VIRUS DISEASES OF CHRYSANTHEMUMS IN THE MANAWATU

A thesis presented in partial
fulfilment of the requirements
for the degree of
Master of Horticulture Science
(in Plant Science)

at

Massey University

by

David William LUNN

1972

ACKNOWLEDGEMENTS

I would particularly like to thank my supervisor, Dr. K.S. Milne, without whose help and encouragement this thesis would not have been completed.

I would also like to thank:

Mr. M. Hollows for the provision of the chrysanthemums used in this study and for his financial support;

Dr. G. Latch of the Plant Diseases Division of the D.S.I.R. (Palmerston North) and Mr. D. Hicks of the Plant Physiology Division of the D.S.I.R. (Palmerston North) for the use of their glasshouse facilities;

Dr. R. Elliott and Dr. P. Fry of the Plant Diseases Division of the D.S.I.R. (Auckland) for their advice.

Thanks also to my fellow students and other members of the Plant Pathology Department for their encouragement, Mr. R. Leitch for his photographic expertise and Mrs. H. Horsfall for typing this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

ABSTRACT

A survey of the virus diseases of chrysanthemums in the Manawatu revealed that tomato aspermy virus (TAV) and chrysanthemum stunt were present in most cultivars grown for cut-flowers, often resulting in unmarketable flowers. Latent infection of both viruses was revealed in most cultivars tested by sap or graft indexing to suitable indicator plants.

Rod-shaped particles of the leaf mottle group were observed in leaf-dip electron microscopic examinations from most cultivars examined, although leaf symptoms were not widespread.

Characterisation of TAV revealed that the virus could be sap and graft transmitted and was aphid transmitted in a stylet-borne manner. Host range and *in vitro* properties of TAV did not differ sufficiently from overseas reports to warrant the designation of a new strain of TAV. The addition of DIECA to chrysanthemum triturates markedly increased the infectivity of TAV in chrysanthemum sap.

Graft indexing for chrysanthemum stunt, using the cultivar 'Mistletoe' was found to be more reliable than sap inoculation to either 'Mistletoe' or cineraria, or the starch local lesion test. Defoliation of the 'Mistletoe' scions reduced the latent period of chrysanthemum stunt from 4 to 2 months.

The presence of rod-shaped particles ca. 690 x 15 nm in leaf-dips from many chrysanthemums, and from inoculated petunia leaves showing yellow local lesions suggested the presence of one or more of the leaf mottle viruses. Graft indexing with 'Good News' chrysanthemums gave symptoms of mosaic infection similar to those described in the U.S.A., suggesting a relationship between the mosaic and leaf mottle groups. The leaf mottle virus infecting chrysanthemums in the Manawatu was found to be aphid transmitted

and of the non-persistent or stylet-borne type. Erratic symptom expression in Petunia hybrida Vilm. necessitated the use of leaf-dip examinations to determine the presence of virus particles in the inoculated petunia leaves during the assessment of the in vitro properties of the leaf mottle group.

Suggested control measures include indexing to obtain virus-tested chrysanthemum stock plants, and the prevention of re-infection from outside sources by the aphid vectors. Preliminary investigations showed that meristem-tip culture is feasible for elimination of TAV and viruses of the leaf mottle group from totally infected chrysanthemum cultivars, but that treatment during "hardening off" is critical for plantlet survival.

CHAPTER 1.

INTRODUCTION

Chrysanthemums are one of the most popular ornamental crops grown. The cultivated chrysanthemum grown for cut-flower production belongs to the species Chrysanthemum morifolium Ram. but most cultivars have been extensively hybridised with Chrysanthemum indicum L. and Chrysanthemum nipponicum Hort. The intensive culture of chrysanthemums has resulted in increased flower numbers and better quality flowers, and attention is now being given to virus diseases and their effect on flower quality and plant vigour.

There are at present some 20 viruses or virus-like agents known to infect chrysanthemums, and some of these are reviewed in the following section.

1.1 A REVIEW OF THE LITERATURE ON VIRUSES RECORDED IN CHRYSANTHEMUMS

1.1.1 Aster Yellows Group */*:*/*:*/*:S/Au

One of the first reports of a disease of chrysanthemums attributed to a virus or virus-like agent, was by Nelson (95). He described symptoms appearing in chrysanthemums, similar to those described for aster yellows, and concluded that one of the yellows-type infectious agents was present. He called this disease chrysanthemum yellows. Kunkel (83) demonstrated that the causal agent of aster yellows was also present in some chrysanthemum cultivars and appeared to cause symptoms similar to those described by Nelson (95). These included the production of small green flowers and weak spindly lateral shoots, while thin

weak basal shoots with shortened internodes were shown to be signs of infection in plants which flowered normally. The infected plants usually died within a few months, but cuttings from recently infected plants often exhibited weak terminal growth and shortened internodes (15).

Similar symptoms have been reported in England by Hollings (51), where green flowers developed on infected 'Balcombe Perfection' chrysanthemums, together with spindly growth and root necrosis. Graft transmission of the causal agent was possible, but Hollings reported no leaf-hopper transmission. When symptoms of flower distortion did not appear on grafting infected plants to 'Blazing Gold', Hollings concluded that the symptoms were typical of the aster yellows group rather than flower distortion and called the disease chrysanthemum green flower (51).

In recent years aster yellows appears to have disappeared from commercial crops, possibly because of efficient control of the leaf-hopper vector, and the self-eliminating tendency (through unsuccessful propagation) of the infected cuttings.

The causal agents of 'yellows-type' diseases have long been thought to be viruses, but now there is considerable evidence casting doubt on this. In 1967, Doi et al (33) in Japan, and subsequently workers in other countries, reported mycoplasma-like organisms (MLO) in the phloem of plants exhibiting a variety of symptoms, including yellowing. There are numerous reports of MLO being associated with aster yellows and with the leaf-hopper vector (after feeding on diseased plants) (5, 88). Symptoms of aster yellows are reported to regress if plants are treated with certain tetracycline antibiotics. Similar reports have been made for a number of other diseases where MLO have been implicated (71).

It would be surprising if chrysanthemum yellows (sensu Nelson) and chrysanthemum green flower (sensu Hollings) do not prove to belong to this category too.

1.1.2 American Flower Distortion */*:*/*:*/*:A/Au

Symptoms of flower distortion, attributed to a virus infection, were recorded in England by Bewley and Richards (4) and Prentice (101), and later in the U.S.A. by Brierley (11).

In England, the organism causing this distortion of flowers was shown in most cases to be a strain of tomato aspermy virus (TAV) usually in association with at least one other virus (46).

In the U.S.A., however, Brierley (11) compared symptoms expressed in chrysanthemums infected with TAV, chrysanthemum mosaic, and those showing flower distortion symptoms, and he reported that the presence of flower distortion symptoms was not always related to infection with TAV and consequently attributed these symptoms to a flower distortion virus. Further work by Brierley (12) demonstrated that the pathogen causing flower distortion was transmissible by grafting and by dodder. Root necrosis, symptomatic of 'yellows-type' diseases, generally resulted in death of the infected plants, and Brierley proposed that this American flower distortion was in fact caused by one of the 'yellows-type' organisms.

Grafting infected plants to the chrysanthemum 'Blazing Gold' was shown to be the best means of identification of flower distortion. Symptoms of tight rosetting and deformation of the younger leaves appeared within 2 months and small distorted flowers were produced (21).

1.1.3 Tomato Aspermy Virus R/I:*/*:S/S:S/Ap

This virus (TAV) causes distinctive symptoms in tomatoes, including apical chlorosis, leaf mottling and distortion, the development of a bushy habit, a reduction in fruit numbers, and as the name of the virus implies, poor seed development (109).

Blencowe and Caldwell (6) demonstrated that these symptoms in tomatoes could be caused by a virus also present in chrysanthemums. Bewley and Richards (4) and Skillman (106)

described symptoms of leaf mottling, flower distortion and colour-breaking in chrysanthemums and named the disease chrysanthemum mosaic. However, Prentice (101) showed that mosaic symptoms were not always associated with distorted flowers and suggested that a better name for this disease would be chrysanthemum flower distortion.

Hollings (46) indexed several hundred chrysanthemums with various flower and leaf abnormalities and showed that many were infected with TAV, often in conjunction with other viruses. He named the disease aspermy flower distortion to distinguish it from the American flower distortion, and suggested that chrysanthemum mosaic (sensu Bowley and Richards) was in fact caused by a combined infection of TAV and one or more viruses of the leaf mottle group.

Smith (109) has called the virus involved in the production of aspermy flower distortion, chrysanthemum aspermy virus, and this is regarded as a strain of TAV (63).

Symptoms attributed to TAV in chrysanthemums include a reduction in flower size, untidy distorted flowers, and colour break in some of the darker flowered cultivars (97,108).

Several workers (23,37,46) have reported that Myzus persicae Sulz., together with other aphid species, in particular Aulacorthum solani Kltb. and Macrosiphoniella sanborni Gill., transmitted TAV from chrysanthemum to chrysanthemum.

The most satisfactory means of identification of TAV is the use of indicator plants, such as Petunia hybrida and Nicotiana glutinosa L. (46).

The properties of TAV in crude plant sap have been recorded overseas by several workers (23,37,46,97,99).

The results of studies on TAV present in chrysanthemums in the Manawatu are shown in chapter 3.

1.1.4 Tomato Spotted Wilt Virus R/*:*/*:S/S:S/Th

This virus, first reported in New Zealand in 1946 by Chamberlain (25), has been reported overseas as infecting chrysanthemums (1,18,80).

Symptoms in young chrysanthemum cuttings include pale chlorotic areas on the leaf tips, later spreading and turning bronze and necrotic (15).

