VG029
Development of methods for the rapid detection and elimination of virus diseases of garlic

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Know-how for Horticulture™

VG029

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Control of Virus Diseases of Garlic

The final report for HRDC Project VG 029.

July 1989 to June 1992

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HORTICULTURAL RESEARCH & DEVELOPMENT CORPORATION

AGRICULTURE VICTORIA

Control of Virus Diseases of Garlic

The final report for HRDC Project VG 029
(originally ASRRF Project NV 15),

"The development of methods for the rapid detection and elimination of virus diseases of garlic and the establishment of a tissue culture based pathogen-tested scheme".

This was project conducted at the Institute of Plant Sciences, Burnley from July 1989 to June 1992.

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1(a). Industry Summary

All garlic varieties in commercial production in Australia are infected with a complex of viruses which are reported to significantly reduce the yield and quality and possibly to increase the susceptibility of the plant and cloves to fungal infection, particularly during storage. This has occurred because garlic can only be multiplied by vegetative means; commercial garlic does not produce fertile true seed. Cloves are separated from the parent bulb and used as planting material for the next generation and consequently any virus-infected parent plant will give rise to virus-infected progeny.

Control of virus diseases of vegetatively propagated plants relies on three important strategies, the elimination of virus from selected stock plants by heat treatment; establishment and multiplication of tissue culture plantlets from heat treated meristems (young growing tips); initial testing and critical monitoring of daughter plants for virus. Daughter plants that test free of all known viruses are assigned "virus-tested" status and are then propagated and supplied to the industry through a pathogen-tested scheme or similar approach.

Prior to this project, no virus-tested garlic plants were being produced in Australia and the methods used to test for the viruses of garlic were relatively laborious and expensive. Some tissue culture of garlic had been attempted, but only on a small scale and it was clear that improved tissue culture methods would be required to enable the rapid, large scale production of the virus-tested stock cultivars.

Initially, various lines of a range of Australian garlic cultivars were tested for virus using a range of conventional tests. Three common viruses were identified as Garlic yellow streak potyvirus (GYSV), Onion yellow dwarf potyvirus (OYDV) and Garlic latent carlavirus (GLV). An improved test known as an ELISA (the acronym for Enzyme-Linked ImmunoSorbent Assay) which is based on the development of an antiserum to the mixture of virus proteins was developed. This test is suitable for the large scale, efficient testing of garlic plants for the presence of any of the three common viruses and provides a result within 32 hours.

Various lines of cultivars considered most suitable for the Australian industry were identified via an associated industry survey (Clarke. pers comm). Sample lines were obtained from key Australian growers and these were subjected to heat treatment and meristem-tip culture for virus elimination. Tissue culture plantlets were produced and exhaustively tested for all known viruses of garlic and were designated "virus-tested" status if the results of all tests were negative. The virus-tested lines were held under secure tissue culture conditions to ensure no virus contamination could occur. These lines acted as nucleus stock from which extra virus-tested material was produced for further multiplication and supply to industry partners. The virus-tested lines produced during this program were: California Early (Virginia; Dri Veg; Murray Bridge), California Late, Marlborough White, New Zealand Purple, Italian White, Schumex and Mexican.

Testing of a range of tissue culture media was undertaken and the optimal formulations were identified for the various stages of growth, multiplication and rooting of garlic plantlets in tissue culture. Good results were obtained for the small scale multiplication of garlic in tissue culture, however these results could not be matched by a commercial tissue culture laboratory which was contracted by the industry partners to this program to pilot the large scale commercial production.

At the completion of the project, nine virus-tested lines were available for commercial use as were the methods to continue monitoring them for freedom from virus infection. Basic tissue culture protocols were also developed for a small scale, tissue culture based production system for garlic.

1(b). Technical Summary

As a consequence of vegetative reproduction all garlic varieties in commercial production in Australia are infected with a complex of viruses. The virus diseases of garlic are reported to significantly reduce the yield and quality and possibly to increase the susceptibility of the plant and cloves to fungal infection, particularly during storage.

Tissue culture techniques for garlic had been investigated prior to this project and it was known that viruses could be eliminated from a proportion of plants by using the meristem culturing technique. Once 'virus-tested' plants have been obtained (ie. tested free of all known viruses of garlic) they can be supplied to the industry through a pathogen-tested scheme or similar approach.

Prior to this project, electron microscopy and inoculation to a range of herbaceous indicator plants were the methods used to test for the viruses of garlic. Whilst these are relatively reliable, they are inefficient and time consuming and are not suitable to be used on the large scale envisaged for the commercial production of pathogen-tested garlic. A rapid and sensitive test, suitable for large scale testing of virus, is the Enzyme-Linked ImmunoSorbent Assay (ELISA).

Initially, various lines of a range of Australian garlic cultivars were tested for virus using electron microscopy, immunosorbent electron microscopy and inoculation to indicator plants. Three common viruses were identified as Garlic yellow streak potyvirus (GYSV), Onion yellow dwarf potyvirus (OYDV) and Garlic latent carlavirus (GLV). The mixture of virus components was separated from the garlic host plants and the protein component was purified. An antiserum was produced in a rabbit against the mixture of garlic virus proteins and when evaluated was found to be of a relatively high titre and of a high enough quality to use for ELISA. Some problems were encountered due to a "background" reaction caused by antibodies developed to host plant proteins.

A number of lines of cultivars considered most suitable for the Australian industry were identified via an associated industry survey (Clarke. pers comm). Sample lines were obtained from key Australian growers and these were subjected to heat treatment and meristem-tip culture for virus elimination. Tissue culture plantlets were produced and exhaustively tested for all known viruses of garlic and were assigned "virus-tested" status if the results of all tests were negative. The virus-tested lines were held under secure tissue culture conditions to ensure no virus contamination could occur. These lines acted as nucleus stock from which extra virus-tested material was produced for further multiplication and supply to industry partners. The virus-tested lines produced during this program were: California Early (Virginia; Dri Veg; Murray Bridge), California Late, Marlborough White, New Zealand Purple, Italian White, Schumex and Mexican.

Improved tissue culture media formulations were investigated to enable the rapid, large scale production of the pathogen-tested (PT) cultivars from the few cultures that comprised the nucleus stock of the program. Various tissue culture media formulations based on previously published literature for garlic or other members of the Allium family were trialled. Good results were obtained for the small scale multiplication of garlic in tissue culture using a formulation based on MS media with various additives including isopentenyl adenine (2-ip). At least 6-fold multiplication and growth to small rooted plantlets ready for deflasking was achieved regularly in 6-8 weeks for most of the virus-tested lines. These results could not matched by a commercial tissue culture laboratory who were contracted by the industry partners to this program to pilot the large scale commercial production.

2. Recommendations:

2(a). Extension/adoption by industry of research findings

Growers and industry have been made aware of the losses caused by the presence of virus disease in the currently grown cultivars of Australian garlic. The industry should aim to encourage all growers to plant only virus-tested garlic lines when they are commercially available and adopt strategies aimed at a long term "pathogen-tested" approach to ensure an improvement in the yield and quality of the Australian grown product. It is further recommended that a system somewhat similar to that used in France be adopted for the production, inspection and distribution of virus-tested garlic in Australia.

2(b). Directions for future research and/or activities supported by the HRDC

- The tissue culture of garlic is notoriously difficult and is highly variable between cultivars. A detailed study is required to ensure that tissue culture protocols are reliable and reproducible when used on a scale sufficiently large to supply the needs of industry.

- Industry guidelines are required for a total quality management system for the production of garlic through a virus-tested/pathogen tested approach in order to ensure industry best practice is adopted by all growers.

- Virus-tested garlic varieties recently developed overseas (eg. in France) should be imported into Australia (under licence if necessary) and trialled to determine their suitability for production under Australian conditions and as potential export varieties to satisfy Southeast Asian markets.

2(c). Financial/commercial benefits of adoption of research findings:

Australian Production

Australia is almost insignificant in the production of garlic. Until the early 1980s, production was mainly in the Murrumbidgee Irrigation Areas (MIA) in New South Wales and the Lockyer Valley in Queensland. In the MIA and surrounding districts 160 hectares were planted in 1983 by about 60 growers. The largest single area was 24 hectares. The annual area grown in the Lockyer Valley varied from 70 to 100 ha. The total production in New South Wales in 1983 was about 600 tonnes and in Queensland 400 tonnes. Other states totalled about 50 tonnes.

Late in the 1980s, South Australia increased its production considerably and by 1990, Victoria was promoted as the area for garlic production with as many as 150 garlic growers.

In 1981 the world wide garlic production was well over 2 million tonnes, of which most was produced in Asia.

Imports: Australian imports of fresh garlic are estimated to be between 1200 to 1500 tonnes (approx. $6 million). The major countries exporting garlic to Australia are:

Country	Available locally mainly
South Africa	Late November to April
New Zealand	February to August
Mexico	May to October
California	May to November
France	August to October
Argentina	January to March

Utilisation

In the 1980s, Australian demand for garlic was estimated to be close to 3000 tonnes and this figure has remained relatively stable (Salvestrin, 1993). Most of the garlic used in Australia is sold as a fresh vegetable in the market (at least 2000 tonnes). Factories utilise at least 300 tonnes and growers at least a further 150 tonnes for planting material.

Economic analysis

An estimated figure for Australian garlic use in 1989/90 was $10.5 million. Prices paid in Australia are probably equal to the highest in the world. Average prices overseas are more in the vicinity of $1.50 to 1.80/kg compared to $3.00 to $3.50/kg for the same product in Australia. It is possible to import garlic into Australia for $1.80 to $2.00/kg and even less from such countries as Argentina, France and Taiwan. Quality of imported garlic has been generally superior to Australian garlic and this has been reflected in the prices.

It is not possible to put any firm figures on the likely benefits to accrue from an industry based on virus-tested garlic producing a regular supply of the highest quality, virus-tested product. Many growers have indicated that they would like to be able to obtain a regular supply of virus-tested planting material, although it is unclear what price would be acceptable for the premium product. Further benefits would accrue to the industry, as increased production of high quality garlic in Australia would probably cause a significant reduction in the quantity of the imported product required by the Australia garlic industry. If production costs could be made sufficiently competitive it is also likely that an export industry to Asian and South Pacific countries could be established. If Australia could capture a mere 1% of the total world market in garlic it would be worth an estimated Aus $200 million per annum.

3. Technical Report

Investigation Team

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The Plant Research Institute provided fully equipped laboratories in virology, tissue culture, electron microscopy and for ELISA technology. Glasshouse and growth room facilities, an animal house, The Plant Sciences Library and computing resources were also utilised for this project.

General Objectives

(i) To identify the viruses present in Australian garlic and to produce antisera for the development of enzyme-linked immunosorbent assays (ELISA).

(ii) To eliminate the viruses of particular garlic cultivars through tissue culture of the meristems and to maintain a nucleus of these cultivars as pathogen-tested stock.

(iii) To develop improved methods for tissue culture production of garlic.

3(a). Introduction

In all areas of the world where garlic is cultivated it has been reported to be universally infected with virus (Bos, 1982). This situation is the result of multiple virus infections occurring over a number of years in a sterile crop that is vegetatively propagated. The viruses have been shown to reduce garlic yields by up to 50% (Delecolle and Lot, 1981), 3-45% (Havranek, 1974) and 25-50% (Messian *et al.*, 1981). Yield improvements of 33-88% have resulted from the use of "virus-free" material (Walkey and Antill, 1989).

Australian garlic crops are infected with a complex of at least three viruses; a carlavirus (Koenig, 1982), probably garlic latent virus (GLV), and at least two potyviruses (Hollings and Brunt, 1981), garlic yellow streak virus (GYSV) and a strain of onion yellow dwarf virus (OYDV)(Sward, 1990). These were identified using decoration electron microscopy with imported antisera to a range of Allium viruses. Undecorated potyvirus-like particles were also observed alongside the decorated particles suggesting the presence of at least one other unidentified potyvirus. Walkey *et al.*,(1987) found that garlic grown in or imported into the U.K. to be infected by several viruses, with individual cultivars containing different viruses or different proportions of the same viruses. In New Zealand, the potyvirus GYSV is widespread (Mohamed and Young, 1981). In Germany, Graichen and Leistner (1987) found a carlavirus they termed

garlic latent virus (GLV). Japanese workers reported complex infections composed of two unidentified garlic mosaic potyviruses and one latent carlavirus (Lee *et al.*, 1979). Van Dijk *et al.* (1991) reported the existence of a mite borne potyvirus in garlic that was not transmitted by aphids and induced no visible or only mild symptoms in garlic. It was tentatively named onion mite borne latent virus-garlic strain (OMbLV-G). Other mite transmitted viruses, such as onion mosaic from Russia and garlic mosaic from the Philippines have also been reported.

The identification of viruses infecting garlic has been hampered by their narrow host range, common occurrence in complexes and similar physio-chemical characteristics which make them difficult to separate, identify and characterise. In the past, garlic virus detection has relied upon electron microscopy (EM) and inoculation to indicator plants but indexing techniques such as decoration and immunosorbent electron microscopy (ISEM) are now also commonly used. Conci and Nome (1991) produced a polyclonal antiserum against a mixture of viruses found in Argentinian garlic which proved to be GYSV, OYDV and a carlavirus serologically related to carnation latent virus (CLV). This antiserum was then used in ISEM to index plantlets which were derived from meristems excised from heat treated cloves.

Virus elimination is most effectively carried out in garlic using meristem culture (Bhojwani *et al.*, 1982, Bertaccini *et al.*, 1986, Pena-Inglesias and Ayuso, 1982) and the application of heat treatment to cloves (Conci and Nome, 1991) and to plants (Walkey *et al.*, 1987) prior to meristem excision has been found to increase the rate of virus elimination in tested plantlets. After virus elimination, healthy clones must be rapidly multiplied to the large number of plantlets necessary to provide the basis for commercial plantings. Traditional vegetative field multiplication, which has to occur geographically isolated from sources of infection, is both slow and expensive.

Rapid multiplication of garlic has been achieved *in vitro* by a number of workers (Bertaccini *et al.*, 1986, Bhojwani, 1980, Matsubara and Chen, 1989, Walkey *et al.*, 1987, Moriconi *et al.*, 1990). However, a media formulation which is optimal for one cultivar may not be so for another (Bertaccini *et al.*, 1986, Bhojwani, 1980). It was considered crucial therefore, to determine the optimal media formulation for rapid multiplication and growth of selected local cultivars so that large scale commercial multiplication could become economically viable. A section of the research was therefore designed to investigate the multiplication and growth of selected garlic cultivars on six media prepared on the basis of previously published formulations.

3(b). Materials and methods

(i) Virus identification and immunoassay development

Plant material

Bulbs of garlic cultivars Californian Late, New Zealand Purple, Mexican, Marlborough White, Italian White and Californian Early were obtained from a range of commercial sources within Australia. The bulbs were refrigerated at 4°C for up to 30 days prior to being stored in a dry, well ventilated shed. Individual cloves were then introduced to tissue culture or planted in 15 cm diameter plastic pots or polystyrene produce boxes containing a pine bark and sand mix under glasshouse conditions (10-30°C). All plants were regularly fertilised with Aquasol®.

Virus indexing using electron microscopy

Serological EM testing was done using antisera kindly provided by Dr A. Mohamed, Ministry of Agriculture and Fisheries, New Zealand (GYSV) and Dr L. Bos, IPO-DLO, Wageningen, The Netherlands (OYDV, leek yellow stripe virus (LYSV) and shallot latent virus (SLV) as well as from Agdia (carnation latent virus (CLV)). Virus decoration tests were done by crushing a leaf

piece from the youngest fully expanded leaf in distilled water, dipping a formvar/carbon coated 300 mesh copper grid into the mixture, draining and drying it. The grids were then floated on antiserum drops diluted 1:100 in 0.05M potassium phosphate buffer, pH 7.5, for 45 min. at room temperature. Grids were washed with distilled water, stained with 2% potassium phosphotungstate, pH 7.0, drained, dried and viewed with an Hitachi H 600-S electron microscope. Controls consisted of leaf material from healthy garlic which had undergone meristem culture for virus elimination several seasons previously.

