Paper 6

PATHOGEN TESTED POTATOES IN NEW ZEALAND. I. PRODUCTION OF PATHOGEN TESTED SEED

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INTRODUCTION

Pathogen testing of potatoes was introduced in New Zealand in 1977 by Crop Research Division, DSIR, Lincoln, with the aim of improving the health state of seed potatoes. It was anticipated that this would lead to improved yields of cleaner, more uniform sized table potatoes. In 1978, a co-operative programme between Crop Research Division, Alex McDonald (Merchants) Ltd and Mr Nelson Pyper was formalised. The programme has now developed to the stage where the Pathogen Tested Potato Scheme is a commercial venture, not just a research project.

Other countries, such as the United Kingdom, Canada, and Australia have pathogen testing programmes as part of their potato certification schemes. (Harrison *et al.*, 1974; Harrison and Marringley, 1977). The certification of pathogen tested potatoes in New Zealand is run concurrently with the existing group certification scheme, but standards required for the pathogen tested potatoes are more rigorous (Table 1).

The New Zealand Pathogen Tested Scheme began with pathogen tested Sebago tubers imported from Australia, and tubers of virus tested stem cuttings of Red King Edward from Scotland. Further cultivars, such as Rua and Ilam Hardy, the two most important cultivars in New Zealand, have subsequently been added to the scheme and new cultivars are added as they become available.

The scheme entails heat treatment, tissue culture, and tests for virus, fungi and bacteria; followed by multiplication both in culture tubes and in the glasshouse; and thence field multiplication for two or three years in low aphid areas near Methven, Canterbury. As in other pyramid type seed certification schemes, seed is not retained at any level, but proceeds through the scheme from mother stock to table ware. New mother stocks of pathogen tested plants are produced each year.

The field multiplications up until basic are laboratory tested for virus, and from basic stage onwards, are visually inspected by Ministry of Agriculture and Fisheries staff.

The scheme aims to produce seed of the best lines available in New Zealand, true to type, with lower levels of disease, more attractive and uniform sized tubers, and improved storage ability.

MATERIAL AND METHODS

Growing plants for culturing

Tubers are planted in a sterilized bark potting mix and placed in a controlled growth cabinet at 25 °C with a 16 hour day and an 8 hour night, until the plants have reached a height of approximately 170 mm. The temperature is then raised gradually over a period of a week to 36 °C. After three weeks, axillary buds are removed for tissue culture.

Production of disease-free mother plants

The axillarly buds are surface sterilised with 70% ethanol and 0.5% sodium hypochlorite. The meristem tip — meristem and primordial leaves (0.5 mm x 0.3 mm) — is then dissected out from the buds under sterile conditions using a binocular microscope within a laminar air flow cabinet. The meristem tip is cultured as it is the fastest growing tissue in the plant and can out-grow disease after

Table 1. Standards required for potato crop certification (D.A. Cleverley, 1982)

	Group scheme			Pathogen tested scheme		
	Gp 1	Gp 2	Gp 3	Basic	1st Gen	2nd Gen
Maximum virus	0.1%	0.5%	1.0%	0.1%	0.1%	0.2%
Maximum rogues	0.0%	0.2%	0.4%	0.0%	0.0%	0.0%
Maximum bolters	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Blackleg	2-10% or over grade Gp 3 20% + reject			0.25%	0.25%	1.0%
Verticillium wilt	2-10% or over grade Gp 3 20% + reject			0.25%	0.25%	1.0%

three weeks heat treatment.

The meristem tissue is placed in a tube, on a modified Murashige and Skoog (1962) growth media containing 27 chemicals. The balance of hormones added to the media is such as to minimize formation of callus which might lead to genetical changes in the potato tissue. The tubes are placed in an incubator for between 2 and 12 months at 25 °C with a 16 hour day and 8 hour night.

When small plantlets have formed, virus testing and multiplication begin. The plantlets are transferred to pots of soil mix in a greenhouse mist bed, then to benches with trickle irrigation and later retested for virus.