Ie (69) described tomato spotted wilt virus (TSWV) as being an isometric particle 70-90 nm in diameter, with a thermal inactivation point of 40-46 C, a longevity *in vitro* of only a few hours and a dilution end point of 10^{-2}-10^{-3}. He also reports that TSWV has a wide host range and is mechanically transmissible, although difficult to purify.

Spread of TSWV in the field can occur by means of the thrips vector (109), although efficient insect control and roguing of young infected cuttings could account for the virtual disappearance of this virus in commercial chrysanthemum crops in the Manawatu.

1.1.5 Leaf Mottle Group

In 1952 Noordam (97) reported the presence of chrysanthemum virus B (CVB) infecting chrysanthemums in Holland, which gave chlorotic local lesions when sap inoculated to *Petunia hybrida*. Electron microscopic examination revealed the presence of rod-shaped particles ca. 600 x 30 nm. Symptoms observed in infected chrysanthemums included both flower and leaf abnormalities. Later work by Brierley and Smith (19) in the U.S.A. showed the presence of a similar virus which also gave chlorotic lesions on *P.hybrida*. They concluded that Noordam's virus B was present in a number of cultivars and was responsible for causing a disease they called chrysanthemum mosaic.

The flower symptoms attributed by Noordam to virus B were also observed by Hollings (46) in England but the presence of TAV in most plants studied made the effect of virus B on

chrysanthemum flowers difficult to determine. Subsequently, Hollings (48) reported that virus B was seldom isolated from TAV-free chrysanthemums with flower abnormalities. In these few instances he attributes the flower distortion to cold weather during bud opening rather than virus B infection. The leaf symptoms in many cultivars prompted Hollings (48) to suggest the name mild mosaic for the disease caused by virus B.

During studies on chrysanthemum virus B Hollings identified a further virus, chrysanthemum vein mottle which gave chlorotic lesions on P.hybrida and of similar particle size to that later accepted for chrysanthemum virus B (ca. 700 x 17 nm). However, vein mottle was distinguished from virus B on the basis of host range and the failure of virus B strains to afford cross protection (48). Chrysanthemum vein mottle also differed from CVB in that graft inoculation to the CVB-infected chrysanthemums 'English Blazing Gold', 'Pink Mistletoe', 'Penrod', 'Balcombe Perfection' and 'Imperial Pink', gave severe leaf mottle symptoms after 4-11 weeks, and no local lesions developed on Vicia faba L. when inoculated with chrysanthemum vein mottle (48).

The justification for such a distinction on these bases could be questioned, at least until further characterisation is conducted and serological studies made.

In 1958 Brierley and Smith (22) distinguished 8 mosaic viruses in the U.S.A. on the basis of slight difference in lesion development on P.hybrida, symptom expression on the grafted 'Good News', 'Mistletoe' and 'Dynamo' chrysanthemums, and aphid transmission characteristics.

Similar differences in symptom expression in naturally infected chrysanthemums and in inoculated plants led Hollings (57) to suggest the formation of a leaf mottle group, comprised of chrysanthemum virus B, chrysanthemum vein mottle, chrysanthemum necrotic mottle, and chrysanthemum dwarf mottle, all of which had rod-shaped particles ca. 700 nm long and gave chlorotic lesions on P.hybrida. The possible inclusion in this leaf mottle group of Brierley and

Smith's mosaic viruses and Keller's virus Q (78), which also gives chlorotic local lesions on P.hybrida (19) could result in up to 13 diseases of chrysanthemums being attributed to viruses of the leaf mottle group.

Studies have shown that "chrysanthemum virus B", i.e. one of the leaf mottle group, is serologically related to the carnation latent virus and potato viruses S and M (42), and serological tests have been used by several workers to detect 'CVB' in chrysanthemums (40,99).

The variability of the symptom expression in naturally infected chrysanthemums (48), test cultivars (22) and in inoculated P.hybrida (48) makes the possibility of these leaf mottle viruses being related to, or even strains of chrysanthemum virus B, a feasible proposition. For the purposes of this study, the viruses in this group were considered as one entity and no attempt was made to distinguish the constituent components of the leaf mottle group.

In 1970, Hollings and Stone (61) reported unusual leaf symptoms in 'Golden Sundance' chrysanthemums. These included leaf flecking and chlorotic spotting, the latter areas becoming necrotic and falling out, causing a "shot-hole" effect. On grafting to 'Mistletoe' and 'Good News', severe vein mottle symptoms developed. Electron microscopic examination revealed rod-shaped particles ca. 700 nm in length, of the vein mottle type.

The relationship of this "chrysanthemum shot-hole" virus to chrysanthemum vein mottle is not known, but it is possible that the symptoms expressed in infected 'Golden Sundance' could be due to a hypersensitive reaction to chrysanthemum vein mottle, or that vein mottle is one constituent of a mixture of viruses acting to produce these symptoms.

1.1.6 Chrysanthemum Virus Q

Another virus infecting chrysanthemums and causing symptoms on P.hybrida similar to the vein mottle group, is chrysanthemum virus Q. This virus was originally reported by Keller (78) and was first seen in the cultivar 'Blanche', where symptoms of severe leaf distortion and white leaf flecking were induced when symptomless 'Blanche' was grafted to stunt-infected chrysanthemums. Grafting stunt-infected chrysanthemums to 'Blanche' gave more severe stunt symptoms and suggested the possibility of a symptomless virus in 'Blanche' which acted synergistically with stunt to cause the leaf distortion and flecking symptoms.

Brierley and Smith (19) and Miller (89) compared CVB and virus Q, and concluded that either CVB was a mild form of virus Q, or virus Q was a complex involving CVB as one of its constituents.

1.1.7 Chrysanthemum Stunt */*:*/*:*/*:S/*

The first report of chrysanthemum stunt was by Dimock (29) in the U.S.A. in 1947. The disease was first thought to be a physiological effect of continuous vegetative propagation resulting in degeneration and debilitation. A Deuteromycete fungus was also suggested as the causal organism, but further investigations revealed no relationship between the fungus and stunt symptoms (79). In 1949 Brierley and Smith (16,17) reproduced the stunt syndrome through graft and mechanical transmission from chrysanthemum to chrysanthemum and suggested that stunt was caused by a virus. Studies by Keller (79) showed that although stunt was highly infectious there were no satisfactory local lesion hosts. The most reliable indexing method involved grafting to 'Mistletoe' chrysanthemums, where distinctive white spots and flecks (measles) developed after 6-8 weeks.

Symptoms of chrysanthemum stunt have been reported in several countries including Holland (97), England (51) and Canada (80) and the disease is undoubtedly present in most countries where chrysanthemums are grown.

The symptoms of chrysanthemum stunt can vary with the cultivar infected and time of year, with symptoms being more easily recognised during periods of rapid growth - in the summer and spring (109). Three diagnostic features of stunt infection in chrysanthemums have been described (51,79,80).

> The whole plant can be up to half the normal size, with leaves and internodes proportionally smaller. The plant does not appear deformed, merely reduced in size. This difference in size is particularly noticeable during rapid forcing of the plants, in comparison with healthy plants.
>
> Flowers on infected plants develop 7-10 days earlier than on healthy plants.
>
> The flowers are smaller than usual.

Other flower symptoms, including bleaching of darker flowered cultivars, have been reported although the bleaching is usually entire rather than streaked as is often the case with TAV-infected flowers (18). In some instances, particularly with winter glasshouse crops, stunted plants may fail to flower because of their reduced vigour (15).

Some chrysanthemum cultivars have been found to give distinctive leaf symptoms when infected with stunt, and these have been used as test plants.

'Vibrant' and 'Seagull' give light pin-point flecking 1 month after graft-inoculation, and 2-3 months after sap-inoculation with stunt (79).

'Blazing Gold' gives a diffuse yellow veining in the young leaves, 6-8 weeks after graft-inoculation (15).

'Mistletoe' produces distinctive chlorotic leaf flecking when infected with chrysanthemum stunt (18).

Virus Q-infected 'Blanche' gives pronounced leaf distortion when infected with chrysanthemum stunt, and was used by Keller (79) in his studies of chrysanthemum stunt. He found that the latent

period in 'Blanche' could be reduced from 3-5 months to 2 months by removing most of the leaves above the graft union.

Hollings (51) reported that symptoms similar to those described by Keller (79) had been observed in chrysanthemums in England since 1952. However, the causal agent of stunt in these plants was less infectious than that described in America. Furthermore, Hollings was able to eliminate stunt from the English chrysanthemums by heat treatment, a result he was unable to achieve with "American" stunt. He also reported an increase in the incidence of the "American" stunt in England and attributed this to the increase in popularity of chrysanthemum cultivars bred in America (51).

Serological studies (51,79) have shown a lack of serological activity of preparations from stunt-infected plants and this fact combined with work on purification of the causal agent of stunt (52,67) have led Hollings to hypothesise that the causal agent is not a virus, although many properties suggest this (113). Using extraction procedures similar to those used by Diener and Raymer (28) in their characterisation of potato spindle tuber, Hollings and Stone (61) have succeeded in extracting infectious material similar in properties to potato spindle tuber (28). Incubation of the infectious extract with ribonuclease destroyed infectivity, the infective principle was not precipitated with 8% polyethylene glycol plus 2% NaCl, and no virus-like particles could be seen in electron microscope examinations of infective preparations. These results support the possibility that chrysanthemum stunt 'virus' is an uncoated nucleic acid (61).

1.1.8 Other "Viruses" Infecting Chrysanthemums

Most of the other reports of virus-like diseases of chrysanthemums involve the occurrence of symptoms in a few chrysanthemum cultivars, and much still remains to be established on the distribution, prevalence and characteristics of the causal agents.