Virus purification

The protocol followed was as outlined by Mohamed and Young (1981). Leaves with mosaic symptoms containing the filamentous virus complex and weighing 300g were harvested from cultivar Mexican plants. In 100g batches they were homogenised and clarified in a mixture containing 200ml 0.5M sodium borate buffer, pH 8.1 with 0.2% thioglycollic acid, 50ml chloroform and 50ml carbon tetrachloride. The homogenate was centrifuged at 10,000g for 10 minutes and the pellet was discarded. The supernatant was collected and polyethylene glycol, MW 6000, was added to 5% (w/v) and NaCl to 1.75% (w/v) and the suspension stirred for 4 hours at 4°C and then centrifuged at 10,000g for 10 minutes. The pellet was resuspended in 0.05M sodium borate buffer, pH 8.1, plus 0.5M urea (resuspension buffer) and was stirred overnight at 4°C. Polyethylene glycol, MW 6000, was added to the supernatant to 8% (w/v) prior to it being stirred overnight at 4°C. The supernatant was centrifuged at 10,000g for 10 minutes. Triton X-100 was added to the supernatant to a final concentration of 1% (v/v) after which it was stirred at 4°C for 30 minutes. After another centrifugation at 10,000 for 10 minutes the supernatant was collected and centrifuged at 78,000g for 90 minutes. The pellet was resuspended in the resuspension buffer and gently stirred overnight at 4°C. This suspension was layered onto 15ml 20% sucrose cushion in resuspension buffer and centrifuged at 24,000rpm for 3 hours in a Beckman SW 28 rotor. The pellet was resuspended in a total of 3ml of resuspension buffer and stirred overnight at 4°C. The sample was layered onto a 10-40% sucrose gradient in resuspension buffer and centrifuged at 25,000rpm for 2.5 hours in a Beckman SW 28 rotor. The virus zones were removed, pelleted at 78,000g for 2 hours and resuspended in 100μl of resuspension buffer.

Electrophoresis and immuno-blotting

Polyacrylamide gel electrophoresis was performed on purified preparations. Molecular weight markers used were carbonic anhydrase (MW=29,000 daltons), egg albumin (MW=45,000 daltons) and bovine serum albumin (MW= 60,000 daltons). Gels were silver stained according to the method of Merrel *et al.* (1982) and immuno-blotting (western blot) was performed using GYSV and OYDV antisera. Staining of bands was done with amido black for total proteins and with a horseradish peroxidase system for the specific viral protein bands.

Antiserum production

A New Zealand long eared rabbit received three intramuscular injections of purified virus preparations emulsified with Freund's incomplete adjuvant at 15 and then 25 day intervals. Three bleeds were done 1, 6 and 10 weeks after the last injection. The IgG component was purified by precipitation with saturated ammonium sulphate and dialysed against 3 changes of ½PBS over 24 hours. The antiserum titre was determined by microprecipitation test.

Virus indexing using ELISA

The polyclonal antiserum to the Australian complex of viruses in conjunction with antiserum to OYDV (commercially available from Sanofi in France) was used in double antibody sandwich ELISA (Clark and Adams, 1977) to index the surveyed cultivars and all plantlets and plants derived from meristem cultures. Paired wells were used in Nunc Maxisorp plates. Samples were taken from the youngest fully expanded leaf and homogenised and diluted 1:20 with 0.05M tris-HCl, pH 6.8, with 0.05M NaCl, 20mM Na_2 EDTA, 0.02% (w/v) Tween 20 and 5% (w/v)

mannose prior to being strained through muslin. The addition of mannose reduced the high background values caused by the highly viscous and reactive sap inherent in Allium species. The plates were coated with antibody at a dilution of 1:500 and the alkaline phosphatase-antibody conjugate was used at 1:750. The substrate p-nitrophenyl phosphate was used at 1mg/ml. Absorbance readings at 405nm (A_{405}) were made using a Titertek Multiskan plate reader blanked on wells with substrate only. The Sanofi OYDV kit was used as described in its instructions except that the mannose supplemented extraction buffer described above was used.

(ii) Virus elimination by meristem tip culture and heat treatment

Heat treatment
Individual garlic plants were established from high quality source bulbs of selected cultivars (Fig. 1) and grown in terracotta pots in an open, well drained soil mix. The plants, which all exhibited typical symptoms of virus disease (Fig. 2), were grown until approximately 20cm tall (3-4 leaf) and then placed into the heat therapy room (Fig. 3), acclimatising the plants first at lower temperatures that were increased daily until the room temperature was 38°C. The plants were maintained at this temperature for up to 8 weeks or until they showed signs of collapse. They were then removed from the soil, topped and tailed, taken to the tissue culture laboratory where they were placed in a laminar flow cabinet on a binocular dissector (Fig. 4) and dissected to the meristem tip which was isolated and established on liquid media in tissue culture (Fig. 5) - see later methods.

Bud excision
Representative, clean, firm bulbs were selected and separated into cloves. Each clove was peeled and thoroughly rinsed in 70% ethanol for 10-20 seconds and lightly flamed on a sterile laminar flow bench where the rest of the procedure was carried out. Each bud was isolated from its clove by removing the surrounding storage leaves. This was achieved by cutting the clove laterally in two between 5 and 10mm above the clove's basal plate, followed by two longitudinal cuts down along both sides of the bud and through the basal plate. The tough dry basal plate material was thinly sliced off and the remaining pieces of storage leaves peeled away to reveal the bud.

In vitro heat treatment of buds and meristem excision
Buds of cultivars Californian Late, New Zealand Purple, Marlborough White and Italian White were placed into 120ml polypropylene vials with 15ml of MS medium (Murashige and Skoog, 1962) supplemented with 1.75mg/l indole acetic acid and 2.15mg/l kinetin and solidified with 3.5g/l Gelrite®. Cultures were incubated in a growth room at 28°C with a photoperiod of 16 hours at 88$\mu Es^{-1}m^{-2}$ for 2-4 days. Viable cultures were transferred to a heat treatment cabinet at 36-38°C with a photoperiod of 16 hours at 52$\mu Es^{-1}m^{-2}$ for 6 weeks. Control plantlets were grown at 22°C. Heat treated and control cultures were removed to a sterile laminar flow cabinet where meristems of between 0.2 and 1.2mm in diameter were excised under a binocular dissecting microscope whilst immersed in liquid medium in a 5 cm diameter plastic Petri dish. The diameter of excised meristems was recorded using an eyepiece micrometer when they were placed in 5cm Petri dishes containing 5ml of liquid medium which was then sealed with a strip of parafilm and incubated at 22°C with a photoperiod of 16 hours at 88$Es^{-1}m^{-2}$ whilst being shaken at 60rpm on a Ratek shaker. Plantlets that developed were transferred to solid medium after 2-4 weeks in liquid media and grown on for another 4-8 weeks before being indexed for the first time.

Meristem excision and in vitro heat treatment of meristems
Buds from cloves of cultivar Californian Early were initiated in liquid media at 28°C for 2-4 days. Viable buds were selected and meristems were excised as previously described and placed in Petri dishes which were then incubated in a heat treatment cabinet as described whilst being shaken at 60rpm on Ratek shakers for 3-6 weeks. Control meristems were cultured at 22°C. Plantlets that

developed were then removed and subcultured on solid medium at 22°C prior to indexing 4-8 weeks later.

Hardening-off tissue culture plantlets and re-indexing

Plantlets were readily induced to form roots by culturing on an auxin and cytokinin free medium (Walkey *et al.*, 1987)- (Fig.6). The media used consisted of Gamborg's B5 mineral salts medium (Gamborg *et al.*, 1968)(Flow Laboratories Ltd) containing 30g/l sucrose, 1mg/l Nicotinic acid, 1mg/l pyridoxin HCl, 10mg/l thiamin, 100mg/l inositol and 3.5g/l Gelrite at pH 5.7. Following root development (2-4 weeks) vials were removed to an insect proof glasshouse for acclimatisation to light and temperature for up to 1 week. Thereafter, the lids were progressively loosened over another week until the plantlets were removed, the media washed off the roots and all the expanded leaves removed prior to planting in pasteurised potting mix in a 3cm diameter plastic pot. Plants were maintained under intermittent mist spray conditions for up to 4 weeks and were transferred to trays (Fig. 7) when sufficiently large. Individual plants were carefully examined to ensure they were free of virus symptoms (Fig. 8) and were re-indexed near maturity just prior to drying off for harvesting and storage of the bulbs (Fig. 9).

(iii) Improved methods for tissue culture based production of garlic

Plant material

Garlic cultivars of known and potential commercial importance (Clarke, pers. comm.) were obtained from a range of commercial sources within Australia. Cultivars Mexican and Californian Late were planted and propagated in 15cm diameter pots and polystyrene produce boxes under glasshouse conditions (10-30°C). The soil used was based on a pine bark and sand mix and all plants were regularly fertilised with Aquasol®. Glasshouse grown plants were harvested, cured, topped, tailed, refrigerated at 4°C for one month and then stored at room temperature in a well ventilated shed prior to being introduced to tissue culture.

Introduction to *in vitro* culture

A literature search revealed a number of media formulations for the growth and multiplication of garlic *in vitro*. Six published formulations (Table 1) were chosen for evaluation (Bertaccini *et al.*, 1986, Bhojwani, 1980, Matsubara and Chen, 1989, Walkey *et al.*, 1987, Abo El-Nil, 1977, Novak *et al.*, 1984). These were chosen because of the widespread reporting of their success rates, encouraging results obtained in preliminary experiments and the ready availability of their components. The formulation based on that of Abo El-Nil (1977) was modified to include MS basal salts with minimal organics medium (Murashige and Skoog, 1962) (Sigma) instead of AZ medium (Abo El-Nil and Zettler, 1976). The gelling agent was altered from agar to Gelrite® in all the formulations.

Clean and firm bulbs were selected and separated into cloves. Each clove was peeled, thoroughly rinsed in 70% ethanol for 10-20 seconds and then lightly flamed on a sterile laminar flow bench. Each bud was isolated from its clove by removing the surrounding storage leaf. This was achieved by cutting the clove laterally in two, between 5 and 10 mm above the clove's basal plate. Following this, two longitudinal cuts were made along both sides of the bud and through the basal plate. The tough, dry basal plate material was thinly sliced off and the pieces of storage leaf peeled away to reveal the bud. The bud was then placed into a liquid form of one of the six culture media (Table 1). All cultures were grown in growth rooms at 18-22°C with a 16 h photoperiod under photosynthetically active radiation of $88\mu Es^{-1}m^{-2}$ provided by cool white fluorescent tubes.

Multiplication and growth experiment
Actively growing buds 5-10 mm in size were placed into 120 ml polypropylene tubes containing 15 ml of one of the six solidified media and cultured as previously described. Cultures were examined after six weeks for the number of shoots (axillary and adventitious), the length of the longest shoot and the presence or absence of roots. Two cultivars were assessed in this manner: virus infected cultivar Californian Late (274 plantlets) and virus tested cultivar Mexican (126 plantlets).

Statistical analysis
The effects of media and presence of roots on the number of shoots produced were assessed by fitting a generalised linear model with a Poisson error distribution. The relationship between the percentage of plantlets with roots and the media used was examined using a similar model, but with a binomial error distribution. These models were chosen to appropriately match the discrete nature of the data. The effect of media on longest shoot length was tested using analysis of variance. The relationship between longest shoot length and number of shoots formed was assessed by examining a scatterplot for each medium and calculating the correlation coefficient.

3(c). Results

(i) Virus identification and immunoassay development

Virus indexing using electron microscopy
When standard sap dip electron microscopy was done on sap of any field collected garlic plants a mixture of flexuous filamentous particles of varying lengths (range approx. 550-800nm) was observed (Fig. 10). In many samples classic pin-wheel or cylindrical inclusion bodies that are usually associated with potyvirus infection were also present.

Decoration electron microscopy tests using imported GYSV and OYDV antisera resulted in both decorated and undecorated virus particles (Fig. 11). In decoration EM tests using the Australian garlic virus complex antiserum all particles observed were decorated. No particles were decorated by antisera to carnation latent virus (CLV), shallot latent virus (SLV) or leek yellow stripe virus (LYSV). Positive decoration was observed only in infected, never healthy, test samples.

Virus purification
Sucrose gradient centrifugation resulted in the presence of one main viral band and a large diffuse zone. Spectrophotometric examination in the UV range showed a slight peak and typical absorbance values were A_{240} = .190, A_{248} = .154, A_{260} = .170, A_{280} = .140. Electron microscopy of the viral band revealed many intact virus particles of varying lengths (Fig. 12).
The diffuse zone contained very few virus particles.

Electrophoresis and immuno-blotting
Silver staining of the electrophoresed samples (approx. 40 ul and 10 ul of purified virus) revealed a complex set of bands in the MW ranges of 27,000-35,000 and 45,000-50,000 daltons approx. (Fig. 13). The higher MW range of bands corresponded mainly to host plant proteins whilst components of the lower MW range of bands presumably corresponded to particular viral proteins from the different virus types (ie. the carlavirus and the potyvirus).

The immuno-blot (western blot) revealed clearer sets of bands with a main band presumed to correspond to the potyvirus protein band of approx. 33,000 daltons and a second, somewhat fainter band presumed to correspond to the carlavirus protein, of 30,000 daltons (Fig. 14).

Antiserum production
When tested using double diffusion in agar the antiserum gave a titre of 1/512 when reacted with leaf sap from a plant that provided tissue for purification.

Virus indexing using ELISA
Initially the antiserum was not entirely suitable for large scale testing of garlic due to a component of 'healthy' garlic proteins that caused high backgrounds in ELISA. Typical early A_{405} results were:

infected positive control sample	=	1.40-1.65*
virus-tested negative control	=	0.45-0.85
buffer only	=	0.05-0.20

* These figures relate to the range of spectrophotometer readings.

The addition of mannose to the extracted sap combined with the use of a Tris-HCl buffer helped to reduce the high background levels. Typical A_{405} results using the refined protocols were:

infected positive control sample	=	1.23-1.67
virus-tested negative control	=	0.12-0.17
buffer only	=	0.01-0.13

The results of ELISA testing of a range of field grown material were sometimes variable and this was thought to reflect the presence of different combinations of the viruses forming the typical three virus complex.

(ii) Virus elimination by meristem tip culture and heat treatment

Meristem excision and in vitro heat treatment of meristems
Heat treatment of excised meristems in liquid shake culture increased the rate of virus elimination (Table 2), although the survival rate of meristems was reduced by this treatment.

Meristems of 0.8mm and above in diameter were more likely to survive culturing and grow into differentiated plantlets no matter what the treatment (Table 3). Virus elimination levels of 50% or higher were only achieved with control meristems smaller than 0.7 mm in diameter but were achieved with heat treated meristems up to and including those of 1mm in diameter. Heat-treatment resulted in a greater frequency of virus elimination in all categories of meristem size.

Greater frequency of virus elimination was measured for heat treated in vitro plantlets compared with untreated controls although the percentage survival was slightly lower than the control treatments (Table 4).

Hardening-off tissue culture plantlets and re-indexing
Rooted plantlets were hardened-off, planted in the glasshouse and grown to maturity with a high degree of success. All plants appeared to grow true to type. ELISA and EM indexing of mature plants showed that only 8% harboured some virus even though none exhibited symptoms. No OYDV was detected in any of these plants.

(iii) Improved methods for tissue culture based production of garlic.

Results for the multiplication and growth of cultivars Californian Late and Mexican are shown in Table 5. The Bertaccini *et al.*, (1986) medium stimulated the development of significantly more shoots for cultivar Californian Late (3.6) than did any other media (1.7 to 2.2). Cultivar

Mexican produced significantly more shoots on both Bertaccini *et al.*, (1986)(6.8) and Bhojwani (1980) media (5.3)- (Fig. 15), than any other (1.0 to 2.1). The presence of roots did not significantly affect the number of shoots produced for either Californian Late or Mexican. Overall, media by Matsubara and Chen (1989) and Walkey *et al.*, (1987) were the most effective in initiating roots on plantlets. A greater percentage of Mexican plantlets developed roots than did Californian Late plantlets on all but the medium by Novak *et al.*, (1984). Plantlets of Californian Late developed the longest shoots on the medium by Abo El-Nil (1977). There was no significant difference between media in the length of the longest shoot for Mexican plantlets with lengths ranging from 9.9 to 11.3 cm. Mexican plantlets produced longer shoots on all media than did Californian Late. There was no consistent relationship between longest shoot length and the number of shoots formed for either cultivar.

3(d). Discussion

(i) Virus identification and immunoassay development

Cultivated garlic in Australia is infected with a complex of filamentous virus. At least two potyviruses were present in most plants tested. These two viruses were GYSV and OYDV which had not previously been recorded as infecting Australian garlic but had been found in onion. At least one unidentified carlavirus was also present in most plants tested. Conci *et al.*, (1992) reported a carlavirus closely related to carnation latent virus (CLV) in garlic in Argentina. It has been determined here that the Australian carlavirus is not closely related to CLV. This unidentified carlavirus may be garlic latent virus (GLV). GLV was first reported by Lee *et al.*, (1979) infecting garlic in Japan and more recently in Germany (Graichen and Leistner, 1987). Van Dijk *et al.*, (1991) found the German and Japanese isolates of GLV to be serologically distinct. There was also no evidence of the presence of LYSV nor SLV in any sample even though these viruses are commonly found in garlic in western Europe (Walkey *et al.*, 1987) and LYSV has been identified as infecting leek in Australia (Sward, 1991).

Given that the complex of viruses infecting Australian garlic is widespread and conserved, the decision was made to purify the complex so that an antiserum could be produced for use in a rapid diagnostic test such as ELISA. It was not feasible within the time constraints of the project to separate the viruses and so produce antiserum to each. A polyclonal antiserum was thought to be the most appropriate for use in a pathogen tested scheme given its broad specificity making it ideal to detect different but serologically-related viruses. The antiserum produced has proven suitable for serological assays and with optimisation and modification of the extraction buffer to include mannose it has provided the basis of a reliable ELISA.

