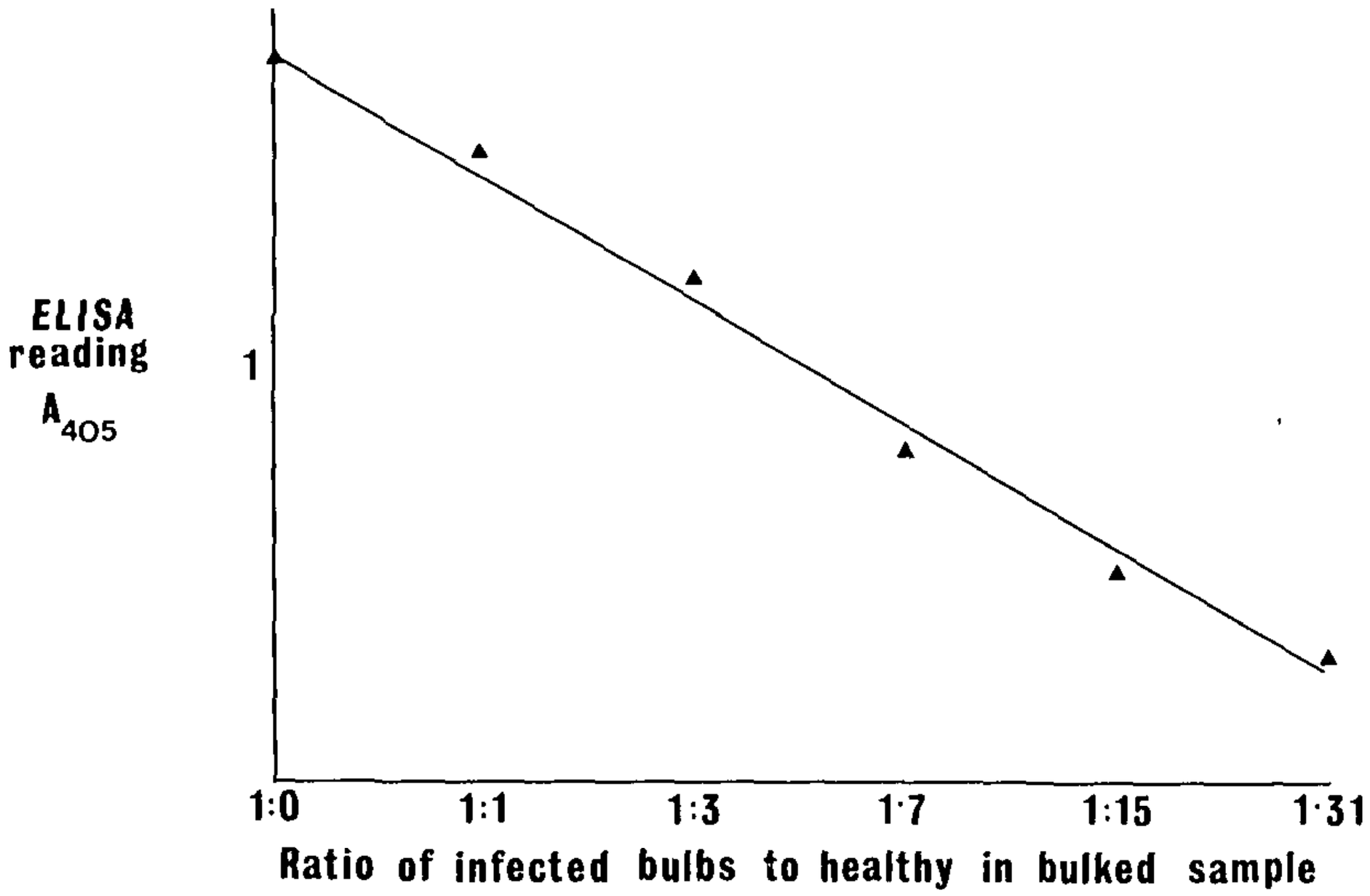

Figure 2. Effect of bulking of samples on virus detection.

The above steps of preparing or obtaining antisera, manufacturing sap extracting equipment and then determining optimal conditions for reliable tests have been the major stages in the development of a plant virus ELISA laboratory in the Tasmanian Department of Agriculture. The laboratory is able to test material for private propagators and thus greatly expand Tasmania's ability to supply virus-tested stock. Tests can be undertaken on hops, stone fruit, potatoes, tulips, *Lilium,* gladiolus, iris, freesia, orchids and roses. The information can then be used by propagators to locate clean stock, to reassure themselves that they are not selling diseased stock or, by labeling stock as virus-tested, to inform the customer of its high quality.

CURRENT DEVELOPMENTS

1. Plant virus antisera are becoming increasingly commercially available.
2. Monoclonal antisera, effective in detecting wider ranges of viruses, are now being produced. They will decrease the outlay on antisera required by ELISA laboratories.
3. Complete ELISA "kits" designed for simplicity of use will permit tests to be carried out by a wide range of users, including propagators.
4. ELISA tests for a range of bacterial and fungal diseases are being developed.

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