Factors influencing the identification of these causal organisms as viruses, include the variability of symptom expression in different chrysanthemum cultivars, e.g. "shot-hole" symptoms in 'Golden Sundance', the possibility of strains of a virus giving different reactions on test plants, e.g. TAV, and the presence of 2 or more viruses acting together to give distinctive symptoms different to those caused by the constituent viruses, e.g. chrysanthemum stunt mottle.

Until the conditions of Koch's Postulates are satisfied as far as is possible with obligate parasites, the identification of these organisms must remain in doubt.

Some of these viruses or virus-like organisms reported to infect chrysanthemums but about which little is known, are recorded below.

Chrysanthemum stunt mottle */*:*/*:*/*:S/*

Symptoms in chrysanthemum of vein mottling, vein clearing and occasional leaf distortion, accompanied by plant stunting were described by Welsh (115). He also showed that the causal agent could be graft transmitted and proposed that the lack of early flowering symptoms, together with leaf mottling symptoms, distinguished the disease from chrysanthemum stunt, and he proposed the name chrysanthemum stunt mottle. Smith (109) however, lists chrysanthemum stunt mottle as a synonym for chrysanthemum stunt, and it is possible that the presence of one of the leaf mottling group, together with chrysanthemum stunt could give the symptoms described by Welsh.

Chrysanthemum latent "virus" */* */* */* S/*

This "virus" is apparently rare in chrysanthemums in England and has not been recorded in the U.S.A. or Europe. Symptoms on P.hybrida are similar to those expressed by TAV, but the "virus" does not go systemic. Latent "virus" differs from the leaf mottle group in host range, physical properties, and is not aphid transmitted (48).

Chrysanthemum ringspot "virus" */* */* */* S/*

This was first reported by Brierley and Smith (20) in the U.S.A. when some cultivars developed large, chlorotic ring patterns. The causal agent was shown to be graft transmitted but not aphid-borne. Grafting infected plants to 'Good News' resulted in yellow spots developing after 6 weeks and yellow blotching with leaf distortion after 6 months. Symptoms on Senecio cruentis D.C. included severe dwarfing, but attempts to seperate the causal agent from that of chrysanthemum stunt failed - the two were always found together - and it is possible that symptoms on S.cruentis were the result of a mixed infection (20). No vector for chrysanthemum ringspot has been reported (102).

Hollings (50) has reported similar symptoms in a few chrysanthemums in England and he has called the causal agent chrysanthemum ringpattern "virus".

Chrysanthemum "virus" C */* */* */* S/*

This "virus" has been reported in Holland by Noordam (97) but was found in only one plant and no further reports have been made. The infected plant showed mottling and circular spotting on the leaves, with slight distortion of the flowers. Sap inoculation to P.hybrida gave grey necrotic spots, characteristic of TAV, and also concentric ring lesions. Systemic symptoms included ringspots and yellow-white line patterns.

Chrysanthemum "virus" D */* */* */* S/*

Symptoms of flower distortion on chrysanthemums, similar to those caused by TAV, were reported by Prentice (102). Inoculation to Chenopodium amaranticolor Coste. & Reyn. gave distinctive chlorotic and ring local lesions after 3 weeks. Later work by Hollings (51) showed that chrysanthemums infected with a mixture of "virus" D and vein mottle, when grafted to 'Good News', gave symptoms of leaf mottling, flower distortion and floret necrosis within 6-8 weeks. In New Zealand, chrysanthemum "virus" D has been recorded in the chrysanthemum cultivars 'Peach Blossom' and 'Actress', by Thompson (113).

Chrysanthemum "virus" E */* */* */* S/*

This "virus" has been reported in England by Hollings (46) and causes symptoms similar to those induced by virus D, including reduction in flower size and short tangled florets often tightly packed in the centre. Flowers can be bleached and may fail to open. Grafting to 'Blazing Gold', 'Good News' and 'Mistletoe' all give similar symptoms of leaf mottling, and no vectors have been reported for this virus (105).

Chrysanthemum rosette group */* */* */* S/*

Brierley and Smith (22) reported the presence of a group of "viruses" infecting chrysanthemums which, when grafted to the cultivars 'Good News' and 'Golden Mistletoe' gave yellow mottling and leaf dwarfing, sometimes accompanied by rosetting.

The first disease in this group was observed in the cultivar 'Ivory Seagull' and the causal agent could be re-isolated to chrysanthemums from inoculated S.cruentis plants which remained symptomless (18). Kemp (80) reported a disease similar to Ivory Seagull rosette in the cultivar 'Wilsons White'.

The other disease in this group, Yellow Rayonante rosette, was first observed in the cultivar 'Yellow Rayonante' by Brierley and Smith (22). They considered that Yellow Rayonante rosette differed from Ivory Seagull rosette in that re-isolation from inoculated cineraria plants was not possible.

Chrysanthemum chlorotic mottle

Chlorotic mottle, first reported in the cultivar 'Yellow Delaware' in 1967 by Dimock and Geissinger (30) caused mild mottling of the young leaves, often followed by general chlorosis and dwarfing. Delayed flower development was also noted. Frequently these symptoms were only transient. The causal organism was shown to be graft transmissible, and electron microscopic examination of infected sap failed to show any virus-like particles (30,31).

Sowbane mosaic virus

Hollings, Stone and Bouttelli (64) have reported the presence of a virus serologically related to sowbane mosaic virus and carnation 689 virus in 26 out of 29 English chrysanthemum cultivars, and suggest that pollen transmission of the virus may occur (58).

Another virus, similar to sowbane mosaic virus has been reported in the chrysanthemum cultivar 'Rose Harrison' which had undergone heat treatment and meristem-tip culture. Purified preparations of this virus contained isometric particles 25-30 nm diameter, and gave local lesions followed by systemic mottling and distortion symptoms in Chenopodium quinoa Willd. This virus was not found to be serologically related to sowbane mosaic virus, but exhibited the same property of being difficult to transmit in crude sap, from chrysanthemum to Chenopodium spp, but in partially purified and concentrated preparations transmission to Chenopodium spp was possible (58).

The salient features of this review are listed in tables 1 and 2.

Table 1 is a summary of virus or virus-like organisms occurring naturally in chrysanthemums, including synonyms, methods of transmission, and therapy of infected plants.

Table 2 is a summary of the properties of the three main virus or virus-like diseases occurring naturally in chrysanthemums, viz. tomato aspermy virus, chrysanthemum stunt and viruses of the leaf mottle group.

TABLE 1. Viruses and virus-like organisms occurring naturally in chrysanthemums; synonyms, methods of transmission, and therapy of infected plants.

Virus or Virus Group - Synonyms		Transmission*		Therapy**	
YELLOWS GROUP					
American flower distortion	(11)	G.	(12)	H.	(21)
Aster yellows virus	(83)	G. L.	(15)	H.	(109)
- Chrysanthemum yellows virus	(95)				
- Chrysanthemum green flower virus	(51)	G.	(51)	H.	(111)
LEAF MOTTLE GROUP					
Chrysanthemum virus B	(97)	G.S.A.	(97)	H.M.	(41)
- Chrysanthemum mild mosaic virus	(48)				
- Noordam's chrysanthemum virus B	(48)				
Chrysanthemum dwarf mottle	(57)	G.S.	(61)		
Chrysanthemum mosaic virus group	(22)	G.S.A.	(22)	H.	(22)
Chrysanthemum necrotic mottle	(48)	G.S.	(61)		
- Chrysanthemum "shot-hole"	(61)	G.	(61)		
Chrysanthemum vein mottle	(48)	G.S.A.	(48)		
CHRYSANTHEMUM VIRUS C	(97)	S.	(97)		
CHRYSANTHEMUM VIRUS D	(102)	G.S.	(51)		
CHRYSANTHEMUM VIRUS E	(51)	G.	(109)		
CHRYSANTHEMUM LATENT VIRUS	(48)	S.	(48)		
CHRYSANTHEMUM RINGSPOT VIRUS	(20)	G.S.	(20)	H.	(55)
- Chrysanthemum ringpattern virus	(50)				
CHRYSANTHEMUM ROSETTE GROUP					
Ivory Seagull rosette virus	(18)	G.S.	(22)		
Yellow Rayonante rosette virus	(22)	G.S.	(22)		
CHRYSANTHEMUM STUNT	(29)	G.S.	(79)	H + M	(60) (62)
- Marmor chrysanthemi sp.nov.	(79)				
Chrysanthemum stunt mottle	(115)	G.S.	(109)		
Chrysanthemum chlorotic mottle	(30)	G.	(31)		
TOMATO ASPERMY VIRUS	(6)	G.S.A.	(63)	H.M.	(14) (55) (91)
- Chrysanthemum mosaic virus	(4)				
- Lycopersicon virus 7	(6)				
Chrysanthemum aspermy virus	(109)	G.S.A.	(46)		
- Aspermy flower distortion	(46)				
- Cucumis virus 1 strain chrys.	(97)				
- Chrysanthemum virus A	(97)				
Chrysanthemum mild mottle	(114)	G.S.A.	(114)		
TOMATO SPOTTED WILT	(18)	S.Th.	(69)	H.	(109)

* G. = graft transmission L. = leaf-hopper transmission
S. = sap transmission A. = aphid transmission
Th. = thrips transmission.

** H. = hot air treatment M. = meristem-tip culture.

TABLE 2. Some characteristics of chrysanthemum stunt, tomato aspermy virus and viruses of the leaf mottle group.