(ii) Virus elimination by meristem tip culture and heat treatment

The heat treatment of *in vitro* plantlets substantially increased the frequency of virus elimination in the three cultivars tested. However, the extreme treatment conditions appeared to have a deleterious effect on the rate of survival of cultured meristems. 54% of control plantlets survived meristem excision with 38% of these having virus eliminated, whereas 42% of meristems from heat treated plants survived with 68% of these having virus eliminated. The use of heat-treatment enables a greater proportion of virus tested plantlets to be obtained. The survival of heat-treated meristems excised from plantlets that have undergone heat-treatment could perhaps be increased through more gradual increases in temperature, trimming of shoots, sub-culturing, decreasing the length of exposure to such extreme conditions and increasing the size of excised meristems.

Heat-treatment of excised meristems in liquid shake culture proved an inefficient means of producing virus tested plantlets. Although the virus elimination rate was increased by using heat-treatment the survival rate of the meristems was so poor as to be impracticable. The therapy appeared to be too extreme for the delicate meristem tissue although factors such as insufficient meristem size, increased contamination rates and the desiccation of cultures also affected survival.

Virus-tested plants were successfully hardened-off and grown true-to-type in the glasshouse. Although they exhibited no symptoms of virus infection, indexing 18 months later using ELISA and electron microscopy revealed that 8% of plants contained some virus particles although OYDV was not detected in any plant. It appears that plants that have undergone heat treatment and meristem tip culture may sometimes contain a virus that takes a very long time to increase to a detectable titre. These results demonstrate the importance of re-indexing meristem derived plants as near mature plants in the glasshouse before certification as virus-tested.

(iii) Improved methods for tissue culture based production of garlic.

Although the media trialled were broadly recommended for garlic shoot proliferation in the published literature, the trials reported here showed cultivar specific responses. Multiplication rates of less than 2.5 fold every 6 weeks are regarded as unacceptable for large scale commercial multiplication (Barlass, pers. comm.). Rates of multiplication that were commercially acceptable were obtained only on media containing the cytokinin isopentenyl adenine (2-ip). These findings support the results of Bertaccini *et al.*, (1986), Pena-Iglesias and Ayuso (1982) and Bhojwani (1980) who found that 2-ip was essential for high levels of clonal multiplication. Choi *et al.*, (1985) linked the presence of this cytokinin to increased rates of survival of garlic meristems in culture.

The multiplication rate of cv. Mexican plantlets on the Bertaccini *et al.*, (1986) medium at 6.8 fold in 6 weeks is comparable to that of Bertaccini *et al.*, (1986) who reported that this medium induced shoot proliferation at a 6 fold rate for plantlets of cv Bianco Piacentino. Garlic shoot proliferation rates of 8 fold in 6 weeks (Bhojwani, 1980) for cv Rose-de-Kakylis and 6 fold in 6 weeks (Bhojwani *et al.*, 1982) for unknown New Zealand cultivars have also been recorded. Moriconi *et al.*, (1990) reported a rate of 6.5 with as many as 27 plantlets per explant using clonal types Blanco and Colarado. We obtained as many as 17 plantlets per explant. Our rates compare favourably with those of Choi *et al.*, (1985) who obtained a rate of 4 fold in 10 weeks by trimming emerging shoots of local cultivars to stimulate further proliferation.

Many of our cultures have shown a decline in shoot proliferation during extended periods in culture. This phenomenon has also been observed by Moriconi *et al.*, (1990) and is most likely due to suppression of adventitious shoot formation by bulblet formation (Fig. 16). Bulblet formation occurs in response to exposure to long day lengths but unfortunately garlic does not multiply rapidly *in vitro* under short days (Brennan & Sward, unpublished). Plantlets that have formed bulblets can usually still be planted out and grown to successfully form mature garlic plants.

Both cultivars readily formed roots on the auxin and cytokinin free medium (Walkey *et al.*, 1987) which supports the findings of Walkey *et al.*, (1987) and Bertaccini *et al.*, (1986). These results showing that the presence of roots had no significant effect on the number of shoots produced for either cultivar, conflicts with the commonly held belief that root formation undermines shoot proliferation (Bhojwani, 1980). Workers multiplying shallots in tissue culture have found that multiplication rates fall if roots are removed (Mullins, pers. comm.).

The present work demonstrates that different garlic cultivars may respond in various ways to the same culture medium. In order to develop a commercially viable rapid micropropagation scheme which utilises tissue culture, the media chosen must be suited to specific cultivars. The correct choice of media can substantially improve clonal propagation rates. There are numerous cultivars and selections of garlic grown worldwide, and they are known by a myriad of common names. Some workers believe that there are only 6 basic garlic types which can be separated on the basis of their required photoperiod (Pena-Iglesias and Ayuso, 1982). Variability of performance is thought to be genetically determined and not due to specific nutritional requirements (Bhojwani *et al.*, 1982, Bertaccini *et al.*, 1986). However, a range of other factors must also be considered in analysing such variability. The period of time between harvest and initiation to culture could influence the initial rate of growth. Cultivar Californian Late is a much later variety than cv. Mexican and may not have fully finished its dormant phase when it was cultured for these trials. The absence of virus in cv. Mexican could also have enabled it to out perform Californian Late (Havranek, 1974, Messiaen *et al.*, 1981, Walkey and Antill, 1989). Periods of cold storage are known to affect the rate and vigour of sprouting in field planted garlic (Mann and Minges, 1958, Rahim and Fordham, 1988) and may have influenced one of these cultivars more than the other.

During the course of this garlic R&D program, some virus-tested cloves of the cultivar "Mexican" were multiplied up and planted in a small scale field trial at the Ovens Research Station, Myrtleford (Fig. 17). Although the details of the trial are not reported here, it is significant to note that many of the plants became reinfected with virus after a few months in the field. In addition, there was a problem with "rough" bulb formation, possibly due to inappropriate mineral concentrations in the soil. In order to set up a scheme for the virus-tested garlic stock it is clear that a total quality management system will have to be developed with specific protocols to ensure that each stage of production is controlled and secure. Only then will the Australian garlic industry have access to the high quality product that is so urgently required.

3(e). Acknowledgments

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Table 1. Composition of tissue culture media for garlic multiplication and growth.

components (mgl^{-1})	Abo El-Nil (1977)+	Matsubara and Chen (1989)+	Bertaccini *et al.*, (1986)++	Bhojwani (1980)++	Novak *et al.*, (1984)++	Walkey *et al.*, (1987)++
$NH_4H_2PO_4$	-	-	-	-	230.06	-
NH_4NO_3	1650	1650	-	-	320.16	-
KNO_3	1900	1900	2500	2500	2500	2500
$MgSO_4.7H_2O$	180.9	180.9	250	250	250	250
KH_2PO_4	170	170	-	-	-	-
NaFe EDTA	37.26	37.26	37.25	37.25	37.25	37.25
$FeSO_4.7H_2O$	27.8	27.8	27.85	27.85	27.85	27.85
$MnSO_4.4H_2O$	16.9	16.9	10	10	10	10
$ZnSO_4.4H_2O$	8.6	8.6	2	2	2	2
$NaH_2PO_4.H_2O$	-	-	130.5	130.5	130.5	130.5
$CaCl_2.2H_2O$	332.2	332.2	150	150	150	150
H_3BO_3	6.2	6.2	3	3	3	3
KI	0.83	0.83	0.75	0.75	0.75	0.75
$CoCl_2.6H_2O$	0.025	0.025	0.025	0.025	0.025	0.025
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025	0.025	0.025
$NaMoO_4.2H_2O$	0.25	0.25	0.25	0.25	0.25	0.25
$(NH_4)_2SO_4$	-	-	134	134	134	134
Inositol	100	100	100	100	100	100
Nicotinamide	-	-	1	1	1	1
Piridoxine.HCL	-	-	1	1	1	1
Thiamine.HCL	0.4	0.4	10	10	10	10
Benzilaminopurine (BAP)	-	0.1	-	-	0.23	-
Kinetin	2.15	-	-	-	-	-
Indole acetic acid (IAA)	1.75	-	-	-	-	-
Isopentenyl adenine (2-ip)	-	-	0.5	0.5	-	-
Naphthaleneacetic acid (NAA)	-	0.1	0.5	0.1	0.19	-
Sucrose	30000	30000	30000	30000	30000	30000
Gelrite(R)	3500	3500	3500	3500	3500	3500

+ Concentration of macro and microelements by Murashige and Skoog (1962).

++ Concentration of macro and microelements by Gamborg *et al.*, (1968)

Table 2.

The effect of heat treatment (36^{0}C, 21-42 days) of excised meristems on the frequency of survival and virus elimination for garlic cultivar Californian Early compared to control plantlets (22^{0}C)

Control		Heat treatment	
Meristems survived (%)	Virus eliminated (%)	Meristems survived (%)	Virus eliminated (%)
38/77 (49)	18/38 (47)	38/153 (25)	22/38 (58)

Table 3.

The effect of meristem size on the frequency of survival and virus elimination of both control (22°C) and heat treated (36°C, 42 days) *in vitro* garlic plantlets of cultivars Californian Late, Italian White, New Zealand Purple and Marlborough White.

Meristem size (mm)	Control		Heat Treatment	
	Number survived (%)	Virus eliminated (%)	Number survived (%)	Virus eliminated (%)
0.2 - 0.4	1/7 (14)	0/1 (0)	-	-
0.5	3/9 (33)	2/2 (100)	0/2 (0)	-
0.6	6/20 (30)	4/6 (67)	1/5 (20)	1/1 (100)
0.7	7/19 (37)	3/6 (50)	2/6 (33)	1/1 (100)
0.8	24/38 (63)	5/17 (29)	5/10 (50)	3/4 (75)
0.9	17/30 (57)	5/12 (42)	4/11 (36)	2/4 (50)
1.0	38/60 (63)	12/30 (40)	12/24 (50)	7/9 (78)
1.1+	22/37 (59)	5/20 (25)	7/16 (44)	1/3 (33)

Table 4.

The effect of heat treatment of *in vitro* plantlets (36°C, 42 days) on the frequency of survival of excised meristems and frequency of virus elimination for four cultivars of garlic when compared to control plantlets (22°C).

Cultivar	Control		Heat Treatment	
	Number survived (%)	Virus eliminated (%)	Number survived (%)	Virus eliminated (%)
Californian Late	40/65 (62)	8/32 (25)	14/42 (33)	5/8 (63)
Italian White	30/45 (67)	10/20 (50)	-	-
New Zealand Purple	26/68 (38)	7/23 (30)	14/22 (64)	8/12 (67)
Marlborough White	22/42 (52)	11/19 (58)	3/10 (30)	2/2 (100)
TOTALS	118/220 (54)	36/94 (38)	31/74 (42)	15/22 (68)

Table 5. Growth of garlic plantlets (cvs. Californian Late and Mexican) examined after six weeks on six tissue culture media. For each cultivar, means within a column followed by the same letter are not significantly different at p=0.05.

Cultivar	Media	No. of viable plantlets	No. of shoots	percentage with roots	longest shoot length (cm)
Californian Late	Abo El-Nil (1977)	42	2.0a	57a	8.5c
	Bertaccini *et al.*, (1986)	61	3.6b	67a	6.5b
	Matsubara and Chen (1989)	46	1.7a	74a	7.0b
	Bhojwani (1980)	31	2.2a	68a	5.2a
	Novak *et al.*, (1984)	50	2.0a	78a	7.2b
	Walkey *et al.*, (1987)	44	1.8a	80a	6.6b
Mexican	Abo El-Nil (1977)	18	2.1b	78a	10.3a
	Bertaccini *et al.*, (1986)	17	6.8c	94ab	9.9a
	Matsubara and Chen (1989)	22	1.2ab	100b	11.3a
	Bhojwani (1980)	23	5.3c	87ab	10.6a
	Novak *et al.*, (1984)	24	1.3ab	75a	10.2a
	Walkey *et al.*, (1987)	22	1.0a	100b	10.9a

Fig. 1 Examples of poor quality (left), high quality (right) and packaged "Gourmet" garlic available on the Victorian market.

Fig. 2 Typical mosaic streaking is visible on the leaves of virus infected garlic plants.

Fig. 3 Garlic plants growing in terracotta pots are placed in heat treatment for up to 8 weeks.

3(h). Figures

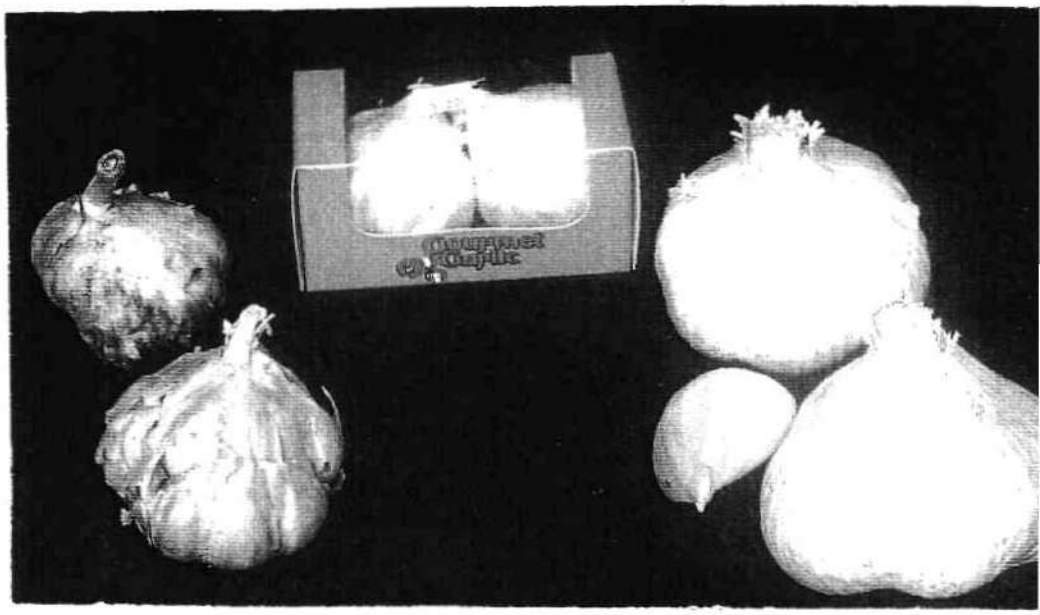

Fig. 1

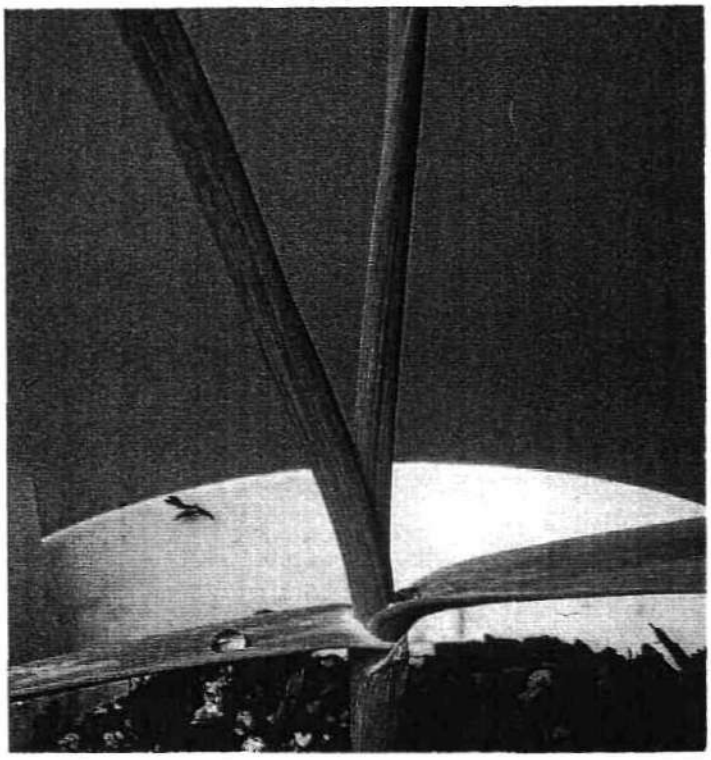

Fig. 2

Fig. 3

Fig. 4 Meristems are dissected under a binocular dissector placed in a laminar flow cabinet.

Fig. 5 The dissected meristem is established in tissue culture in a liquid medium and soon starts producing shoots and leaves.

Fig. 6 Tissue culture plantlets are readily induced to form roots when established on Gamborg's B5 mineral salts medium.