New mother plants are produced each year, from material cycled through heat treatment, from buds of sprouting tubers of the previous season's mother plants, or from tissue cultured plantlets that have been disease tested.

Disease testing

Various techniques are used in virus testing (de Bok, 1972). They include:

- The use of indicator plants.
- Serological tests such as the microprecipitin test — involving droplets of antisera to the viruses, and Elisa test — double anti-body sandwich with an enzyme and substrate which has a colour intensity which can be measured.
- Electron microscope inspection, where the virus can be seen.

Fungal and bacterial testing is carried out on tubers using potato dextrose agar and Stewarts agar for blackleg, and potato dextrose agar for verticillium wilt and fusarium.

Multiplication of disease-free stock for field planting

Plants which have tested negative form the motherstock for further multiplication. This is initially done in tissue culture.

The tissue cultured plantlets are transferred to soil and grown for one cycle in the greenhouse to produce tubers for field planting.

Field multiplication

Year one — isolated from other potato crops at a relatively aphid-free site around 365 m altitude. The plants are spaced one metre apart to prevent possible cross infection. Clonal identity is maintained so that genetic identity can be checked against the original material. All plants are virus tested using leaf samples.

Year two — grown at a similar altitude near the Rakaia Gorge. Year two covered about 2 hectares in 1984/85, all 20,000 plants were virus tested. Clonal identity is retained, but plants are grown at commercial spacings. At harvest clonal identity is dropped and only cultivar identity is retained.

Years three and four — also grown in a seed area near the Rakaia Gorge. In 1984/85, the third year of multiplication covered an area of 6.7 hectares. Virus testing is less intensive; a representative test pattern is used for virus sampling. Depending on the numbers required for 2nd generation plantings, year three or four is harvested and entered into certification as basic seed.

Material from the Pathogen Tested Scheme occupied about 332.9 hectares in the higher areas of the Canterbury Plains in 1984/85.

RESULTS

The production of pathogen tested seed requires considerable care and precision in all phases of the operation.

- The heat treatment places stress on both plant and disease — if a plant has a significant amount of disease, virus, bacterial or fungal, it may collapse in the heat treatment.
- Cultivar differences in response to the growth media are very strong.
- Up to one hundred meristem tips of a cultivar need to be cultured to guarantee survival of at least one virus free plant.
- Care must be taken to ensure that the genetic makeup of the pathogen tested plants is not altered by the tissue culture process. In particular, callus formation must be prevented.
- Experience has shown that the best time to carry out the initial heat treatment and tissue culture is during spring and summer.
- Multiplication in culture and greenhouse is carried out at any time of the year, provided light (to extend day length) and heat can be supplied, once the cultivar has been tested and found free of pathogens. Dormancy and maturation factors have to be taken into account when timing field planting.
- The time taken from introduction of new tubers of a cultivar, to pathogen tested motherstock is 12-18 months.

Several new techniques are being assessed that may assist in speeding up the programme and reducing the risk of introducing variability and disease. These include electrophoretic mapping of potato protein — to test that varietal identity has been maintained; virus testing in culture; and the production of minitubers in culture.

In addition to producing pathogen tested seed of New Zealand cultivars, disease free material from quarantined overseas cultivars is also being multiplied for faster assessment in New Zealand.

Assessment trials (Ovenden *et al.*, 1985) have shown the improved performance of the pathogen tested potatoes over normal commercial seed. Research (Ovenden and Martin, 1981; Ovenden *et al.*, 1983) has shown that the scheme is producing cultivars which are true to type, have lower levels of disease, have more uniform sized tubers and are healthier and more vigorous with higher yeilds.

ACKNOWLEDGEMENTS

The author acknowledges the technical assistance of Mrs Frances Andrews since 1983, other Alex McDonald

(Merchants) Ltd staff for assistance with field work, Plant Diseases Division of DSIR for supervision and help in virus testing, the Applied Genetics Section of Crop Research Division for summer assistance with field and glasshouse work.

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