Characteristic	Chrysanthemum stunt	Tomato aspermy virus	Leaf mottle group
Cryptogram	*/* */* */* S/*	R/1 */* S/S S/Ap	*/* */* E/E S/Ap
Symptoms on chrysanthemums	Stunting, early flowering, bleaching in darker flowers (51,79)	Flower distortion (46,97,108)	Occasional leaf mottle or leaf fleck (19,48,57,97)
Diagnostic species	'Mistletoe' - yellow leaf fleck 2-8 months after grafting (79)	N.glutinosa-leaf mottle C.amaranticolor-dot lesions P.hybrida-leaf mottle (37,46,63)	P.hybrida-yellow local lesions T.expansa-yellow lesions (19,48,89)
Physical properties	TIP - 96-100 C DEP - 10^{-2}-10^{-3} LIV - 55 days @ 21 C (79)	TIP - 50-60 C DEP - 10^{-4}-10^{-5} LIV - 2-6 days (63)	TIP - 65-70 C DEP - 10^{-2}-10^{-4} LIV - 24 hours (48,97)
Particle characteristics	Possible uncoated RNA particle (60,61)	RNA - single stranded (?) isometric 25-30 nm diam. (42,65,114)	Rod-shaped particles ca. 700 x 17 nm (48,61)
Control	Heat - 14-37 weeks @ 35 C then meristem-tip culture (60,62)	Heat - 4 weeks @ 37 C or meristem-tip culture (14,55,91)	Heat - 8 weeks @ 37 C or meristem-tip culture or both (41)

1.2 CHRYSANTHEMUM VIRUS SITUATION IN NEW ZEALAND

There is very little information published on the viruses present in chrysanthemums in New Zealand. Tomato aspermy virus has been recorded in tomatoes in New Zealand (113) and Dingley (32) lists TAV as infecting chrysanthemums in New Zealand. Thomson (113) has reported the development of symptoms attributed to chrysanthemum virus D on sap inoculated C.amaranticolor leaves. These results were obtained with inoculations from two chrysanthemum cultivars, 'Peach Blossom' and 'Actress'.

The introduction of cultivars from overseas, and the prevalence of virus infection reported overseas, (54,97,99) particularly of TAV, chrysanthemum stunt and viruses of the leaf mottle group, suggest that these viruses in particular could be present in cultivars grown in the Manawatu.

1.3 OBJECTIVES OF STUDY

A study of viruses naturally infecting chrysanthemums was undertaken to determine:

a) the incidence of viruses in chrysanthemums grown commercially for cut-flowers in the Manawatu;

b) the identity and characteristics of the more commonly occurring viruses in chrysanthemums in the Manawatu;

c) approaches to control of the viruses found in chrysanthemums in the Manawatu, based on the general methods of chrysanthemum virus control outlined in chapter 6 and the virus characteristics determined in this study.

CHAPTER 2.

GENERAL SURVEY OF CHRYSANTHEMUM VIRUSES INFECTING CHRYSANTHEMUMS IN THE MANAWATU

2.1 TOMATO ASPERMY VIRUS

2.1.1 Visual Survey

Examination of a number of chrysanthemum cultivars was made to determine the incidence of symptoms attributed by overseas workers to virus infection. The plants examined were grown as a commercial cut-flower crop and the chrysanthemums were planted in blocks of 4 rows at a spacing of 6" x 6". This gave a plant population of ca. 1000 plants/40 yard block.

The results of the visual survey of chrysanthemums made to determine the incidence of flower distortion symptoms attributed to TAV are shown in table 3.

2.1.2 Indexing for TAV

An indexing program was undertaken to determine the extent of symptomless TAV infection (46) and to confirm that the causal organism was TAV not American flower distortion, which gives similar flower distortion symptoms in chrysanthemums.

The plants to be indexed were selected by using a table of random numbers (93) to determine the distance (in yards) between samples. From each sample plant, 5 leaves were taken from various positions on the plant to eliminate the influence of any erratic virus distribution within the plant (46,99).

TABLE 3. Incidence of TAV in chrysanthemums in the Manawatu as determined by symptom expression.

Chrysanthemum cultivar	Number of plants with flower distortion symptoms (per 1000 plants)
'Madame E. Rogers'	76
'Shantung'	75
'Fred Shoesmith'	68
'Alec Bedser'	30
'Sunburst Mefo'	24
'Golden Favorite'	23
'Rivalry'	22
'Snowshine'	21
'Dark Dawn'	16
'Red Kitchener'	16
'Sussex Amber'	16
'Sussex Pink'	16
'Pink Pride'	15
'Loula'	15
'Apricot Pride'	14
'Mauve Dawn'	13
'Bessie Rowe'	12
'Violet Kitchener'	9
'Yellow Madonna'	9
'Durham Glow'	8
'Purple Mayflower'	8
'Regalia'	8
'Glorietta'	7
'White Dawn'	7
'Bronze Shoesmith'	6
'Ethyl Edmonds'	6
'Festival'	6
'Kathleen Dowd'	6
'Amber Perfection'	5
'Maestro'	4
'Sampford'	4
'Yellow Dawn'	3
'Beige'	0
'Copper Queen'	0
'January Gold'	0
'Mistletoe'	0
'Nightingale'	0

Eight samples were tested from each cultivar. Leaf discs were triturated in 0.033M Na_2HPO_4 + 0.05 DIECA (sodium diethyl-dithiocarbamate), pH 7.4, and rubbed onto carborundum-dusted leaves of 2 plants each of N.glutinosa, C.amaranticolor and P.hybrida 'Rose of Heaven'.

The presence of TAV was shown by the development of small yellow-white local lesions on C.amaranticolor after 4-6 days (47,49), diffuse chlorotic lesions on N.glutinosa after 7-10 days followed by systemic mottling and leaf distortion after 2 weeks (46), and diffuse chlorotic-grey local lesions within 1 week on P.hybrida followed by systemic mottling and distortion (46). These symptoms distinguish TAV from American flower distortion. Results are shown in tables 4 and 5.

TABLE 4. Incidence of TAV in chrysanthemums in the Manawatu as determined by indexing to selected indicator hosts.

Chrysanthemum cultivar	Visual symptoms	Indicator hosts		
		C.amaranticolor	N.glutinosa	P.hybrida
'Madame E. Rogers'	2	2*	2	1
'Shantung'	1	2	2	1
'Fred Shoesmith'	1	2	2	0
'Sussex Pink'	1	2	2	2
'Sussex Amber'	1	1	1	1
'Dark Dawn'	1	2	2	2
'Pink Pride'	1	2	2	1
'Mauve Dawn'	0	1	1	1
'Apricot Pride'	1	2	2	1
'Regalia'	1	2	2	2
'White Dawn'	0	2	2	1
'Festival'	0	1	0	0
'Ethyl Edmonds'	0	2	2	2
'Mistletoe'	0	1	1	0
'Nightingale'	0	1	1	0
'Copper Queen'	0	0	0	0

* Figures are plant numbers/8 samples.

TABLE 5. Comparison of symptom expression and indexing tests for determining the incidence of TAV in chrysanthemums in the Manawatu.

Chrysanthemum cultivar	Symptoms of TAV	Indexing tests for TAV
'Shantung'	4*	5
'Madame E. Rogers'	2	5
'Apricot Pride'	1	3

* Figures are plant numbers/20 samples.

A wide variation in the severity of symptom expression was observed in this survey of TAV infected chrysanthemums and this is in agreement with overseas reports (37,46). A conservative estimate of the incidence of flower symptoms attributed to TAV (97,108) is shown in table 3. The presence of TAV in chrysanthemums showing these flower distortion symptoms was confirmed by indexing tests (table 4) and symptomless TAV infection was observed in 15 of the 16 cultivars tested, again confirming overseas results (46,69). The low sample number (8 plants per cultivar) was necessitated by the lack of glasshouse space, and table 5 shows the results of further TAV indexing tests using a larger sample size (20 plants) and reducing the number of cultivars selected. These results showed that 10% of the plants tested were symptomless carriers of TAV.

2.2 CHRYSANTHEMUM STUNT

2.2.1 Visual Survey

The results of the visual examination of chrysanthemums, at flowering time, to determine the incidence of plant stunting and early flowering symptoms attributed to chrysanthemum stunt, are shown in table 6.

TABLE 6. Incidence of chrysanthemum stunt in chrysanthemums in the Manawatu as determined by symptom expression.

Chrysanthemum cultivar	Number of plants showing stunt symptoms (per 1000 plants)
'Shantung'	40
'Madame E. Rogers'	39
'Fred Shoesmith'	32
'Bronze Shoesmith'	24
'Apricot Pride'	22
'Alec Bedser'	20
'Durham Glow'	20
'Pink Pride'	17
'Mauve Dawn'	16
'Sussex Pink'	16
'Ethyl Edmonds'	14
'Bessie Rowe'	12
'Kathleen Dowd'	10
'Golden Favorite'	6
'Red Kitchener'	6
'Rivalry'	5
'Yellow Dawn'	5
'Violet Kitchener'	4
'Regalia'	3
'Amber Perfection'	3
'Snowshine'	1
'Yellow Madonna'	1
'Sunburst Mefo'	0
'Maestro'	0
'Sampford'	0
'Purple Mayflower'	0
'Copper Queen'	0

2.2.2 Indexing for Chrysanthemum Stunt

Although the stunting and early flowering symptoms are indicative of stunt infection, indexing for chrysanthemum stunt was undertaken to confirm the presence of the causal organism, and to determine the incidence of symptomless infection of stunt in chrysanthemums in the Manawatu. Four plants were selected from each cultivar, using random number tables and these were cut back to 3-4 buds on each lateral shoot. The plants were then transferred to the glasshouse where they were grown in 9" 'planter' bags. Two further plants, showing symptoms of stunting and early flowering were also selected.