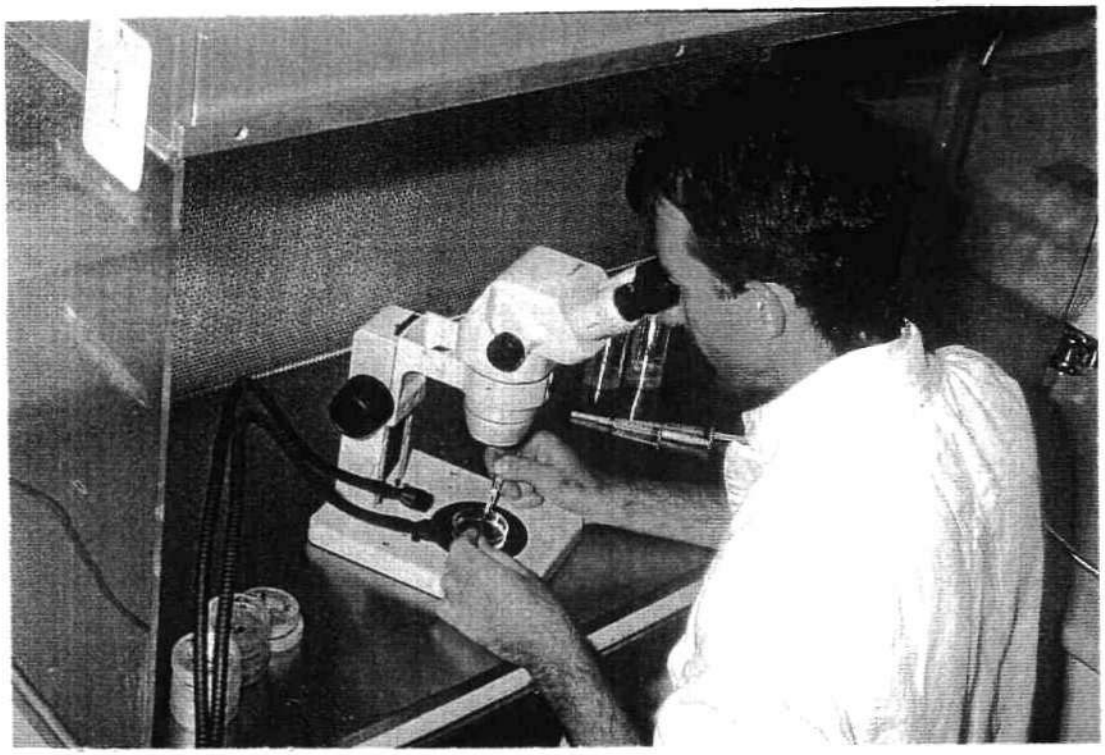

Fig. 4

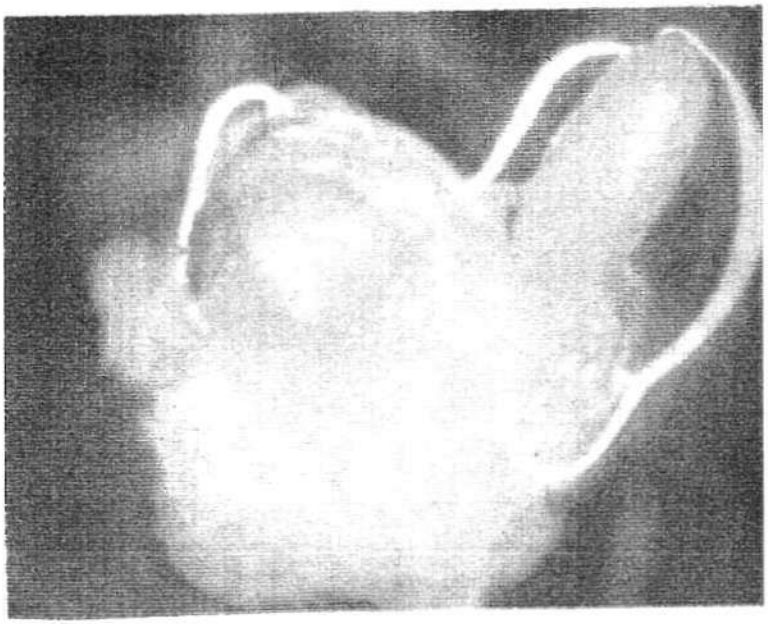

Fig. 5

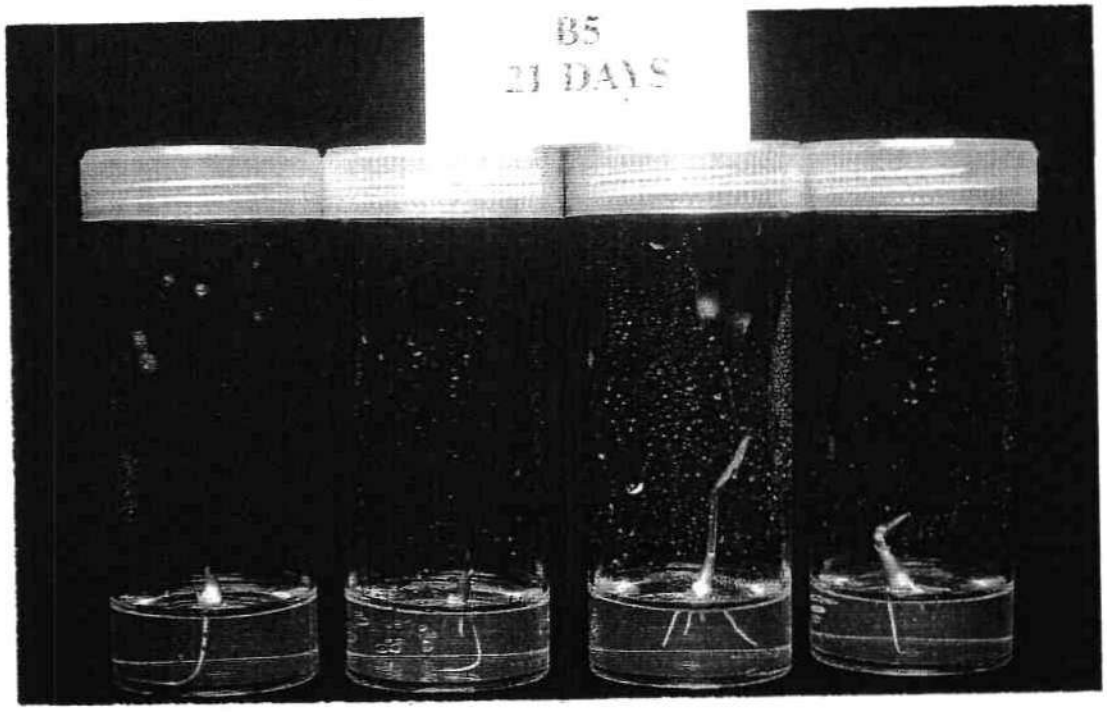

Fig. 6

Fig. 7 Virus-tested plants are hardened off, deflasked, then eventually established in the glasshouse in an open soil medium in small trays.

Fig. 8 The mature virus-tested plants are visually examined to ensure that no virus symptoms are present. Visual examination is followed up by stringent virus indexing using a range of test procedures.

Fig. 9 Mature virus-tested plants are re-indexed prior to drying off. Bulbs are then harvested and are strung up and allowed to dry in the glasshouse prior to storage.

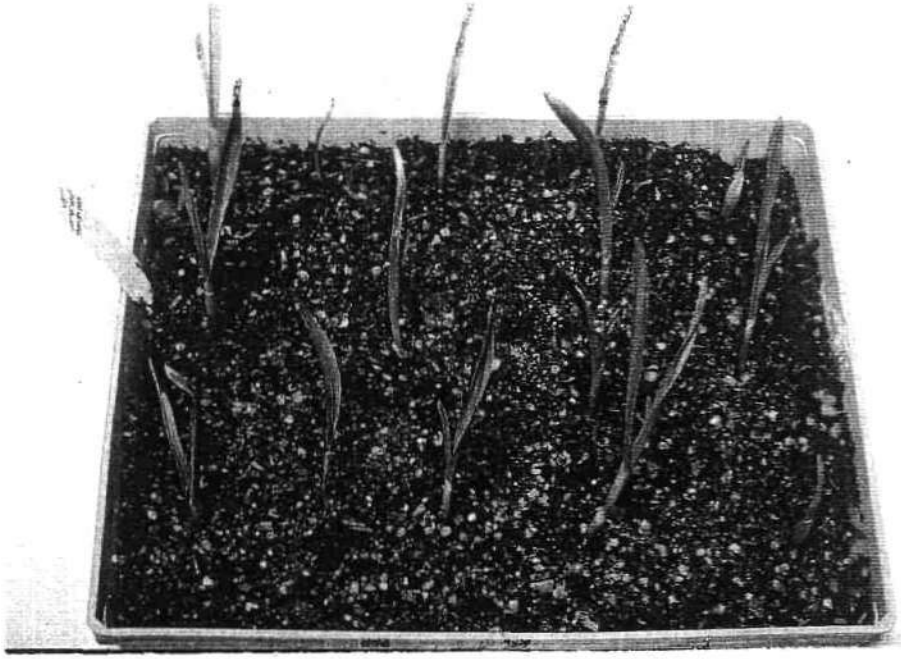

Fig. 7

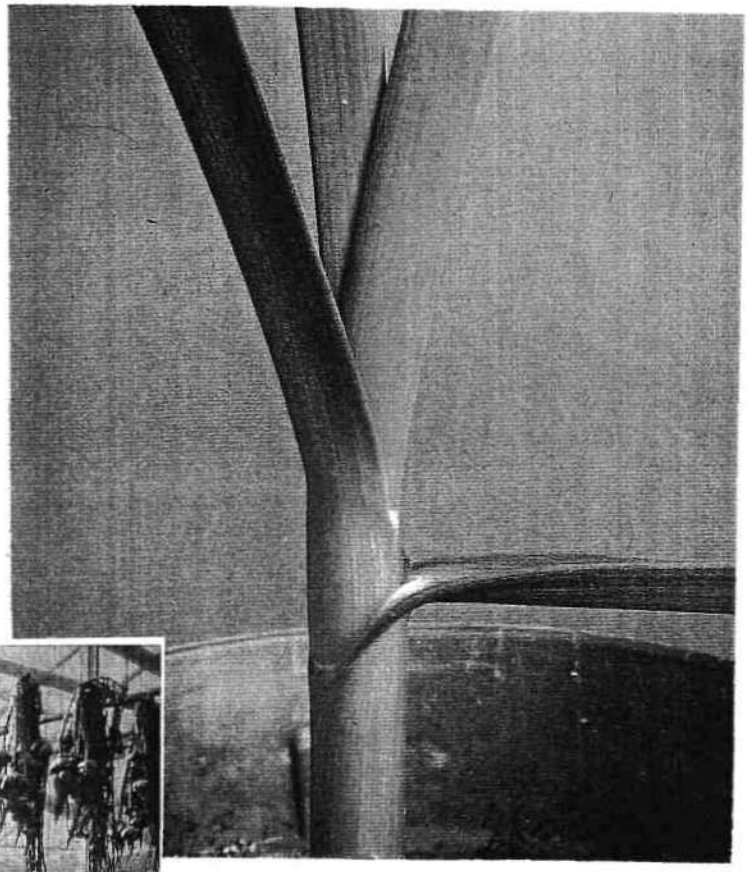

Fig. 8

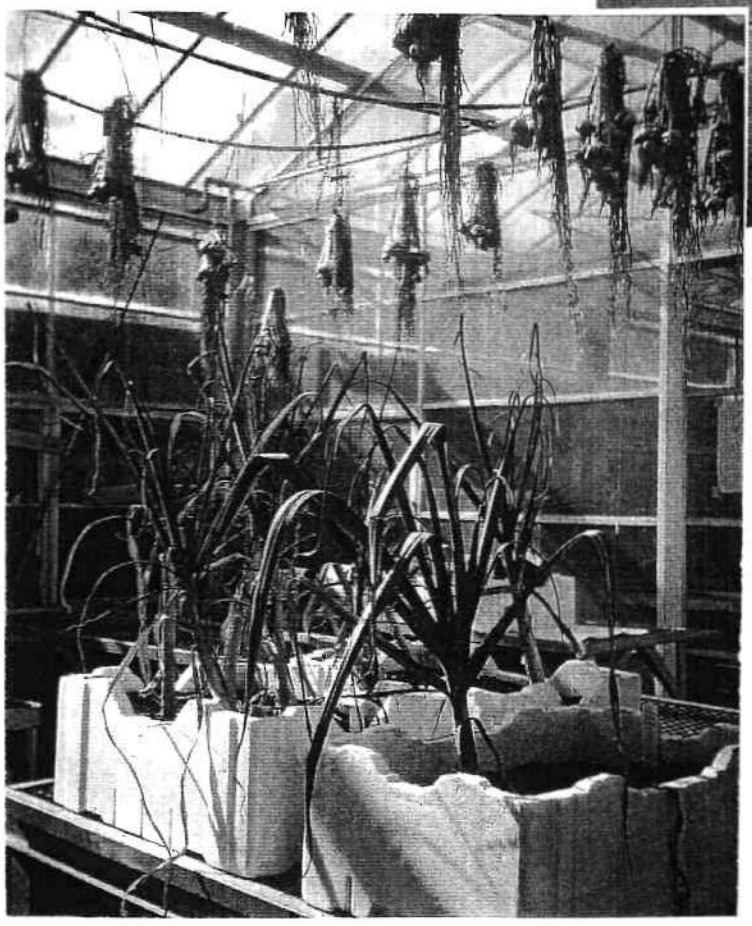

Fig. 9

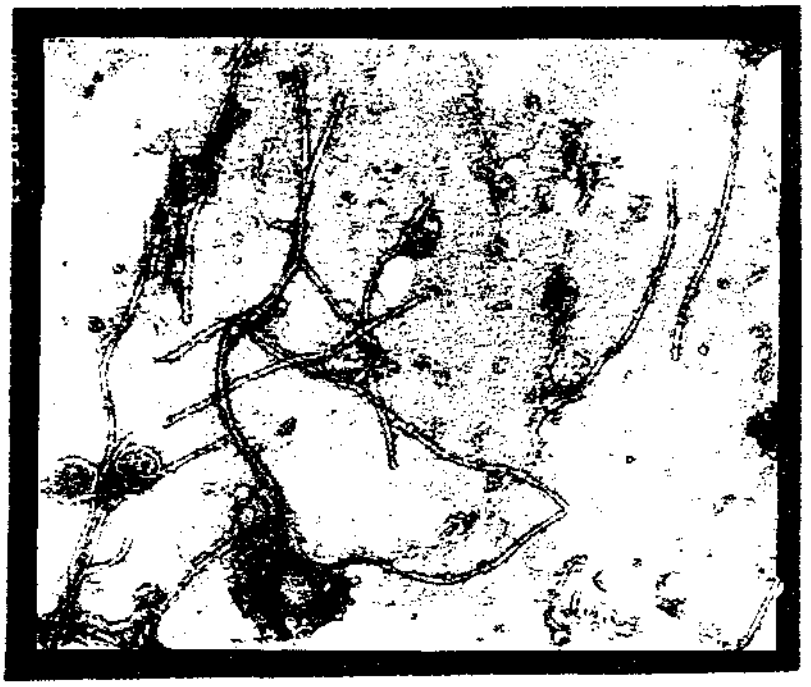

Fig. 10 Sap dip preparation of infected garlic plant showing a complex of filamentous virus particles varying from approx. 550-800 nm .

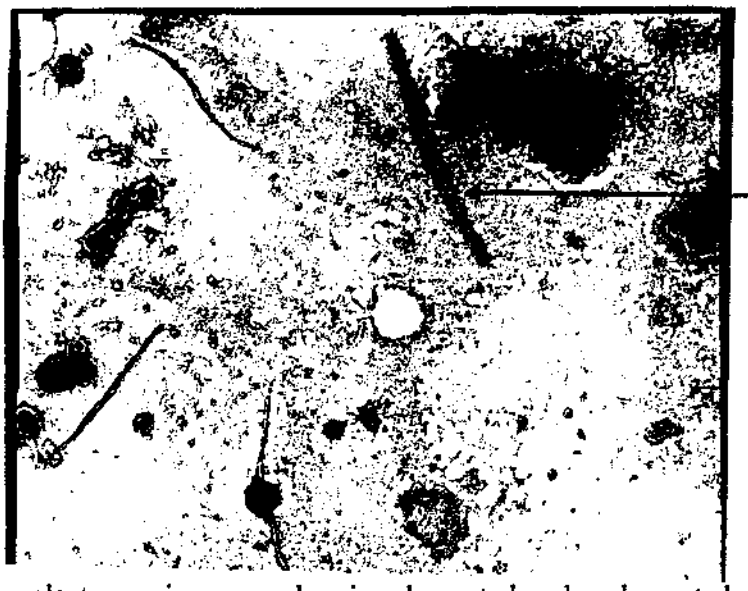

Fig. 11 Decoration electron microcopy showing decorated and undecorated virus particles.

Fig. 12 Electron microscopy of a partly purified preparation of garlic viruses.