Several methods of grafting have been used in indexing for chrysanthemum stunt, including approach or inarch grafting (67) and splice grafting (15).

In this study a modification of Brierley and Olsen's technique was used (15). Virus-tested* 'Mistletoe' scions were grafted to several of the young developing lateral shoots to provide replication. The splice-grafts were made close to the lateral tips, before secondary thickening developed and the scions were held in place with grafting tape ensuring that the cambial layers of the scion and the stock coincided (fig. 9). All cuts were made with a flamed scalpel to minimise cross-infection. Grafted plants were then placed under mist for 10-14 days before being transferred to the glasshouse.

The presence of chrysanthemum stunt was shown by the development of chlorotic-white flecking on the young 'Mistletoe' leaves within 6-14 weeks (79) (fig. 10). The results are shown in tables 7 and 8.

---

* The source of the virus-tested 'Mistletoe' chrysanthemums was: The Nuclear Stock Association (Ornamentals) Ltd., Littlehampton, England.

TABLE 7. Incidence of chrysanthemum stunt in chrysanthemums in the Manawatu as determined by graft-indexing to 'Mistletoe'

Chrysanthemum cultivar	Visual stunt symptoms	Stunt symptoms on 'Mistletoe'	Number of symptom-less plants
'Shantung'	2*	3	1
'Madame E. Rogers'	2	3	1
'Fred Shoesmith'	2	2	0
'Apricot Pride'	2	3	1
'Pink Pride'	2	2	0
'Ethyl Edmonds'	2	3	1
'Bessie Rowe'	2	2	0
'Kathleen Dowd'	2	2	0
'Regalia'	2	4	2
'Copper Queen'	0	3	1

* Figures are plant numbers/6 samples.

TABLE 8. Comparison of symptom expression and indexing tests for determining the incidence of chrysanthemum stunt in chrysanthemums in the Manawatu.

Chrysanthemum cultivar	Number of plants with stunt symptoms	Number of plants with chrysanthemum stunt
'Shantung'	3*	13
'Madame E. Rogers'	3	11
'Apricot Pride'	2	8

* Figures are the number of plants/20 samples.

Chrysanthemum stunt symptoms described by Keller (79) and Hollings (51) were observed in 22 of the 27 cultivars examined (table 6). There was a wide variation between cultivars in the incidence of stunt symptoms, with up to 4% of some cultivars showing symptoms. Graft-indexing with 'Mistletoe' confirmed the presence of chrysanthemum stunt in plants showing symptoms and showed that symptomless infection occurred in 6 of the 10 cultivars examined (table 7). The incidence of symptomless chrysanthemum stunt infection is shown in table 8, where a larger number of plants (60) were indexed from 3 cultivars. Fifty per cent of the indexed plants gave a positive reaction for chrysanthemum stunt, although stunt symptoms were observed in only 13% of the indexed plants.

2.3 LEAF MOTTLE VIRUSES

2.3.1 Visual Survey

Symptoms of leaf mottling were observed in chrysanthemums in the Manawatu, but only in very low number and in a few cultivars (6 out of 20) (figs. 11, 12).

TABLE 9. Incidence of leaf mottling symptoms in chrysanthemums in the Manawatu.

Chrysanthemum cultivar	Leaf symptoms	Number of plants showing symptoms
'Shantung'	vein clearing	6
'Loula'	chlorotic mottle	1
'Daily Mirror'	chlorotic mottle	2
'Rivalry'	chlorotic mottle	1
'Waltz Time'	chlorotic mottle	2
'William Duckham'	chlorotic mottle	2

The variable nature of these symptoms (table 9), seen mainly in young plants or young growth, cast doubts on the effectiveness of symptom expression as a means of determining the incidence of the leaf mottle group in chrysanthemum crops.

2.3.2 Indexing for Leaf Mottle Viruses

Electron microscopic examination of sap from the leaves showing leaf mottle symptoms revealed the presence of rod-shaped particles ca. 700 x 15 nm, typical of the leaf mottle group (fig. 13) (40,44,48).

Electron microscopic examination of leaf-dip samples from a number of chrysanthemum cultivars was used to determine the incidence of the leaf mottle group of viruses in chrysanthemums in the Manawatu.

Random selection of 10 leaves per cultivar was made using the method outlined in chapter 2.1.2. Sap was expressed from each leaf into 2-3 drops of 1% phosphotungstic acid (PTA), using a spotting plate as a mortar and a small glass rod (2 mm diameter) as a pestle. A drop of "extract" was transferred with capillary tubing to an electron microscope grid. After the excess liquid had been removed with filter-paper strips, the grid was dried and examined using a Philips EM 200* microscope, at a magnification of 18,000 X.

If no particles were observed within 2 minutes, the leaf was assumed to be free from viruses of the leaf mottle group (40). Results are shown in table 10.

---

* Trade name.

TABLE 10. Incidence of the leaf mottle viruses in chrysanthemums in the Manawatu as determined by leaf-dip electron microscopy.

Chrysanthemum cultivar	Number of plants with rod particles present
'Madame E. Rogers'	8*
'Fred Shoesmith'	8
'Shantung'	8
'Sussex Pink'	8
'Sussex Amber'	7
'Rivalry'	7
'Ethyl Edmonds'	7
'Dark Dawn'	7
'Apricot Pride'	7
'Pink Pride'	6
'Bessie Rowe'	6
'Regalia'	6
'Golden Favorite'	5
'Nightingale'	5
'Bronze Shoesmith'	5
'Alec Bedser'	4
'Glorietta'	4
'Waltz Time'	4
'William Duckham'	4
'Amber Perfection'	3
'Snowshine'	3
'Violet Kitchener'	3
'Kathleen Dowd'	2
'Loula'	2
'Daily Mirror'	2
'Mauve Dawn'	0
'Yellow Dawn'	0

* Figures are plant numbers/10 samples.

Sap inoculation to P.hybrida from selected chrysanthemum cultivars known to contain rod-shaped particles of the leaf mottle group, using 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, gave the results presented in table 11.

TABLE 11. Confirmation of the identity of rod-shaped particles seen in chrysanthemum leaves by inoculation to P.hybrida.

Chrysanthemum cultivar	Presence (P) or absence (A) of rod-shaped particles in chrysanthemum cultivars*	Symptoms on P.hybrida
'Shantung'	P 8	3
	A 2	0
'Fred Shoesmith'	P 8	2
	A 2	0
'Regalia'	P 6	2
	A 4	0
'Madame E. Rogers'	P 8	3
	A 2	0
'Sussex Pink'	P 8	1
	A 2	0

* Sample number of 10 plants/cultivar.

Symptoms of leaf mottling, attributed to viruses of the leaf mottle group (48,97), were observed in only a few plants (table 9) suggesting that viruses of the leaf mottle group are present in chrysanthemums in the Manawatu. Electron microscopic leaf-dip examinations revealed the presence of rod-shaped particles ca. 700 x 15 nm of the leaf mottle group (57) in 24 of the 27 cultivars examined (table 10), with several cultivars being heavily infected.

P.hybrida inoculated with chrysanthemum sap containing rod-shaped particles, developed chlorotic local lesions after 2-5 weeks. Symptoms did not develop in those P.hybrida inoculated with sap from chrysanthemums where rod-shaped particles were not observed (table 11). These results confirmed that the rod-shaped particles were infective virus particles of the leaf mottle group. The erratic development of chlorotic local lesions on inoculated P.hybrida, due to the adverse conditions under which the plants were grown, and the occasional development of mottling caused by TAV infection, made leaf-dip examinations for the presence of rod-shaped leaf mottle particles a more efficient method of indexing for viruses of the leaf mottle group. Hakkaart (40) has also shown that electron microscopy is adequate for leaf mottle indexing. However, one drawback of this technique is that differentiation between the various leaf mottle viruses - vein mottle, dwarf mottle, necrotic mottle and virus B - is not possible, but for routine indexing, this is not important because of the similar properties of the viruses in the leaf mottle group.

Rod-shaped particles were observed in a number of chrysanthemums showing flower distortion symptoms but in all cases, TAV was also present; and although Noordam (97) describes flower abnormalities in chrysanthemums infected with chrysanthemum virus B, indications are that viruses of the leaf mottle group found in the Manawatu do not cause significant flower abnormalities, confirming overseas results (48).

2.4 OTHER CHRYSANTHEMUM VIRUSES

Although most attention was given to the major virus or virus-like organisms infecting chrysanthemums, i.e. TAV, chrysanthemum stunt and the leaf mottle group, tests were also undertaken to determine the presence of other chrysanthemum viruses in chrysanthemums grown in the Manawatu.

Those plants grafted with 'Mistletoe' to index for chrysanthemum stunt were also grafted with the cultivar 'Good News'* in order to test for the presence of several other viruses or virus-like organisms. Although yellow leaf spot symptoms developed in 18 of the 60 plants grafted with 'Good News' the presence of rod-shaped particles of the leaf mottle group precluded any diagnosis of either chrysanthemum virus Q (78) or chrysanthemum ringspot virus (20). Symptoms of chrysanthemum rosette (18) and chrysanthemum virus E (51) were not observed on any of the 'Good News' scions.

Sap inoculation of 178 plants from 16 cultivars to P.hybrida failed to show symptoms of chrysanthemum virus C (97), tomato spotted wilt (109), or chrysanthemum latent virus (48), and routine screening for TAV by sap inoculation to C.amaranticolor failed to show the presence of chrysanthemum virus D (47,113) or tomato spotted wilt (49).

Visual examination of the chrysanthemums failed to reveal symptoms of the aster yellows group (15), tomato spotted wilt (109), chrysanthemum ringspot virus (20), or chrysanthemum virus C (97).

2.5 MIXED VIRUS INFECTION

The presence of more than one virus in chrysanthemums was observed in many cases, particularly the combination of TAV and viruses of the leaf mottle group. In all cases, flower distortion symptoms were observed but no difference was observed between these symptoms and those expressed in plants infected only with TAV. In no plant however did TAV and viruses of the leaf mottle group occur without symptom expression.

---

* Virus-free plants obtained from the Nuclear Stock Association.

Stunted plants with distorted flowers shown to be infected with both chrysanthemum stunt and TAV were observed in only 6 plants.

Electron microscopic examination of chrysanthemums infected with chrysanthemum stunt revealed the presence of leaf mottle type particles in 18 of the 60 plants examined but no difference in symptom expression was observed when compared to plants infected with chrysanthemum stunt alone.

CHAPTER 3.

TOMATO ASPERMY VIRUS

3.1 SYMPTOMS ON CHRYSANTHEMUMS

3.1.1 Natural Infection

Flower symptoms varied from slight distortion of the florets, giving and untidy appearance, to severe floret tubing, twisting and a reduction in flower size (fig. 2). Often the floret distortion was found to be more pronounced on one side of the flower, producing an almost kidney-shaped flower. Indexing to N.glutinosa and P.hybrida gave symptoms diagnostic of TAV (chapter 2.1.2).

Leaf symptoms, including mottling and flecking, were also observed in some plants showing flower distortion, but subsequent electron-microscopic examination revealed the presence of rod-shaped particles typical of the leaf mottle group of viruses, suggesting that TAV does not play an important part in the production of leaf abnormalities (46).

3.1.2 Sap Inoculation

Virus-free 'Mistletoe' and 'Good News' chrysanthemums were inoculated with sap from Nicotiana tabacum L. 'Samsun' plants infected with TAV originally isolated from the chrysanthemum 'Madame E. Rogers' showing severe flower distortion symptoms. It was found that only 4 plants out of the 10 inoculated became infected with TAV but no flower distortion symptoms were observed. Back inoculation to 'Samsun', N.glutinosa and

P.hybrida gave symptoms diagnostic of TAV (figs. 3,4,5,6). Rod-shaped particles characteristic of the leaf mottle group were not observed.

3.1.3 Graft Transmission

Scions from virus-free 'Mistletoe' and 'Good News' were grafted onto stocks of chrysanthemums showing symptoms of flower distortion and giving symptoms diagnostic of TAV on N.glutinosa and C.amaranticolor. After three months, sap inoculation from the 'Mistletoe' and 'Good News' scions to N.glutinosa and C.amaranticolor showed that TAV was present in 17 out of the 20 scions tested.

3.2 SEPARATION

The results of the indexing survey carried out on chrysanthemums in this study showed that in many plants TAV was present as part of a mixed infection, particularly with viruses of the leaf mottle group (chapter 2).

Isolates of TAV alone were obtained from 'Samsun' previously inoculated with sap from chrysanthemums showing flower distortion symptoms (48,63,79).

Electron-microscopic examination of sap from those 'Samsun' plants showing leaf mottling and distortion confirmed the absence of any leaf mottle group viruses, while inoculation to N.glutinosa and C.amaranticolor and P.hybrida ensured that TAV was present.

3.3 HOST RANGE AND SYMPTOMS

Randomly selected leaf discs from the inoculated 'Samsun' (chapter 3.2) were triturated with 0.033M Na_2HPO_4 + 0.5M DIECA, pH 7.4. The triturate was then rubbed with cotton wool pads onto carborundum dusted leaves of the test plants. All the tests plants were raised from seed (appendix IV) and inoculated at the 4-8 leaf stage when most plants were 3-9" high. Controls, inoculated with buffer solution only, were also included.

The following symptoms were observed on plants inoculated with TAV. Re-inoculation to N.glutinosa and C.amaranticolor confirmed the presence of TAV. Nomenclature based on: Bailey, L.H. Manual of Cultivated Plants (1949).

Antirrhinum majus L. 'Tom Thumb' (snapdragon).

Chlorotic spots after 3 weeks, later necrotic.

Callistephus chinensis Nees. 'Mixed Colours' (aster).

Systemic leaf mottle and flower distortion.

Capsicum frutescens L. 'Yolo Wonder' (sweet pepper).

Mild mottle followed by veinal necrosis and leaf distortion.

Celosia argentea L. 'Painted Ostrich' (celosia).

Faint diffuse chlorotic lesions.

Chenopodium amaranticolor Coste. & Reyn.

Small discrete yellow lesions on inoculated leaves after 4-5 days (fig. 7).

Chenopodium quinoa Willd.

Small discrete yellow lesions on inoculated leaves after 1 week.

Cucumis sativus L. 'Moneymaker' (cucumber).

Local chlorotic-necrotic lesions on young inoculated cotyledons after 2 weeks.

Gomphrena globosa L.

Local red-brown necrotic lesions after 3-4 weeks followed by faint systemic mottle.

Helichrysum bracteatum Andr. (strawflower).

Systemic leaf mottle and distortion.

Lycopersicon esculentum Mill. 'Potentate' (tomato).

Severe leaf mottle, slight distortion, inhibition of apical growth resulting in a bushy plant.

Nicotiana clevelandii Gray.

Yellow necrotic lesions after 7 days followed by interveinal chlorosis and subsequent general leaf chlorosis.

Nicotiana glutinosa L. x Nicotiana clevelandii Gray.

Faint chlorotic spots after 1 week followed by severe systemic mottle and leaf distortion.

Nicotiana glutinosa L.

Diffuse local chlorotic lesions after 2 weeks followed by systemic mottle, severe leaf distortion and tendril-like leaves (figs. 3,4).

Nicotiana tabacum L. 'Samsun' (tobacco).

Local chlorotic lesions after 1 week followed by systemic leaf mottle and distortion (fig. 6).

Nicotiana tabacum L. 'White Burley' (tobacco).

Local chlorotic lesions after 1 week, followed by systemic leaf mottle and distortion.

Petunia hybrida var. nana compacta Vilm. 'Rose of Heaven' (petunia).

Diffuse grey chlorotic lesions after 2 weeks followed by systemic mottle and bleached flowers (fig. 5).

Primula obconica Hance.

Systemic leaf mottle after 3 weeks.

Primula malacoides Franch.

Systemic leaf mottle after 3 weeks.

Senecio cruentis D.C. 'Yates Large Flowered' (cineraria).
Faint pink tinge appearing along the main leaf veins and slight plant dwarfing. Some flowers showed petal distortion.

Tagetes erecta L. 'Orange Mum' (marigold).
Local chlorotic lesions after 10 days.

Tetragonia expansa Murr. 'Everlasting' (N.Z. spinach).
Discrete local chlorotic/white lesions after 2-3 weeks.

Vigna sinensis Endl. 'Black-eye' (cowpea).
Local red-necrotic lesions after 3-7 days but no TAV was recovered from infected leaves.

Zinnia elegans var. dahliiflora Jacq. (zinnia).
Faint systemic leaf mottle after 2 weeks.

No symptoms were observed, nor was TAV re-isolated from the following inoculated species:

Brassica oleracea var. capitata L. 'Succession'
'Drumhead'
'Flower of Spring' (cabbage).

Brassica oleracea var. botrytis L. 'Deep Heart'
'Phenomenal 4 Month' (cauliflower).

Brassica pekinensis Rupr. 'Chi Hi Li' (chinese cabbage).

Cheiranthus cheiri L. 'Paris Market' (wallflower).

Dahlia pinnata Cav. 'Unwins Ideal' (dahlia).

Dianthus barbatus L.

Helianthus annus L. (sunflower).

Lactuca sativa L. 'Great Lakes'
'Imperial Triumph'
'Yatesdale' (lettuce).

Lupinus polyphyllus Lindl. 'Russell Mixed' (lupin).

Pisum sativus L. 'Green Feast' (pea).

Vicia faba L. 'Atlas Early'
'Coles Early'
'Exhibition Longpod' (broad bean).

Studies on the host range of TAV have been undertaken by several workers (23,37,46,97) and minor discrepancies have been recorded. Govier (37) reported that Antirrhinum majus, Senecio cruentis and Vigna sinensis were not susceptible to TAV, but results of this study show that the three species give symptoms similar to those described by Hollings (46), and were also recorded as susceptible to TAV by Brierley et al (23). In this study, however, TAV could not be re-isolated from the inoculated V.sinensis plants and only 1 of the 3 S.cruentis plants gave symptoms of TAV infection. Brierley et al (23) reported that Lactuca sativa was susceptible to TAV, but no symptoms were observed in this study, nor could TAV be re-isolated from the inoculated plants. In general, however, the host range of TAV determined in this study agrees with overseas reports for TAV, with small differences attributable to either the influence of environment and the variation in susceptibility between cultivars, as suggested by Govier (37), or the occurrence of different strains of TAV as suggested by several workers (63,70, 104). Such biological variants have also been reported to other plant viruses (26,27).

3.4 APHID TRANSMISSION

N.glutinosa inoculated with infected 'Samsun' sap was used as a source of TAV for the determination of the aphid transmission characteristics. Green peach aphids (M.persicae) were reared on Brassica pekinensis (chinese cabbage) seedlings in aphid cages, and the procedures used in determining the aphid transmission characteristics of TAV were based on those outlined by Bos, Hagedorn and Quantz (7). The results of these tests are shown in tables 12, 13 and 14.