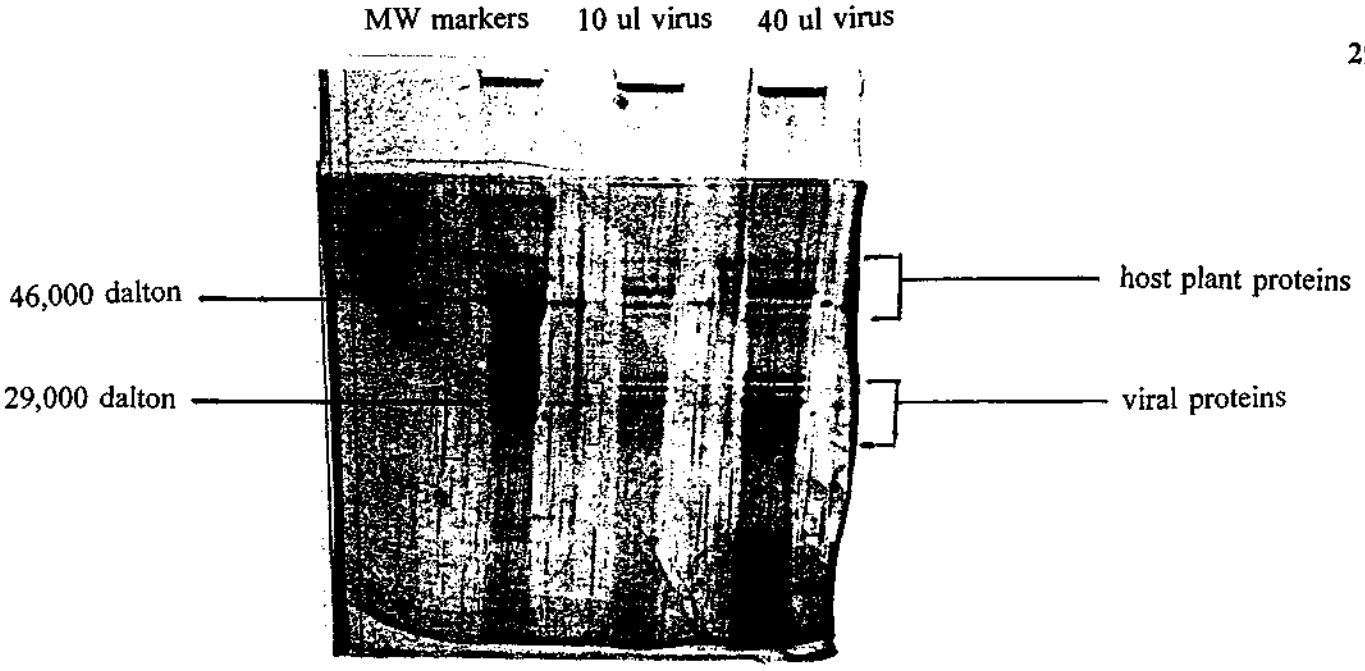

Fig. 13 Silver stained gel of electrophoresed preparation of purified garlic viruses showing a complex set of bands corresponding to 27,000-35,000 daltons (viral proteins) and 45,000-50,000 daltons (host plant proteins).

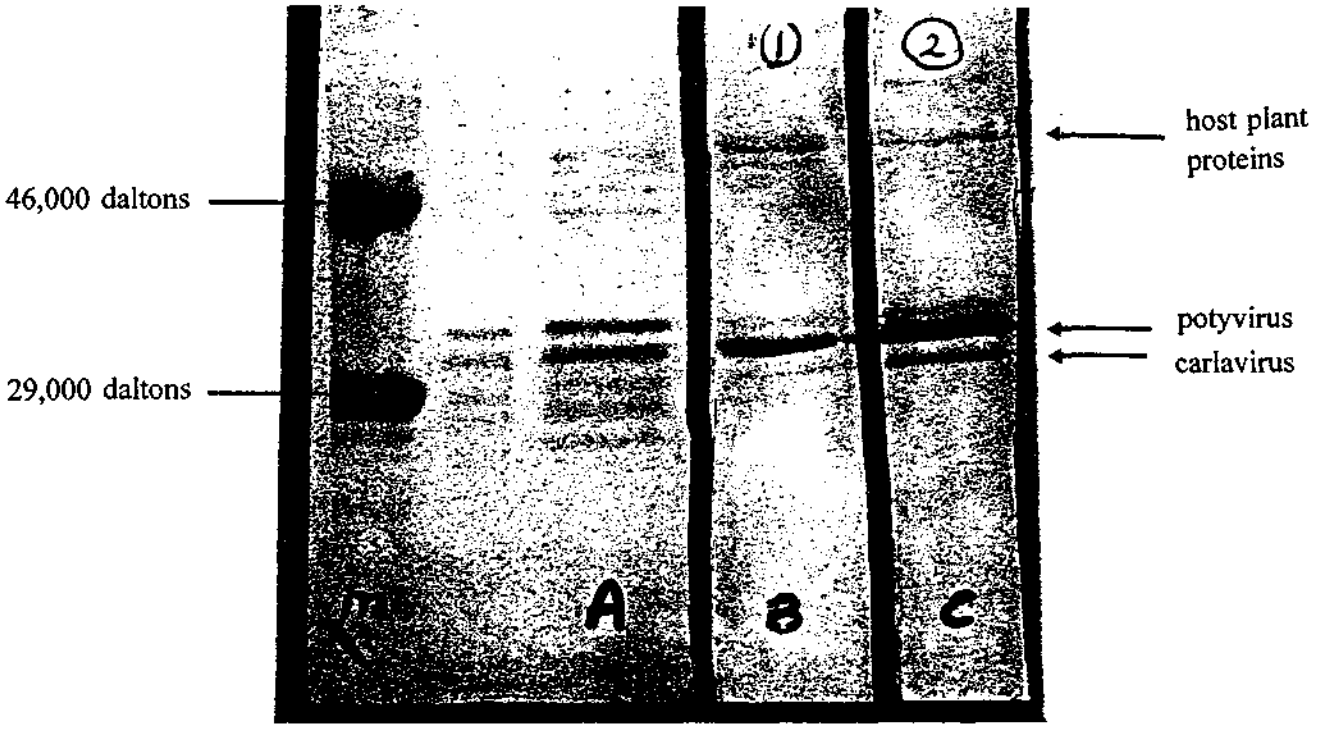

Fig. 14 Western blot of purified garlic preparation:

Lane M: MW markers of 46,000 daltons (upper) and 29,000 daltons (lower)- silver stained.

Lane A: 40 ul sample- stained with amido black for total proteins.

Lanes B and C: 40 ul samples probed respectively with GYSV antiserum (NZ) and OYDV antiserum (France). The darkest band correspond to the potyvirus-related coat proteins whilst the faint bands below correspond to the carlavirus-related coat proteins.

Fig. 15 Tissue cultured garlic showing high rates of multiplication. Those shown here have approximately ten side shoots per plantlet.

Fig. 16 Formation of "bulbiets" in tissue culture tends to suppress multiplication via the production of side shoots. However, these plants can still be successfully deflasked and established in soil.

Fig. 17 Field trial at the Ovens Research Station, Myrtleford, showing virus-tested garlic plants growing in the open.

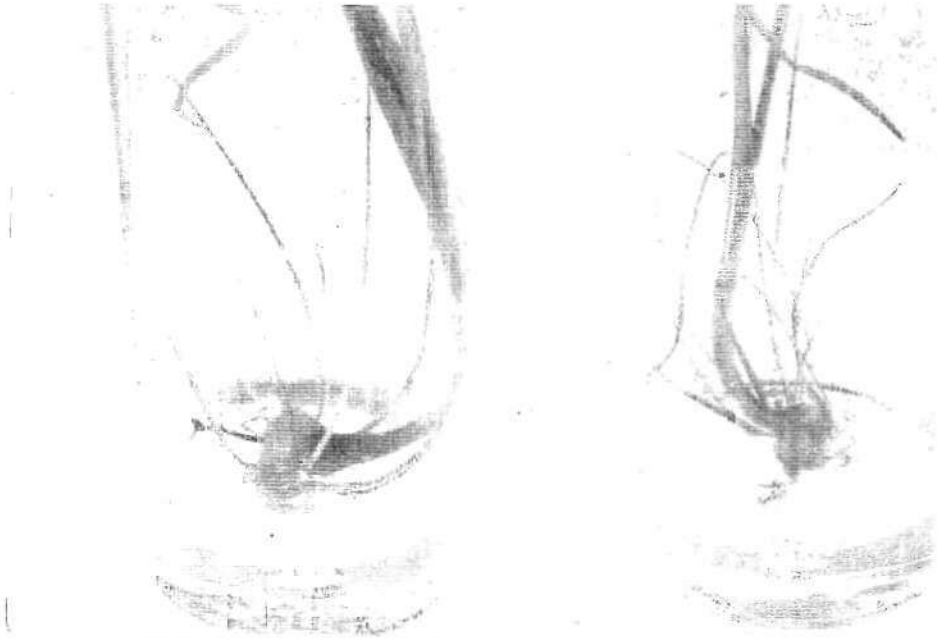

Fig. 15

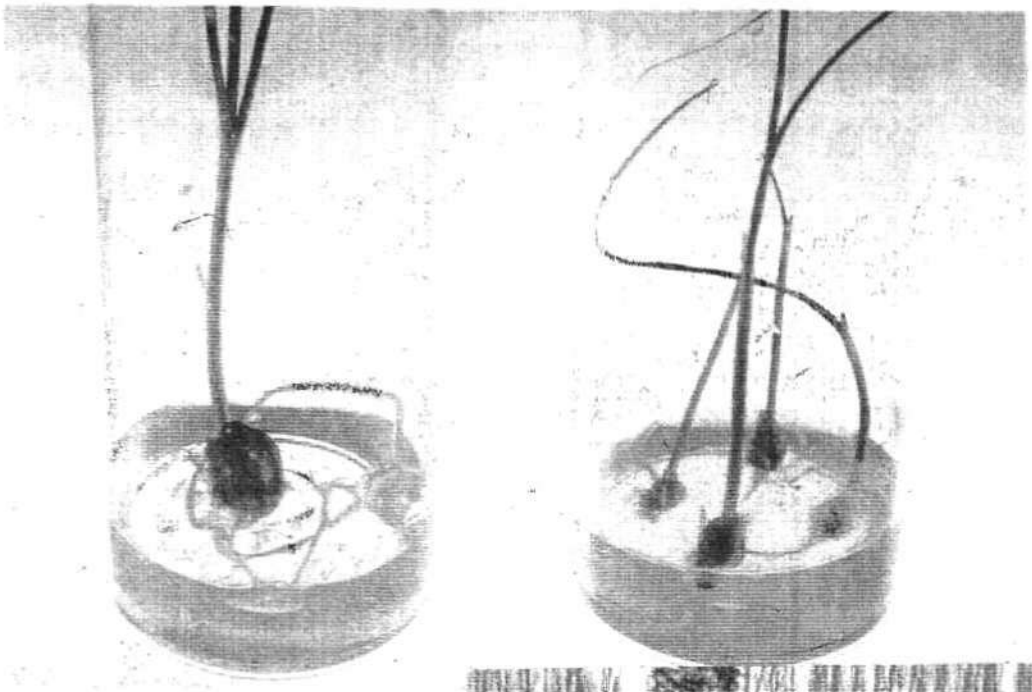

Fig. 16

Fig. 17

4. Appendix 1:

Technology Transfer Activities

- A range of formal and informal talks were presented to growers and industry representatives at field days and during visits to The Institute of Plant Sciences, Burnley.
- Talks were presented and articles prepared for growers at field days at Loxton (17 October, 1989) and Tooleybuc (30 May, 1990).
- Three articles were prepared and published in the "Crop Protection Bulletin".
- Papers were presented at scientific conferences including the Australasian Plant Pathology Conference, the National Conference of the Australian Society for Horticultural Science and the International Symposium on Alliums for the Tropics.

Publications arising from this program:

- Brennan, A.P. and Sward, R.J. (1989). Garlic Viruses; The Victorian Research Program. In : Proceedings of the Garlic Field Day and Seminars. Loxton Research Centre. South Australia. 17 - 18 October, 1989.
- Brennan, A.P. and Sward, R.J. (1989). Virus diseases in garlic : their control and elimination. Crop Protection Bulletin 5 Nov. 1989, 22 - 33.
- Sward, R.J. (1989). Virus disease in leek crops : A cause for concern. Crop Protection Bulletin. 4, Oct, 1989, 14-15.
- Sward, R.J. (1989). Leek Yellow Stripe Virus recorded in leek crops in South East Victoria. Australian Plant Virus Newsletter No. 4. pp. 1-2.
- Sward, R.J. (1990). Lettuce necrotic yellows rhabdovirus and other viruses infecting garlic. Australasian Plant Pathology 19 (2), 46-51.
- Brennan, A. P. (1991). Garlic - Harvesting and post-harvest handling. Crop Protection Bulletin 28 Nov. 1991, 22 - 23.
- Brennan, A.P. and Sward, R.J. (1991). A program to control viral disease in Australian garlic. In: Sustainable Management of Pest, Diseases and Weeds; Procs. of First National Conference, Australian Society of Horticultural Science. Macquarie University, NSW, 1-4 Oct 1991. 293-4.
- Brennan, A.P. and Sward, R.J. (1991) A program to control viral disease in Australian garlic. In Proc. of 8th Conference of the Australasian Plant Pathology Society. Sydney University, NSW. 8-11 Oct 1991.
- Brennan, A.P. and Sward, R.J. (1991). A program to control viral disease in Australian garlic. Poster presentation at the Australian Garlic Growers Association, Toolybuc, NSW. 15 Oct. 1991.
- Brennan, A. P. (1992). Garlic and the wheat curl mite. Crop Protection Bulletin 32 Apr. 1992, 12 - 14.
- Sward, R.J. and Brennan, A.P. (1993). Diagnosis and control of Allium virus diseases in Victoria, Australia. Acta Horticulturae 358, 295-8. Proc. of the International Symposium on Alliums for the Tropics, Bangkok, 19-24 February, 1993.

5. Appendix 2:

Copies of publications arising from this program

Number 358
March 1994

International Society for Horticultural Science in Cooperation with the Asian Vegetable Research and Development Center

AVRDC

International Symposium on Alliums for the Tropics

Editor
D.J. Midmore

Asian Regional Center - AVRDC
Bangkok, Thailand
15–19 February 1993

DIAGNOSIS AND CONTROL OF ALLIUM VIRUS DISEASES IN VICTORIA, AUSTRALIA

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Abstract

A number of virus diseases have been identified in garlic and leek crops in Victoria. In garlic, the three most common viruses identified are garlic yellow streak potyvirus, onion yellow dwarf potyvirus, and garlic latent carlavirus. A mixture of these viruses was purified and a polyvalent antiserum was produced. The antiserum was used to develop an ELISA which was used in conjunction with other conventional tests to rigorously index for viruses in tissue cultured plantlets produced following heat treatment and meristem tip culture. Plantlets that indexed negative for all known viruses of garlic formed the basis of a high health scheme that provides virus-tested material to industry. In leek, the only virus problem identified in Australia to date is leek yellow stripe potyvirus. The virus has caused disease problems in crops where cultural practices facilitate the spread of the virus from infected to healthy plants. Control has been achieved through stringent hygiene measures adopted as part of the cultural practices.

1. Introduction

Garlic (*Allium sativum*) is grown in a number of localities in Australia and all crops examined are infected with one or a number of viruses (Sward, 1990). Virus complexes occur in garlic wherever it is grown throughout the world, except for cultivars treated by meristem tip culture to eliminate viruses. The near-universal virus infection of garlic is a consequence of its method of propagation which is by vegetative means only, garlic being sterile. Thus, virus infection occurring in one generation is passed to all progeny in future generations. This has important consequences for any virus strategy control in garlic.

Leek (*A. ampeloprasum* var. *porrum*), on the other hand, is propagated mainly through seed and so does not suffer from the build up of viruses through the generations as observed in garlic. However, leek yellow stripe virus causes disease in certain leek crops because of its mode of transmission and certain cultural practices adopted by growers (Bos, 1982; Sward, 1991).

This paper describes the diagnosis of the viruses infecting these two members of the *Alliaceae* family and the varied strategies for effective virus control under the different cultural conditions required for each crop.

2. Materials and methods

2.1. Electron microscopy

Negatively stained, sap dip preparations of leaf pieces of garlic and leek were made using 2% w/v potassium phosphotungstate (pH 7.0) and were examined at a magnification of 40 000 times using an Hitachi H600S electron microscope.

2.2. Virus transmission tests of meristem-cultured garlic plants

Garlic leaf tissue was ground in 0.05 M sodium phosphate buffer, pH 7.2 and the extracts rubbed on carborundum-dusted leaves of *Chenopodium quinoa*, *C. amaranticolor*, *C. murale*, and *Allium cepa*.