3.4.1 Acquisition Threshold

This is the minimum time necessary for a vector to feed upon, or have access to, a virus source in order to transmit the virus.

After two hours starvation, aphids were given access to infected source plants for periods of half a minute to 60 minutes and transferred (5/plant) to healthy N.glutinosa plants for an inoculation-access period of 30 minutes. The aphids were then killed by spraying the plants with a 2.2 g/litre solution of 'Lannate'.*

TABLE 12. Effect of virus acquisition-access period on transmission of TAV by M.persicae.

N.glutinosa plants	Acquisition-access period (minutes)				
	0.5	1	5	30	60
Infected/Exposed	0/10	2/10	4/5	5/5	4/5

These results showed that M.persicae required an acquisition-access period of between 0.5 and 1 minute to transmit TAV from N.glutinosa to N.glutinosa.

* Trade name for Methomyl.

3.4.2 Inoculation Threshold

Following 2 hours starvation, aphids were given an acquisition-access period of 2-5 minutes on N.glutinosa infected with TAV, and then transferred (5/plant) to healthy N.glutinosa for inoculation periods of between 1 and 120 minutes before being killed.

TABLE 13. Effect of virus inoculation-access period on transmission of TAV by M.persicae.

N.glutinosa plants	Inoculation-access period (minutes)				
	1	5	30	60	120
Infected/Exposed	0/10	3/10	4/5	5/5	4/5

These results showed that after acquiring TAV from N.glutinosa, M.persicae required an inoculation-access period of between 1 and 5 minutes in order to transmit TAV to N.glutinosa.

3.4.3 Effect of Pre-acquisition Starvation

To determine the effect of starvation on the acquisition of TAV by M.persicae the aphids were starved for periods of 0-8 hours before being transferred (5/plant) to infected N.glutinosa for acquisition-access periods of 1 and 5 minutes. An inoculation-access period of 30 minutes was permitted before the aphids were killed.

TABLE 14. Effect of pre-acquisition starvation on transmission of TAV by M.persicae.

Acquisition-access period (minutes)	N.glutinosa plants	Starvation Period (hours)				
		0	1	2	4	8
1	Infected/Exposed	0/10	2/10	1/5	2/5	2/5
5	Infected/Exposed	5/10	6/10	4/5	5/5	4/5

These results showed that starvation of M.persicae for 1-2 hours increased the efficiency of TAV transmission as determined by the number of N.glutinosa plants developing symptoms of TAV infection.

3.4.4 Transmission to Chrysanthemum

Using infected N.glutinosa as a source of TAV, aphids, after starvation for 2 hours, were given an acquisition-access period of 2-5 minutes before being transferred to healthy 'Mistletoe' chrysanthemums for an inoculation-access period of 2 hours. Indexing the chrysanthemums showed that 3 out of 8 plants became infected with TAV.

Aphid transmission tests, using M.persicae confirmed that TAV is non-persistent or stylet-borne, in that transmission occurred after an acquisition-access period of less than 1 minute (table 12) and after 5 minutes inoculation-access feeding (table 13), with no apparent latent period characteristic of persistent or circulative viruses (81). Aphid transmission to chrysanthemum, using M.persicae, under experimental conditions, occurred but with less than 50% efficiency. Similar results have been obtained overseas (23,37,46).

3.5 PROPERTIES IN VITRO

The assay host used in the determination of the *in vitro* properties of TAV was *C.amaranticolor*. In all experiments, 4 replicates were used, and control inoculations of buffer were also included. Random tissue samples were taken from the source plants with a 5 mm cork borer. The tissue samples were triturated with a phosphate buffer at a ratio of 1 : 1 and rubbed with cotton wool pads onto carborundum dusted half-leaves of *C.amaranticolor*. Local lesions were counted after 6-8 days. Several sources of TAV and several buffer solutions were used to determine their effect on the physical property determinations.

3.5.1 Thermal Inactivation Point

The conventional method of determining the thermal inactivation point of TAV was followed using 0.033M Na_2HPO_4, pH 7.3, viz. two 1 ml aliquots of triturated tissue were taken for each temperature-treatment and these were exposed to the appropriate temperature for 10 minutes in thin-walled test-tubes, and then cooled in iced water. The treated sap was subsequently assayed for TAV infectivity.

TABLE 15. Effect of different source plants on the thermal inactivation point of TAV.

Source	Temperature (°C)							
	20	50	55	60	65	70	75	80
'Samsun'	40*	110	84	45	38	1	0	0
N.glutinosa	53	176	148	81	54	2	0	0
'Mistletoe'	264	217	183	92	49	2	0	0

* Figures are mean lesion counts/half-leaf.

Infectivity was lost when the triturate was heated to 70 C but not 65 C. The source of the virus did not appear to influence the thermal inactivation point.

3.5.2 Dilution End Point

The dilution end point of TAV was determined by diluting infective sap with a phosphate buffer until no infection (i.e. no local lesions on C.amaranticolor) were observed (fig. 7).

TABLE 16. Effect of different source plants and buffer solutions on the dilution end point of TAV.

Source	Buffer (adjusted to pH 7.2)	Dilution						
		1:1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
'Samsun'	0.05M K_2HPO_4	32*	16	12	4	2	0	0
	0.033M Sörensen Buffer	398	258	183	9	4	1	0
	0.033M Sörensen Buffer			504	69	2	0	0
'Mistletoe'	0.05M K_2HPO_4	40	26	15	3	0	0	0
	0.033M Sörensen Buffer	178	86	37	2	2	0	0

* Figures are mean lesion counts/half-leaf.

The dilution end point of TAV in tobacco sap was found to be between 10^{-4} and 10^{-5}, regardless of which buffer solution was used as a diluent, but the Sörensen phosphate buffer (Na_2HPO_4 + NaH_2PO_4) gave a more distinct drop in lesion numbers towards the end point. In chrysanthemum sap the dilution end point occurred between 10^{-3} and 10^{-4} when young tissue was used. A sharp drop in lesion numbers was noted in all treatments at 10^{-3}, with relatively fewer lesions appearing at higher dilutions.

3.5.3 Longevity in vitro

This experiment was designed to determine the infectivity of TAV in chrysanthemum and tobacco tissue triturates. The inclusion of DIECA in the triturate was made to determine the effect of a chelating agent on TAV infectivity.

TABLE 17. Effect of different source plants and buffer solutions on the longevity in vitro of TAV.

Source	Buffer (adjusted to pH 7.3)	Time (hours)								
		0	2	6	12	24	48	96	192	384
'Samsun'	0.033M KH_2PO_4	100*	98	83	42	34	10	0	0	0
	0.033M KH_2PO_4 + 0.05M DIECA	152	301	307	286	302	212	156	0	0
'Mistletoe'	0.033M KH_2PO_4	64		12	6	0	0			
	0.033M KH_2PO_4 + 0.05M DIECA	66		30	21	6	5	0	0	

* Figures are mean local lesion counts/half-leaf.

In tobacco sap TAV lost its infectivity after 48 hours when stored at room temperature while the presence of DIECA extended the longevity in vitro to 96 hours.

In chrysanthemum sap TAV lost its infectivity after 12 hours at room temperature while DIECA extended the infective period to 48 hours.

The results of these tests on the *in vitro* properties of TAV are summarised and compared with overseas reports in table 18. Although different phosphate buffer solutions had no effect on the results of these tests, the addition of DIECA significantly increased the longevity *in vitro* of TAV in chrysanthemum sap (table 17). This suggests the presence of a virus inactivator in chrysanthemum sap. The difference in dilution end points observed between 'Samsun' and chrysanthemum sap (table 16) also suggests the presence of such an inactivator in chrysanthemum sap, while the lack of inactivation in the heat treated sap (table 15) could be explained by the heat denaturation of the virus inactivator, suggesting the involvement of an enzyme in the inactivation process. These results are confirmed by overseas work on virus inactivators and the effect of DIECA on virus infectivity. It is thought that DIECA acts by chelating the copper necessary for the quinone-producing polyphenoloxidase activity, and that the quinones inactivate the virus by combining with the viral protein coat (77,82,86).

TABLE 18. Properties of TAV *in vitro*

Source	Noordam (97)	Hollings (46)	Govier (37)	Brierley (13)	Oertel (99)	Hollings & Stone (63)	Lunn
	Dilution end point						
Chrysanthemum	10^{-3}-10^{-4}	10^{-3}-10^{-4}				10^{-2}-10^{-3}	10^{-3}-10^{-4}
'Samsun'	10^{-3}-10^{-5}	10^{-3}-10^{-4}	10^{-3}-10^{-4}	10^{-3}-10^{-4}	10^{-3}-10^{-4}	10^{-4}-10^{-5}	10^{-4}-10^{-5}
	Longevity *in vitro* (days)						
Chrysanthemum	Less than $\frac{1}{2}$						$\frac{1}{2}$-1
'Samsun'		7-14	2	3-6	6-12	2-6	2-8
	Thermal inactivation point (°C)						
Chrysanthemum							65-70
'Samsun'	65-70	65-70	65-70	65-70	55-60	50-60	65-70

3.6 INFECTIVITY

3.6.1 Buffer Solution

A number of phosphate buffers have been used overseas in the study of TAV, and these are often not elaborated on, other than to cite 'Phosphate buffers' (38,50,114). Experiments were therefore undertaken to determine the effect of different phosphate buffers on the infectivity of TAV in 'Samsun' and chrysanthemum 'Mistletoe' sap, as determined by local lesion development on C.amaranticolor half-leaves.