2.3. Serological tests

Antisera to the following viruses were used to determine virus identity by the virus decoration technique of Milne and Luisoni (1977): garlic yellow streak virus (GYSV), kindly supplied by Dr. N. Mohamed (MAF, Lincoln, New Zealand); onion yellow dwarf virus (OYDV); leek yellow stripe virus (LYSV); and shallot latent virus (SLV) kindly supplied by Dr. L. Bos, The Netherlands; OYDV from Sanofi, France; and carnation latent virus (CLV) from Agdia, USA.

2.4. Virus purification and antiserum production

A polyvalent antiserum was prepared to a mixture of GYSV, OYDV and garlic latent virus (GLV) as it was not feasible to separate the viruses infecting the garlic source material (cultivar Mexican) within the time constraints of the project. The protocol for virus purification was as outlined by Mohamed and Young (1981). Antiserum to the Australian garlic virus complex (AGVC) was produced in a rabbit.

2.5. Virus indexing using ELISA

The double antibody sandwich ELISA of Clark and Adams (1977) was used with some modification to the extraction step as follows: samples were homogenized and diluted 1:20 with 0.05M tris-HCl, pH 6.8, plus 0.05M NaCl, 20mM Na_2 EDTA, 0.02% (w/v) Tween 20, and 3% (w/v) mannose prior to being strained through muslin. The addition of mannose reduced the high background values caused by the highly viscous and reactive sap inherent in *Allium* species (Sward and Brennan, unpublished). Routine ELISA indexing of garlic plantlets and plants derived following heat treatment and meristem culture was done with the AGVC antiserum. In a further set of tests, the AGVC antiserum was compared with the Sanofi OYDV antiserum to see if the same or a different series of viruses was detected. ELISA testing of leek samples was done using the LYSV antiserum.

3. Results

Sap-dip preparations of field-collected garlic samples examined in the electron microscope were mainly comprised of two classes of virus-like particles: flexuous filamentous potyvirus particles and shorter filamentous carlavirus particles. In one set of samples of cultivar Italian White, bacilliform or bullet-shaped rhabdovirus particles were observed. Sap-dip preparations of leek samples with yellow stripe symptoms contained typical potyvirus particles. Indicator plants inoculated with sap from plants derived from meristem cultures that indexed negative for all other tests did not show any symptoms.

Decorated and undecorated particles were observed in tests of infected garlic sap using GYSV and OYDV antisera. In tests using the AGVC antiserum, all particles were decorated, while no reaction occurred with the CLV, SLV, or LYSV antisera. Infected leek samples contained virus particles that reacted positively to the LYSV antiserum.

Purified garlic virus preparations examined in the electron microscope contained high concentrations of typical potyvirus and carlavirus particles.

In ELISA, an absorbance value greater than twice the mean of that of healthy plant material was regarded as a virus positive. The absorbance value for virus-infected garlic samples at a dilution of 1:20 in extraction buffer was in the range of 0.6-1.2, while the healthy garlic control value was 0.05-0.05 at this dilution. All commercially grown cultivars surveyed tested positive for virus with both ELISA systems. Infected leek samples gave an absorbance value of 0.3-0.4, and healthy leek grown from seed gave a value of 0.08-0.09. The set of methods developed for virus testing of garlic was used to confirm the presence or absence of viruses in plants and plantlets produced through heat

treatment and meristem tip culture. The treated garlic was tested at intervals throughout its growth cycle starting with freshly deflasked plantlets through maturity just prior to harvest. When only meristem culture was used, virus was eliminated from 38% of treated plants. When plants were heat treated for 42 days at 36°C and then meristem-cultured virus was eliminated from 68% of treated plants. Using the latter method, it was possible to eliminate virus from 10 selections of garlic required by Australian growers (Brennan and Sward, unpublished).

4. Discussion

The results of the various tests on Australian cultivars of garlic indicated that, most commonly, they were infected with a complex of three virus types, onion yellow dwarf potyvirus (Bos, 1976), garlic yellow streak potyvirus (Mohamed and Young, 1981), and garlic latent carlavirus (Lee et al., 1979). It is generally agreed that the garlic carlaviruses) are not responsible for the major mosaic virus symptoms usually observed (Bos, 1982), the allium potyviruses presumably being the primary cause. Conci et al. (1992) reported a carlavirus closely related to carnation latent virus (CLV) in garlic in Argentina. It has been determined here that the Australian carlavirus is not closely related to CLV. LYSV and SLV were not detected in any sample of garlic tested. Van Dijk et al. (1991) have recently defined a number of serologically distinct, mitebornе potyviruses. It is not known if these viruses are present in Australian garlic cultivars.

The bacilliform or bullet-shaped rhabdovirus particles present in a set of samples of one garlic cultivar were diagnosed by a range of serological and mechanical inoculation tests (Sward, 1990) as lettuce necrotic yellow virus (LNYV). This is apparently the first record of LNYV infecting garlic and emphasizes the fact that no one single test is satisfactory for objective virus testing. If a single test, such as ELISA, is used, there is a distinct possibility that an unusual virus or variant strain of a common virus may not be detected.

Due to the method of propagation of garlic which is by vegetative means only, and the presence of virus in all Australian cultivars, the only appropriate method for virus control is through initial virus elimination by meristem culturing of important cultivars and their subsequent maintenance in a pathogen tested (PT) scheme. PT or certification schemes have been used successfully since the 1930s to control viruses in vegetatively propagated crops (Ebbels, 1979). They are operated under a relatively standard set of stringent conditions. Elite, virus-tested material is maintained in screenhouses to exclude aphids that can transmit many of the viruses. Further generations are multiplied in areas chosen for their isolation or minimize the chance of reinfection, and careful roguing of any suspect plant is carried out. Regular indexing is undertaken to ensure that early generation material remains essentially free of virus. Commercial crops are planted with high health material arising from the PT scheme and although some virus reinfection may occur, a considerable yield advantage can still be expected compared to non-PT material.

Leek yellow stripe potyvirus is the only virus to be detected in Australian leek crops to date. LYSV is both aphid and mechanically transmitted, but is not seedborne (Bos, 1981). Thus, virus infection diagnosed in leek crops, all of which are grown from seed, has occurred during the same growing season. In the infected crops examined in southeast Australia, seedlings were being raised in seedbeds situated near mature crops, and aphids moving from the mature crops were transmitting LYSV into the seedlings. Prior to transplanting into the field, seedlings were being pulled in bunches and the roots and tops trimmed with a blade. This practice makes it easier to transplant the seedlings, but it also facilitates the sap transmission of virus from any infected seedling to most of the other seedlings in the same or subsequent bunches. The control measures recommended to growers include isolating seed beds from mature crops and insecticide spraying of seedlings for aphid control. The blade for trimming of seedlings should be dipped in disinfectant between bunches and plant with symptoms in the field crops should be removed so they do not remain as a potential source of infection for other

plants. If it is feasible, growers in a local area should be informed of the advantage of implementing a common period each year when leeks are not to be grown, in order to provide a break in the continuous cycle of virus reinfection.

Acknowledgment

The authors thank the Horticultural Research and Development Corporation and Biofresh Australia Pty. Ltd. for funding components of the garlic research presented here.

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SUSTAINABLE MANAGEMENT OF PESTS, DISEASES AND WEEDS

Australian Horticulture Clean and Green in the 90's

Proceedings

1st National Conference

Australian Society of Horticultural Science
Macquarie University, Sydney

September 30 - October 3 1991

A PROGRAM TO CONTROL VIRAL DISEASE IN AUSTRALIAN GARLIC (*ALLIUM SATIVUM*)

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Garlic yield and quality are significantly reduced by viral disease. Mosaic symptoms are typical of virus infection. However, yellow streaking, dwarfing and leaf distortion in the form of rolling and curling are common. Since garlic is sterile it is vegetatively propagated and consequently any virus infected plant will give rise to virus infected progeny. The aim of this project is to provide a continuous supply of pathogen tested planting material.

Surveys have established that the incidence of onion yellow dwarf potyvirus (OYDV), garlic yellow streak potyvirus (GYSV) and garlic mosaic carlavirus (GMV) in Australian grown garlic is high (Table 1).

These viruses are commonly transmitted by aphid species. Viruses were identified by particle morphology, decoration with specific antiserum, immunosorbent electron microscopy and mechanical transmission to herbaceous indicators. These three viruses were purified. An enzyme linked immunosorbent assay (ELISA) was developed and it's activity validated.

TABLE 1. Cultivars examined and found to be infected with OYDV, GYSV and GMV

Cultivar	Source
Californian Early	Virginia, South Australia Murray Bridge, South Australia Riverland, South Australia
Californian Late	Riverland, South Australia
Italian White	Riverland, South Australia Ancona, Victoria
New Zealand White	Marlborough, New Zealand
New Zealand Purple	Riverland, South Australia Sunraysia, Victoria
Mexican	Hay, New South Wales Riverland, South Australia
South African	Riverland, South Australia

A combination of heat treatment of tissue culture plantlets followed by meristem excision and culture was employed to eliminate viruses and successfully obtain virus tested garlic. Plantlets in tissue culture were tested using the ELISA and electron microscopy. Protocols were developed for rapidly multiplying this virus tested garlic in tissue culture. These resultant plantlets were then potted up in a glasshouse, re-indexed for virus and grown to maturity in a screenhouse. The next season the virus tested 'seed' cloves were planted in an '*Allium*-free', high altitude area with a low incidence of aphids while a proportion were further multiplied in tissue culture.

This project has enabled the development of assays, techniques and protocols that facilitate the production of virus tested garlic. It provides an environmentally sustainable strategy for disease management. A range of virus tested cultivars will soon be commercially available that should enable growers to improve their economic viability through obtaining increased yields of better quality garlic.

Lettuce necrotic yellows rhabdovirus and other viruses infecting garlic

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Abstract

Four viruses were detected in severely diseased plants of white Italian garlic from a property in central Victoria. The symptoms included curling, chlorosis, streaking and mosaic of the leaves and stunting of the whole plants. The rhabdovirus, lettuce necrotic yellows virus (LNYV), was one of the viruses isolated from the infected garlic but it was not clear if any specific symptoms could be attributed to it. This is the first record of LNYV infecting a monocotyledon. A probable carlavirus, similar in morphology to garlic latent virus (GLV), was observed under the electron microscope, but produced no symptoms in indicator plants. The potyvirus, garlic yellow streak virus (GYSV), was identified, and was accompanied by at least one other unidentified potyvirus, as undecorated, potyvirus-like particles were observed under the electron microscope alongside those decorated by GYSV antiserum.

Introduction

Virus diseases of garlic (*Allium sativum* L.) have been recorded from most areas of the world where garlic is grown (Brierley and Smith 1946; Ahlawat 1974; La 1973; Pons Iglesias and Ayuso 1982; Lastra *et al.* 1979; Messiaen and Amiour 1960; Mohamed and Young 1981; Lee *et al.* 1979). In most instances, total crop infection with a complex of viruses has been reported. This results because multiple virus infections occur over many years and all garlic is propagated by vegetative means only, the plants being sterile and not producing fertile seed. Virus-like particles most commonly found in garlic are filamentous and range in length from 600 to 650 nm, similar to those of the carlavirus group (Cadilhac *et al.* 1976), or flexuous, filamentous and about 750 nm long, similar to those of the potyvirus group (Mohamed and Young 1981).

Garlic is grown in a number of localities in Australia and all crops examined have shown symptoms of virus infection. However, there are no published reports on the identity of viruses in Australian garlic.

Plants of white Italian garlic from a property at Gladstone in Victoria were severely affected, and leaf curling, chlorosis and stunting of whole plants were observed in addition to the more commonly observed symptoms of leaf streaking and mosaic (Figure 1). This report describes the viruses identified in this severely affected crop and discusses some of the implications of these findings.

Methods

Electron microscopy Negatively stained, sap-dip preparations of leaf pieces of eight infected plants were made with 2% w/v potassium phosphotungstate (pH 7.0).

Tissue pieces from areas of leaf showing symptoms were fixed at room temperature (20°C) in 3% (v/v) glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.0) for 4 h with the final 30 min of this period under vacuum. They were then washed for 2 h at 20°C in three changes of buffer, post-fixed in 2% w/v osmium tetroxide in sodium cacodylate buffer for 1 h at 20°C, washed for 30 min in three changes of buffer, dehydrated for 1 h through a graded ethanol series followed by 20 min in three changes of acetone. This was followed by three changes of Spurr's resin (Spurr 1969) and acetone (1:1) for a total period of 20 min and then by four changes of pure Spurr's resin for a total period of 24 h. The resin was polymerized at 68°C for 12 h. Sections were cut on an LKB Ultratome III and stained for 15 min in a saturated solution of uranyl acetate in 40% (v/v) ethanol and then for 5–7 min in 0.05% (w/v) lead citrate in 0.02M sodium hydroxide (Venable and Coggeshall 1965). Sections were examined in an Hitachi H600S electron microscope at 75 kv.

Virus transmission tests Leaf tissue was mashed in 0.05M sodium phosphate buffer, pH 7.2 and the extracts rubbed on carborundum-dusted leaves of *Nicotiana glutinosa* L., *N. clevelandii*, A. Gray., *N. tabacum* L., *N. rustica* L., *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyne., *C. murale* L., *Cataceum annuum* L., *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicon esculentum* L., *Lactuca sativa* L., *Allium cepa* L., *A. porrum* L., and *Zea mays* L. At least eight plants of each species were inoculated with plant extract and two plants of each species were rubbed with buffer only. A second round of testing was done on 50 seedlings of lettuce (*L. sativa*) by the method described above.

Serological tests Antiserum to garlic yellow streak virus (GYSV), kindly supplied by Dr N. Mohamed (MAF, Lincoln, New Zealand), was diluted 1:100 in 0.05M sodium phosphate buffer, pH 7.0. Clarified sap extracts were dried onto electron microscope grids and the grids were then inverted and floated on the diluted antiserum for 15 min at 20°C. The grids were washed three times in distilled water, then negatively stained with 2% w/v potassium phosphotungstate (pH 7.0), dried and examined in the electron microscope for antiserum decoration of virus particles.

Dr J.W. Randles (Waite Institute, South Australia), kindly applied serological tests for lettuce necrotic yellows virus (LNYV) on samples from Victoria. Test samples were leaf of *N. glutinosa* with either mild or severe systemic symptoms. Double diffusion tests (Randles and Carver 1971) were done with antiserum against the SE3 isolate of LNYV (Stubbs and Grogan 1982; McLean *et al.* 1971).

Figure 1 Garlic leaf showing severe mosaic and streaking.

Figure 2 Sap-dip preparation of infected garlic showing a potyvirus (P) and rhabdovirus (R) particles. Negatively stained in PTA. Bar marker = 100 nm.

Figure 3 Sap-dip preparation of infected garlic showing carlavirus (C) and potyvirus (P) particles. Negatively stained in PTA. Bar marker = 100 nm.

Figure 4 Sap-dip preparation of infected garlic showing scrolled inclusion bodies (SI) typical of a potyvirus infection. Negatively stained in PTA. Bar marker = 100 nm.

Figure 5 Ultrathin section of infected garlic showing scrolled (SI) and pinwheel (PIN) inclusion bodies within the cytoplasm of a mesophyll cell. Bar marker = 100 nm.

Figure 6 *Nicotiana glutinosa* plants 4 weeks post inoculation: control, healthy (left), infected with mild LNYV isolate (middle) and severe LNYV isolate from garlic (right).

Figure 7 *N. glutinosa* inoculated with sap extract from garlic. Large membrane-bound clumps of particles typical of LNYV were observed in transverse (TS) and longitudinal section (LS) in the cytoplasm. Bar marker = 100 nm.

Figure 8 Sap-dip preparation of infected garlic, showing potyvirus particles decorated with antiserum to GYSV (arrowheads). Undecorated potyvirus particles are also present (arrowhead). Negatively stained in PTA. Bar marker = 100 nm.

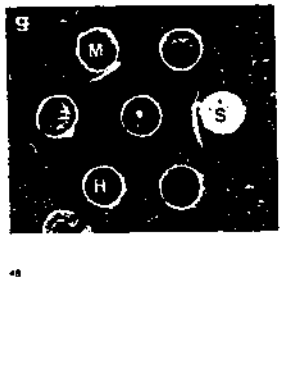

Figure 9 Double gel-diffusion test done by Dr J.W. Randles (Waite Institute, SA) with antiserum prepared to the SE3 isolate of LNYV (centre well) and the two rhabdovirus isolates from garlic inoculated into *N. glutinosa*. Precipitin lines indicate a positive reaction. M = mild, S = severe isolate from garlic, H = healthy *N. glutinosa*.

Results

In sap-dip preparations of each garlic plant examined in the electron microscope, three classes of virus-like particles were observed: flexuous filamentous particles with a mean length of 750 nm (range 700–800 nm), shorter filamentous particles with a mean length of 600 nm (range 550–650 nm), and either bacilliform particles approximately 60 nm × 230 nm or bullet-shaped particles, 60 nm diameter and of various lengths up to 330 nm (Figures 2 and 3). Scrolled inclusion bodies were also observed (Figure 4). In ultrathin sections, the most obvious features were scrolled and pinwheel inclusion bodies throughout the cytoplasm (Figure 5).

Of the plants mechanically inoculated with sap from infected garlic plants, only four species developed symptoms. Two *C. murale* plants developed a few chlorotic local lesions on inoculated leaves only. *D. stramonium* developed systemic interveinal chlorosis and *N. glutinosa* and *N. clevelandii* developed two classes of symptoms, mild and severe, depending on the garlic source plant. The mild symptoms consisted of a systemic mosaic and downward rolling of the younger leaves 20 days post inoculation. The more severe symptoms consisted of local necrotic lesions on inoculated leaves 14 days post inoculation, followed by systemic veinal necrosis progressing to become lethal in most leaves and leaving a few severely rolled leaves in a distorted bunch at the apex of the stem (Figure 6).

Virus-like particles were not observed in sap-dip preparations of those species that did not develop symptoms. In *C. murale* plants showing symptoms, 750 nm filamentous particles were present in local lesions.

In each of the other three species where symptoms were apparent, rhabdovirus-like particles were observed in sap-dip preparations. In fixed and sectioned preparations of *N. glutinosa*, typical rhabdovirus particles were observed in transverse and longitudinal section in the cytoplasm, accumulating in vesicles associated with the endoplasmic reticulum (Figure 7).

In sap preparations from garlic and *C. murale*, where each was treated with GYSV antiserum, decoration of the 750 nm particles was observed under the electron microscope. Undecorated particles of 750 nm were also present in the garlic, but not in *C. murale* (Figure 8).

Specific precipitin bands were observed in the double diffusion test in agar when both mildly infected and severely infected *N. glutinosa* material was tested against LNYV antiserum (Figure 9).

Discussion

The results of the various tests on this particular crop of garlic indicate that it was infected with a complex of at least four virus types.

As well as the flexuous, filamentous particles ranging from 700–800 nm in length observed by electron microscopy, there were scrolled and pinwheel inclusion bodies indicating these viruses belonged to the potyvirus group (Hollings and Brunt 1981). The local lesions observed on *Chenopodium murale* indicator plants, the lack of symptoms on *C. quinoa* and *C. amaranticolor*, and the massive decoration of virus particles with antibodies to garlic yellow streak virus, indicate that GYSV was one of the viruses present. Mohamed and Young (1981) found that this was the main virus affecting the varieties of garlic that they examined in New Zealand. However, in these same preparations examined under the electron microscope, a proportion of the particles of approximately the same length remained undecorated. It is probable that another or a number of other potyviruses were also present. Dellacecle and Lot (1979) considered that the potyvirus they observed in France was onion yellow dwarf virus (OYDV), which, had it been present in the Victorian sample, should have induced symptoms on onion (*Allium cepa*). Similarly, leek yellow stripe virus (LYSV), another potyvirus which is reported to infect other *Allium* species (Bos 1982), should have induced symptoms on leek (*A. porrum*). *C. quinoa* and *C. amaranticolor* had it been present, it is likely, therefore, that another as yet unidentified potyvirus was present. Clarification of this will require further research.

The shorter, filamentous particles, approximately 600 nm in length, were typical of members of the carlavirus group (Koenig 1982). Cadilhac *et al.* (1976) detected carlavirus-like particles in French garlic and named the virus garlic mosaic virus (GMV), whereas Lee *et al.* (1979) detected similar particles in Japanese garlic and named it garlic latent virus (GLV). Lee *et al.* (1979) also reported that GLV produced local lesions on *C. amaranticolor* and *C. quinoa* and had no serological relationship with carnation latent virus and chrysanthemum virus B. It is generally agreed that the garlic carlaviruses are not responsible for the major mosaic virus symptoms usually observed (Bos 1982), the *Allium* potyviruses presumably being the primary cause. The viruses present in the Victorian samples, unlike the Japanese isolate, induced no local lesions on either *C. amaranticolor* or *C. quinoa*.

The bacilliform and bullet-shaped particles observed in sap-dip preparations and ultrathin sections were typical of rhabdoviruses. The symptoms induced on *N. glutinosa*, *N. clevelandii* and *D. stramonium* were typical of LYNV (Francki and Randles 1970). The fact that no sap transmission to lettuce occurred in either the first or second round of testing was consistent with the finding of Stubbs

and Grogan (1963) '. . . that the virus is sap transmissible to lettuce only with great difficulty'. The two classes of symptoms induced on *N. glutinosa* and *N. clevelandii* were typical of the mild and severe (SE3) isolates described by Stubbs and Grogan (1963). Electron micrographs of fixed and sectioned *N. glutinosa* clearly showed accumulations of the rhabdovirus particles in membrane-bound vesicles associated with the endoplasmic reticulum. This is typical of sub-group I of the plant rhabdoviruses (Peters 1981) of which LNYV is the type member. The results of the double diffusion test in agar further supported the contention that these two rhabdovirus isolates from garlic were in fact LNYV. This was later confirmed by Dietzgen and Francki (1988) in an analysis of the structural proteins of the severe LNYV isolate from garlic, to which they produced specific monoclonal antibodies. This is a very interesting finding in itself, as Francki and Randles (1980) stated that 'to our knowledge, no true rhabdovirus is known to have hosts among both dicotyledons and monocotyledons'. This is the first record that LNYV can infect species from both plant groups.

LNYV is mostly spread in nature by the aphid, *Hyperomyzus lactucae* L., which colonises sowthistle (*Sonchus oleraceus* L.) and spreads the virus to secondary host species such as lettuce. Sowthistle is widespread in Victoria and possibly acted as a source of infection for the garlic. At this stage, it is not known if the LNYV is inducing any specific symptoms in garlic as all the garlic examined was also infected by the complex of filamentous viruses. However, it did appear that these samples displayed more severe leaf curling, chlorosis, and stunting than other crops of locally grown, Italian garlic in which LNYV was not detected.

Yield losses caused by the individual viruses or by the virus complexes infecting garlic are impossible to calculate unless 'virus-free' material can be produced, inoculated, and grown in replicated trials with 'virus-free' plants. Quiot *et al.* (1972) selected by visual examination what they regarded as a virus-free French variety and in field trials estimated the yield loss due to virus to be 27%–35%. Messiaen *et al.* (1981) estimated yield losses of up to 50% in comparisons of inoculated and non-inoculated plants of the French cultivar Thermidor. More recently, Walkey and Antill (1989) found that the mid-season leaf growth, clove size and yield of bulbs was substantially increased (33%–56% yield increase) in five cultivars for which they compared virus-free and virus-infected stocks. Further losses due to virus infections in garlic may also occur as a result of reduced longevity of the bulbs in storage. This has been shown to be the case with other *Allium* species such as onion infected with OYDV (Graichen 1978) and leek infected with LYSV (Bos *et al.* 1978).

Acknowledgements

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Leek yellow stripe virus recorded in leek in Australia

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Abstract

Leek yellow stripe virus in leek was identified by morphology of the virus particles, enzyme-linked immunosorbent assay, decoration with specific antiserum, and mechanical transmission to healthy leek seedlings. Although related potyviruses have been identified in other *Allium* species, this is the first confirmed record of leek yellow stripe virus in Australia.

Introduction

Garden leek (*Allium porrum* L.), a cultivated form of *A. ampeloprasum* L. (McCollum 1976), is grown commercially in Victoria, primarily in an area south-east of Melbourne. In August 1989, plants collected from three properties had disease symptoms including wilting, necrotic or chlorotic streaks (Figure 1) and a general grey lustreless colour.

No pathogenic bacteria or fungi were isolated, but virus-like particles were observed in all sap dip preparations examined in the electron microscope. The virus-like particles were flexuous filaments approximately 800–820 nm in length (Figure 2) resembling leek yellow stripe virus (LYSV) (Bos 1981).

Methods

Serological testing was undertaken using antiserum to LYSV. Virus decoration tests were done by crushing a leaf piece in distilled water (approximately 1:1), placing the extract onto a formvar/carbon-coated grid, draining and drying. The grids were then treated with antiserum diluted 1:100 in 0.05 M potassium phosphate buffer, pH 7.5, for 45 min at room temperature. They were then washed with distilled water, stained with 2% potassium phosphotungstate (pH 7.0), drained, dried and viewed with an Hitachi H 600-S electron microscope. Controls consisted of leaf material from healthy leek seedlings processed as above and a serum control in which rabbit pre-immune serum was substituted for the anti-LYSV serum. Positive decoration was observed only in infected test samples with all particles being decorated (Figure 3).

Double antibody sandwich ELISA (Clark and Adams 1977) was used to further test the infected samples. Paired wells were used in a Dynatech Immulon (M-129 B) plate. The extraction buffer was modified by the addition of 5% w/v mannose to overcome the highly viscous and reactive sap that typically causes high background values with ELISA of *Allium* species (Sward and Brennan, unpublished). The plates were coated with antibody at a dilution of 1 μg/mL and the alkaline phosphatase-antibody conjugate was used at 1:500. The substrate p-nitrophenyl phosphate was used at 1 mg/mL. Absorbance readings at 405 nm were made with a Titertek Multiskan plate reader blanked on wells with substrate only.

Results

An A_{405} value greater than twice the mean of the four healthy leek control wells was regarded as a virus positive. The mean A_{405} value for test samples at a dilution of 1:10 in extraction buffer was 0.367 and at 1:50 was 0.335. The mean healthy leek control value at a dilution of 1:10 was 0.076.

Leaf material from an infected field plant was ground in a mortar with 0.1 M sodium phosphate buffer (pH 6.8) and the sap extract was inoculated to 20 healthy leek seedlings. Five leek seedlings were rubbed with buffer only. After 8 weeks, 4/20 test plants developed symptoms similar to those observed in the field (viz. wilting, chlorotic streaks and a general grey lustreless colour) and the presence of particles typical of LYSV was confirmed in the electron microscope. Plants treated as buffer controls did not develop symptoms and did not contain LYSV particles.

Discussion

LYSV, a member of the potyvirus group (Bos 1981), has not been previously reported in Australia, although in other species of *Allium*, related potyviruses such as garlic yellow streak virus in garlic (Sward 1990) and onion yellow dwarf virus in onion (McLean 1985) have been reported. The only other viruses reported to infect leek naturally are shallot latent carlavirus (Bos *et al.* 1978) and tomato blackring nepovirus (Calvert and Harrison 1963). Neither of these viruses is serologically related to LYSV.

In addition to the symptoms described above, infected plants may have a lower moisture content and may weigh up to 54% less than uninfected plants (Graichen 1978), keeping quality may be drastically reduced, and there is usually a greatly increased predisposition of overwintering crops to secondary rotting (Bos *et al.* 1978). Infected plants produce less seed and germination can be reduced by as much as 15% (Graichen 1978).

A major epidemic of LYSV developed in the Netherlands in 1972 as a consequence of the introduction of year-round cultivation of leek crops (Bos 1982). Earlier epidemics had been reported in West Germany with 100% of plants infected (Kupke 1957). LYSV rarely infects other species of *Allium* and is symptomless in crops such as onion (Bos 1982).

The virus is spread naturally by certain aphid species such as the green peach aphid, *Myzus persicae*, and by mechanical means (Bos 1981). Both these modes of transmission could have been responsible for the Victorian epidemic. Seedlings are raised in seed beds sited near old, infected plants, and could be infected by aphids. Prior to transplanting, seedlings are pulled in bunches and the roots and leaves trimmed without disinfection of secateurs between each bunch, and could thus be exposed to mechanical infection. LYSV is not seed transmitted, but aerial bulbils from infected plants are a source of infection (Bos *et al.* 1978) and may be a means by which LYSV is moved over large distances and possibly entered Australia.

Figure 1 Disease symptoms associated with leek yellow stripe virus (LYSV) in leek. Healthy leaf (left) compared with infected leaf (right) showing typical chlorotic stripes.

Figure 2 Sap dip preparation of infected leek showing typical LYSV particles. Negatively stained in PTA. Bar marker = 200 nm.

Figure 3 Sap dip preparation of infected leek showing virus particles decorated with antiserum to LYSV. Negatively stained in PTA. Bar marker = 200 nm.

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Garlic and the Wheat Curl Mite

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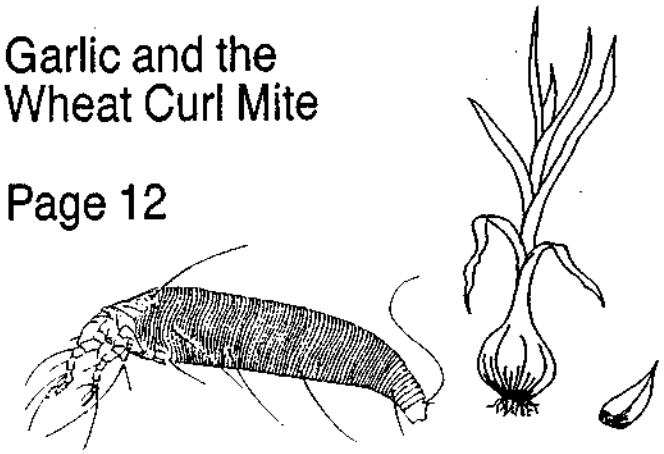

Institute of Plant Sciences
Working Towards Cleaner, More Cost-Efficient Agriculture

GARLIC AND THE WHEAT CURL MITE

Introduction

The incidence of wheat curl mite in garlic samples, presented to the IPS diagnostic services laboratory, has increased dramatically over the past 18 months. This article highlights the problem, outlines the biology of the mite and explains the control and eradication measures.

The Pest and Problem

The wheat curl mite, *Aceria tulipae* (Keifer), also known as dry bulb mite and sometimes simply as garlic mite, was first recorded in Australia in July 1980, colonising wheat near Melbourne. In New Zealand, North America and Europe, it is frequently found infesting wheat. The mite is a known vector of wheat streak mosaic virus which has not been recorded in Australia.

Figure 1.

Since 1980, wheat curl mite has been found in garlic bulbs in Victoria and New South Wales. Feeding by large numbers of wheat curl mites amongst the leaf sheaths of garlic plants can cause twisting, stunting and delayed unfolding of young leaves. This condition is known as "tangle top" (Figure 1).

Frequently mites only become a recognised problem post-harvest as they cause the bulbs to dry out during storage. Infested cloves usually show some tissue breakdown in the form of one or more sunken brown spots.

In addition to direct feeding damage, the wheat curl mite has been shown to transmit virus particles which produce mosaic symptoms in garlic. Virus infection in garlic has been proven to substantially lower yield.

Biology

Wheat curl mite belongs to a family of mites commonly known as eriophyid mites. They differ from other mites in that they only have two pairs of legs instead of four pairs and the legs are found at the "head end" of the body (Figure 2). Wheat curl mite is a tiny, white, worm-like mite about 0.25 mm long, too small to be seen with the naked eye (Figure 2). Even with a strong magnifying glass, it can be difficult to see it against the white fleshy background of a garlic clove.

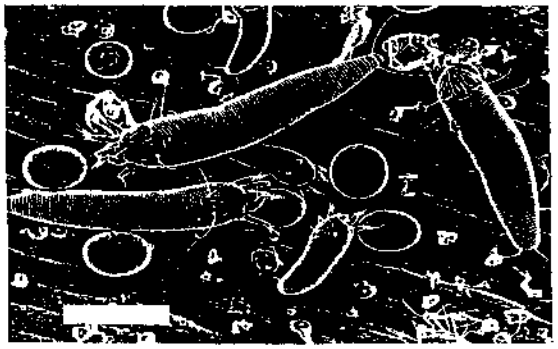

Figure 2.

The mite can complete its full life-cycle on the garlic bulb. The life-cycle consists of an egg, two nymphal stages and an adult. The almost circular eggs are colourless and about 0.6 mm in diameter. The two nymphal or immature stages are similar in appearance to the adults. One female can lay about 12 eggs and the complete life-cycle from egg to egg takes 8-10 days at 24-27°C. Mites can also survive temperatures as low as 4°C, but the time taken to complete the life-cycle increases.

Control

Presently there are no chemicals registered for the control of this pest in the field. Overseas, dusting of the leaves with sulphur, or spraying the leaves with wettable sulphur, mevinphos, chlorobenzilate or a mixture of parathion and endrin have met with varying degrees of success. In the field, the mites are extremely difficult to control as they get into the folds of the leaves which offer them protection from chemicals.

In some countries, post-harvest fumigation with methyl bromide is recommended for the control of mites on bulbs. However, this chemical is an extremely toxic substance and therefore a reliable pest control contractor should be engaged.

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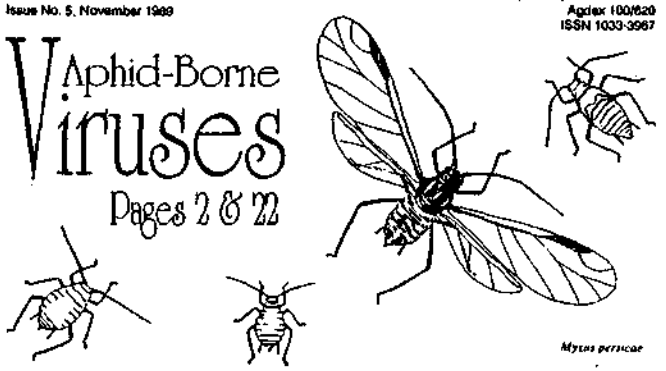
Aphid-Borne Viruses

Pages 2 & 22



Myzus persicae

Plant Research Institute, Burnley

Working Towards Cleaner, More Cost-Efficient Agriculture

VIRUS DISEASES IN GARLIC; THEIR CONTROL AND ELIMINATION

Australian Garlic Industry

The Australian garlic industry is small by world standards, and much more garlic is imported than produced locally. Australian production is estimated at 1000 tonnes annually compared with world production estimated at over 2 million tonnes in 1981. The estimated value for Australian garlic in 1989/90 is $10.5 million. Processing accounts for 250 tonnes, about 200 tonnes is kept for seed and the remainder is for the fresh market.

The major areas of production are the Murrumbidgee Irrigation Area, NSW and the Riverland, SA. In most cases, yields in Australia are well below the world average.

Effects of Virus Infection

Viruses are common in commercially grown garlic in Australia; at least 8 viruses are known to infect garlic. The most obvious symptoms produced by viruses in garlic are colour changes in the leaves. Leaves often show yellow streaks, mosaics or a general chlorosis. These symptoms may be similar to those produced by nutrient deficiencies. Symptom intensity and expression may also vary with weather conditions.

Leaves can also be severely distorted by curling and rolling. These distortions are more noticeable in the immature plant.

The reduction in growth and yield exhibited by infected plants is economically important. Infections of garlic by more than one virus can intensify symptoms and cause substantial yield losses. Trials in the UK have shown yield improvements of up to 88 per cent for virus-tested garlic of certain cultivars.

Virus Spread

Plant viruses can be spread in a number of ways. Garlic, a sterile plant, is propagated vegetatively by dividing bulbs into individual cloves, hence, viruses can easily be disseminated.

Sucking or chewing insects, such as aphids or leafhoppers, may transmit a range of plant viruses. The green peach aphid (*Myzus persicae*), for instance, is a demonstrated vector of onion yellow dwarf virus and garlic yellow streak virus. Various soil dwelling fungi and nematodes can also transmit certain viruses.

Viruses can also spread by mechanical means. This can occur from damage inflicted by machinery and tools that have been in contact with infected material. Also, the cut surface of a leaf of an infected plant contacting the cut surface of a healthy plant may result in virus transmission.

Virus Control

Once a plant or crop in the field is infected by a virus it cannot be cured. Control measures for virus diseases must be preventative.

Insecticides can sometimes be used to control aphid vectors. Weeds that harbour vectors should be controlled. Hygiene is important with regard to both workers and equipment. Work should not be performed on uninfected crops soon after contact with infected plant material.

In certain crops, some virus control can be achieved by early roguing of infected plants or planting away from infected sources. However, in garlic where crops are 100% infected these measures are not applicable. Ideally, garlic should be propagated from virus-tested stock.

Certified Virus-Tested Garlic

Research is being conducted at PRI, Burnley on the viruses infecting garlic. Rapid and sensitive diagnostic tests are being developed for these viruses. Meristem tip dissections and tissue culture techniques followed by stringent virus-testing of cultured plants are being used to produce a range of virus-tested cultivars of garlic.

This improved material will be made available through Biofresh Australia Pty. Ltd., a company owned jointly by DARATECH and agribusiness people from NE Victoria.

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Department of Agriculture

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Armyworm & Budworm Pages 1 & 4

Part A: Field Crops, Pastures and General Articles

Part B: Fruit, Vegetable and Ornamental Crops

Institute of Plant Sciences
Working Towards Cleaner, More Cost-Efficient Agriculture

GARLIC - HARVESTING AND POST-HARVEST HANDLING

Growing high quality garlic is a labour intensive operation, however, great rewards can be achieved through hard work, knowledge and care. Firstly, it is important to select the appropriate cultivar from a reputable source. Fungal, bacterial and viral diseases will drastically reduce the quality of any cultivar. Presently all Australian garlic is infected with virus. Viral disease significantly reduces crop yield. A virus elimination program at the Institute of Plant Sciences, Burnley, has produced virus-tested garlic which should be commercially available within two years. Once source material is suitably established, the treatment of the crop is critical. The following aspects are worth considering.

Fertiliser

Nitrogen fertiliser should not be applied to the crop after bulb initiation which usually occurs from September to October.

Irrigation

The amount of irrigation needed is dependent on rainfall and soil type. The crop must not be stressed for water from late winter onwards as this is the time of heavy foliage growth. The last irrigation should be 5–10 days before harvest, depending on soil type.

Harvest

The time to harvest will depend upon location, climate, the soil type and the cultivar grown. For example, cv. Californian Early is usually 3–4 weeks earlier than cv. Californian Late. Indicators that the crop is ready for harvest include the bulb being fully formed and the neck above the bulb beginning to soften and the tops starting to dry. Representative plants should be pulled up and cut open midway through the bulb for inspection. At maturity the cloves should be well formed, there should be air spaces between the cloves and at least three skins on the bulb. Quality is often determined by the number of skins on a bulb. The plants should still be in a green but mature state. Many crops are harvested one to two weeks too late and therefore quality is reduced.

Traditionally, garlic is hand harvested although mechanisation is becoming more prevalent. A blade knife or rodweeders attached to deep rippers on a tractor can be used to loosen the soil and cut the roots. Ideally the garlic should be lifted as soon as it is cut. In dry arid climates, however, it can be left in the ground for a week or two, providing it does not rain. Rain at this stage can spoil the crop. Lifting is also labour intensive and it is therefore best to have ten or more people working per hectare at this stage.

Drying

The crop should be cured as soon as possible after harvest. As mentioned above, in dry arid climates, the plants can be left lying in the field for 10–14 days with the leaves of one plant lying over the bulb of another in order to provide protection from sun scald.

Crops can also be cured in a shed by spreading the crop over the floor and providing adequate ventilation. In countries such as New Zealand, artificial driers set at 34°C provide ideal drying conditions. Tops should not be removed for several weeks if the crop is destined for the fresh market. Early removal of tops will lessen storage life and bulbs may shrivel. Eventually plants are "topped and tailed" 20–50 mm above the bulb.

After harvest it is normal for bulbs to lose moisture. This loss can be almost 20% of weight in the first month but less in subsequent months. It is not uncommon for an overall weight reduction in the order of 30% to occur.

After drying, the bulbs are cleaned, trimmed of all loose skins, graded and packed ready for sale. This again is often done by hand although mechanisation is speeding up this process.

Storage

Given the correct conditions garlic will store very well for at least 6 months. Good ventilation is important to prevent post-harvest rots and overheating of the bulbs. A high relative humidity (> 70%) should be avoided. Most growers simply store their crop in well ventilated sheds.

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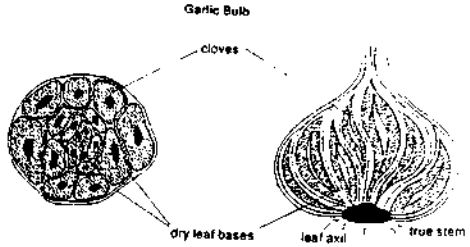

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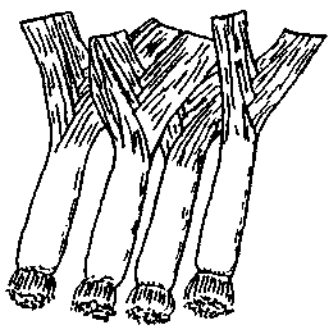
Plant Research Institute, Burnley
Working Towards Cleaner, More Cost-Efficient Agriculture

VIRUS DISEASE IN LEEK CROPS: A CAUSE FOR CONCERN



Recently, samples of diseased leeks, *Allium ampeloprasum*, have been submitted to PRI, Burnley for diagnosis and advice. The samples have had a variety of symptoms including wilting, necrotic or chlorotic streaks and a general grey lustreless colour.

CAUSAL ORGANISM:

Leek yellow stripe virus (LYSV) has been diagnosed in all affected samples and is apparently the primary cause of the observed symptoms. LYSV, a member of the potyvirus group, has not been previously reported in Australia, although related potyviruses have been reported in other species of *Allium* such as garlic and onion.

An explosive epidemic of LYSV developed in the Netherlands in 1972 as a consequence of the introduction of year-round cultivation of leek crops. Earlier epidemics had been reported in West Germany with 100% of plants infected. LYSV rarely infects other species of *Allium* and is symptomless on crops such as onion.

SPREAD:

The virus is spread by certain aphid species such as the green peach aphid, *Myzus persicae*, and by mechanical inoculation (infected cut surface contacting the cut surface of a healthy plant). Both these modes of transmission are apparently responsible for the local epidemic. Seedlings are raised in seed beds sited near old, infected plants and prior to transplanting, seedlings are pulled in bunches and the roots and leaves trimmed without disinfection of secateurs between each bunch. LYSV is not seed transmitted, but aerial bulbils from infected plants are a source of infection and may be a means by which LYSV is moved over large distances.

EFFECTS:

The visible symptoms of LYSV on the plant are described above, but less obvious and more important effects are also usually present. The infected plants have a lower moisture content and weigh less than uninfected plants (yield reductions of up to 34% have been reported), keeping quality may be drastically reduced and there is usually a greatly increased predisposition of overwintering crops to secondary rotting. Infected plants produce less seed and germination can be reduced by as much as 15%

CONTROL:

Measures to control virus diseases must be preventative. Once a plant or crop is virus-infected it cannot be cured. Sources of infection should be avoided or removed, virus spread should be reduced or prevented if possible and plant resistance increased.

The only source of LYSV is apparently older infected leeks grown nearby. Therefore, it is important to isolate seed beds and have them as far as practicable from older crops. Rogoing of infected plants is recommended, particularly in spring and early summer. LYSV is prevalent only in areas with year-round cultivation of leeks. In some European countries, growers have adopted the practice of a "leek free" period to break the cycle of infection, however, this must be adopted by all growers in a district to be successful.

Control of aphids in the seed beds may limit the early and often rapid spread of virus from plant to plant. A number of insecticides are registered for general aphid control on vegetables including dimethoate (withholding period (WHP) 7 days), malidson (WHP 3 days) and pyrethrum (WHP 1 day).

Disinfection of secateurs or cutting implements used to trim the pulled bunches is recommended. A solution of 2% sodium hypochlorite, made up fresh each day, should be used to disinfect the secateurs between trimming each bunch. Growers can prevent the corrosive action of the sodium hypochlorite on tools by dipping them in a mixture of vinegar, water and emulsifiable oil after finishing each session of trimming.

Robert Sward
PRI, BURNLEY

MINUTES OF THE AUSTRALIAN GARLIC INDUSTRY ASSOCIATION

MEETING HELD AT TOOLEYBUC ON 30TH MAY 1990

Present: John Salvestrin, Andrew Ward, Richard Elliott, Alan Brennan, Greg Ajanz, Chris Bennett, Leon Caccaviello, M. G. Caccaviello, Phil Ward, Heather Rundell, Kevin Coughlin, Graeme Stewart, Sam Mancini, J. Angus Lawson, John Sutherland, Roger Schmitke, Raelene Schmitke, Gerry Kelly, Peter Wynne, Jeff Wynne, Peter Hackett, Richard Blennerhassett, Steven Freckleton, Cindy Freckleton, Dilich Enderson, Hanna Muatonen, Gail Wilson, Geoff Hobson, Ian Boyd, Ivan Dettloff.

Apologies: Phil Bennetts, Chris Durkin, Richard Stott, Peter Halett, John Scarvelis, Ken Johnson, Chris Ball, Shirley Sylvin, Robert Horsefall, Valda Horsefall, Jim Geltch, Michael Schultz, Martin O'Laughlan.

Minutes: Moved John Salvestrin, seconded Geoff Hobson that the minutes be accepted as a true and correct record.

Carried

Treasurer's Report: $296.85. It was agreed that accounts should be sent out to members for the 90/91 season, payable in October/November 1991. Also that State Associations are to inform the Australian Garlic Industry Association of current membership.

It was moved by John Sutherland, seconded by Dick Henderson that the current list of membership be sent to the secretary by the States and be updated before the Annual General Meeting in 1990.

Carried

Correspondence: Outgoing:

Letters were sent to numerous organisations requesting comment on standards that should be set for garlic sold as fresh produce in Australia.

Letter to Dr. John Radcliffe of commendation for the work of Shirley Warriner and Dick Henderson on the work they have carried out in the Association.

GARLIC VIRUSES: VICTORIAN RESEARCH PROGRAM

A.P. Brennan and R.J. Sward
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This project is being carried out under contract to Biofresh (Australia) Pty Ltd. A matching grant from the Horticultural Research and Development Corporation is also acknowledged.

Garlic is a demanding crop to grow and a difficult base on which to make a living. It is prone to a number of diseases - the best known being fungal. However, like most plant genera under cultivation it is also commonly infected by one or a number of plant viruses. All the garlic grown in Australia is thought to be infected with at least one virus.

Plant viruses are basically a thread of genetic information, DNA or RNA, which is enclosed in a protein coat. Once inside a plant cell, the virus uses the plant cell components to replicate and multiply itself. The infected plant cell is therefore not functioning to the plant's best advantage but to the viruses'.

Virus infection disrupts the host plant's metabolism and diverts energy and building materials away from plant growth to virus replication. The results can range from a slight lack of vigour through severe wilting to a drastic reduction in growth and yield.

The most obvious symptoms and effects of plant viruses are the colour changes that occur particularly on the leaves. These include flecking, streaking, mosaics, mottling, vein banding, spotting, ringspotting or a general yellowing or chlorosis. Leaf distortions such as curling, rolling, narrowing, puckering and rosetting may also occur. Stunting and dwarfing are common symptoms of virus infections in plants. Also, as the virus multiplies it may block the vascular tissue of the plant and interfere with the transfer of water and nutrients around the plant. Wilting is thus another prevalent symptom.

We have found that, in garlic infected with one virus (type), symptoms are not always easily discernible. In fact symptoms such as colour changes in the leaves may only be visible with changes in the weather to days with more intensive sunlight. However, it appears that when the garlic plant is infected with two types of virus symptoms are very much more severe and drastic. We refer to such plants as having a virus complex. Most of the different garlic cultivars we have examined at Burnley have been found to contain a complex of viruses. In trials conducted in the UK, yield improvements up to 88% were recorded in cultivars from which viruses had been eradicated. In addition, improved clove size and keeping quality was measured. We have a trial in progress at Myrtleford, Victoria, to compare the performance of virus-tested and locally-grown (infected) garlic of the 'Mexican' type. Results of this trial will be available later in the season.

Plant viruses are given a two part name. The first part designates the host plant in which the virus was first recognised (e.g., garlic) and the second part is the main symptom produced on the host plant (e.g., yellow streak). Some of the viruses known to infect garlic are as follows:

Garlic Yellow Streak	}	We have found these three to be the most common
Garlic Mosaic	}	
Onion Yellow Dwarf	}	
Garlic Latent		
Cucumber Mosaic		
Tobacco Mosaic		
Tobacco Rattle		
Lettuce Necrotic Yellows		

Garlic, being a sterile plant, is propagated vegetatively by the division of the bulb into individual cloves. If the 'seed' clove that the bulb formed from was infected with virus then all the resultant cloves in that bulb will contain virus. This explains how garlic-infecting viruses have been so readily spread around the world.

Sucking or chewing insects such as aphids or leathoppers may also transmit a range of viruses. The green peach aphid *Myzus persicae*, for instance, is a demonstrated vector of Garlic Yellow Streak and Onion Yellow Dwarf viruses. Mechanical contact such as rubbing of leaves or grafting between two plants may also aid the spread of certain viruses. Viruses can also be spread through cuts and wounds inflicted upon plants from tools and machinery. Some viruses may also be transmitted by fungi or nematodes in the soil.

At Burnley we are currently examining a range of garlic cultivars in order to determine which viruses are present. Plant virology involves a number of specialised techniques. Initially the plant sap is examined in an electron microscope using one or a combination of methods. Often characteristic particles are readily visible and this identification may be sufficient for many purposes. The virus may also be inoculated into a number of indicator plants and the symptoms produced here may also aid identification. We are aiming at developing a highly sensitive and rapid diagnostic test for all the viruses of importance to the garlic industry. The production of 'virus-tested' cultivars through meristem tip dissections and tissue culture procedures is also a major goal of our project. Once virus-tested material is obtained this can be rapidly multiplied using tissue culture techniques. Tissue culture involves growing the plantlets in sterile conditions on artificial media containing all nutrients and growth regulators that they need. Virus eradication will see the quality of the crop increase, and yields increase. Ultimately we hope to see a replacement of imports with high quality, locally-grown garlic and even the export of our improved product.