The four buffer solutions tested were:

i) disodium hydrogen phosphate (Na_2HPO_4)

ii) dipotassium hydrogen phosphate (K_2HPO_4)

iii) sodium dihydrogen phosphate (NaH_2PO_4)

iv) Sörensen's phosphate buffer (1 : 1 Na_2HPO_4 : KH_2PO_4)

The results of the physical property determinations (chapter 3.5) indicated the presence of a virus inactivator in chrysanthemum sap and DIECA was therefore included in this experiment, the results of which are shown in table 19.

In chrysanthemum sap, very little difference was observed in lesion counts between the various phosphate buffers or between the different concentrations of any one buffer solution but the addition of DIECA markedly increased the infectivity of the chrysanthemum triturate, confirming the presence of a virus inactivator of the phenol type in chrysanthemum sap. Small differences were observed between the various buffer solutions when DIECA was present, with 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, giving the highest number of local lesions.

TABLE 19. Effect of different buffer solutions on infectivity of TAV in 'Mistletoe' and 'Samsun' sap.

Buffer	Lesion Counts	
	'Mistletoe'	'Samsun'
Water	14*	17
0.066M K_2HPO_4 pH 7.2	20	24
0.033M Sörensen buffer pH 7.2	24	85
0.033M Na_2HPO_4 pH 7.2	26	138
0.05M Na_2HPO_4 pH 7.4	32	165
0.066M Na_2HPO_4 pH 7.3	36	135
0.033M KH_2PO_4 pH 7.3	32	109
0.066M Sörensen buffer + 0.05M DIECA pH 7.4	29	124
0.033M Na_2HPO_4 + 0.05M DIECA pH 7.4	188	361
0.05M Na_2HPO_4 + 0.05M DIECA pH 7.4	148	307
0.066M Na_2HPO_4 + 0.05M DIECA pH 7.3	152	216
0.033M KH_2PO_4 + 0.05M DIECA ph 7.3	139	182

* Figures are mean lesion counts/C.amaranticolor half-leaf.

In 'Samsun' sap the effect of DIECA was less marked but more variation was observed between the different phosphate buffers with Na_2HPO_4 giving the best results. The variation in infectivity between the different Na_2HPO_4 buffers used could be due to the different pH values, for those Na_2HPO_4 buffers with pH values of 7.4 showed higher lesion counts than did those with pH values of 7.2 or 7.3.

The phosphate buffer giving the best results in terms of lesion numbers on C.amaranticolor was 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, and unless otherwise stated, this was used for all sap inoculations in this study.

3.6.2 Pre-inoculation Dark Period

There are numerous reports of increased susceptibility of plants to mechanically inoculated virus, following a pre-inoculation period in darkness (3). The effect of darkness on TAV lesion development was determined by comparing the dilution end points of TAV in 'Samsun' sap using 0.033M Sörensen buffer, pH 7.2, as determined by local lesion number on C.amaranticolor with, and without pre-inoculation dark periods (table 20).

TABLE 20. Effect of a pre-inoculation dark period on the dilution end point of TAV.

Pre-inoculation dark period	Dilution					
	10^{-2}	$5x10^{-3}$	10^{-3}	$5x10^{-4}$	10^{-4}	$5x10^{-5}$
24 hours light	450*	113	51	3	1	0
24 hours dark	544	81	68	4	2	0

* Figures are mean lesion counts/C.amaranticolor half-leaf.

Although no difference was observed in the dilution end point of TAV following a pre-inoculation dark period, the lesions developed 1 day earlier in the dark-treated plants, confirming reports that such treatment influences the susceptibility of the inoculated plant rather than the infectivity of the tissue triturate (3).

3.7 MULTIPLICATION

The rate of virus increase in N.glutinosa and the chrysanthemum 'Mistletoe' was determined by inoculation to C.amaranticolor at various intervals and the results are shown in table 21.

TABLE 21. The rate of TAV increase in N.glutinosa and chrysanthemum 'Mistletoe'.

Source	Time (days)										
	0	3	7	9	13	17	19	21	24	27	30
'Mistletoe'	34*	58	121	181	186	207	226	205	320	531	478
N.glutinosa	30	15	24	40	65	74	123	164	195	221	251

* Figures are mean local lesion counts/half-leaf.

These results indicate that TAV increases at a faster rate in chrysanthemum than in N.glutinosa plants.

Results of TAV infectivity tests by Grogan, Uyemoto and Kimble (38) show that the infectivity titre of TAV in N.glutinosa, as determined by lesion development on V.sinensis (cowpea), increases to a maximum after 10 days and then falls rapidly until after 14 days only a few local lesions are produced. Although the results of this study (table 21) show that the virus titre increases after 17 days, the two results need not conflict because of the difference in sampling methods. In this experiment, random leaf discs were taken from inoculated and un-inoculated leaves in order to obtain an assessment of the virus multiplication in the plant in toto. This would mask any localised particle increase (or decrease) in the inoculated leaves. Although it is not stated, Grogan et al (38) probably sampled tissue only from inoculated N.glutinosa leaves.

CHAPTER 4.

CHRYSANTHEMUM STUNT

4.1 SYMPTOMS ON CHRYSANTHEMUMS

Chrysanthemums shown to be infected with chrysanthemum stunt by graft indexing with 'Mistletoe' were often stunted in growth. The infected plants showing these symptoms were usually up to two-thirds the size of healthy chrysanthemums. The other distinctive feature of chrysanthemum stunt infection was the early development of flowers in the stunted plants. Flowering occurred up to 10 days earlier than in healthy plants, and they were often smaller in size (fig. 8). Flower distortion in stunted plants was observed in several plants but indexing tests showed the presence of TAV in these cases.

4.1.1 Graft Transmission

The lack of a suitable local lesion host for chrysanthemum stunt has necessitated the use of grafting as a means of detecting the presence of chrysanthemum stunt in chrysanthemums. Several experiments were undertaken to determine a more efficient and more rapid method of graft indexing for chrysanthemum stunt.

A comparison was made between the grafting technique described in chapter 2.2.2 and the reciprocal method used by Keller (79) and Brierley and Olsen (15), where infected scions were grafted to virus-tested indicator stock plants.

There was no difference in the number of successful grafts or in symptom development, for in both cases infected plants gave symptoms within 6-8 weeks when the leaves above the graft union were removed.

4.1.2 Defoliation

The latent period for chrysanthemum stunt in grafted chrysanthemums can be from 2-10 months (51), but Keller (79) has shown that in 'Blanche' this latent period can be shortened to 6-8 weeks by removing most of the leaves from the grafted scion (fig. 9).

By removing most of the leaves from one of the two 'Mistletoe' scions grafted to each infected plant, the effect of defoliation on the latent period of chrysanthemum stunt was determined. Three cultivars, 'Shantung', 'Madame E. Rogers' and 'Kathleen Dowd' (5 plants/cultivar) were used (table 22).

TABLE 22. Effect of defoliation on the latent period of chrysanthemum stunt in 'Mistletoe' scions.

Scion Treatment	Latent period (weeks)									
	4	5	6	7	8	9	10	12	14	16
	Number of scions with symptoms									
Defoliation	2	5	2	5	1	0	0	0	0	0
No defoliation	0	0	0	1	1	1	3	3	1	2

The three cultivars used, showed no obvious differences in symptom expression, and these results showed that removing the scion leaves (except for the top 2-3 young developing leaves) considerably reduced the latent period of chrysanthemum stunt in 'Mistletoe' scions.

Defoliation was therefore used in all tests where graft indexing for chrysanthemum stunt was undertaken (chapter 2.2.2).

4.1.3 Aphid Transmission

Brierley and Smith (16) reported that Rhopalosiphum rufomaculatum Wilson was a vector for chrysanthemum stunt, with an efficiency of 30%, and that occasional transfer by 4 other aphid species could occur. However, in later studies they reported that these results were the result of contamination of the control and test plants (the nature of which was not discussed) and that aphid transmission of chrysanthemum stunt was not known (79). No other vector has been reported for chrysanthemum stunt (15).

In this study, because R.rufomaculatum aphids were not available, M.persicae was used to determine the aphid transmissibility of chrysanthemum stunt. After starvation for 2 hours, green peach aphids were given an acquisition-access period of 5 minutes on stunt infected 'Madame E. Rogers'. The aphids were then transferred to 'Mistletoe' plants for an inoculation-access period of 30 minutes. No symptoms of stunt developed on the 'Mistletoe' plants and these were discarded after 6 months.

4.1.4 Sap Transmission

Keller's work on the properties of chrysanthemum stunt has shown that symptom expression following mechanical transmission to virus Q-infected 'Blanche' was sufficiently reliable to be used in determining the physical properties of chrysanthemum stunt (79). Sap transmission of stunt to the cultivars 'Seagull' and 'Mistletoe' has also been reported (10,67).

In this study, the cultivar 'Mistletoe' was selected because of the distinctive fleck symptoms produced when inoculated with chrysanthemum stunt.

Plants were partially defoliated above the inoculated leaves in order to reduce the latent period of stunt (79). Three chrysanthemum cultivars, known to be infected with stunt, were used as source plants, and random discs of leaf tissue were taken to minimise the effect of any uneven distribution of stunt particles within the plant. The leaf tissue was triturated with 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, and rubbed onto carborundum dusted 'Mistletoe' leaves (table 23).

TABLE 23. Symptom expression in 'Mistletoe' after sap-inoculation from stunt-infected chrysanthemums.

Source	Latent period (weeks)								
	4	6	8	10	12	14	16	18	20
	Number of plants with symptoms								
'Madame E. Rogers'	0	1	2	1	1	0	0	0	0
'Shantung'	0	1	2	0	1	1	0	0	0
'Kathleen Dowd'	0	1	0	1	1	0	0	0	0

Since only 13 out of the 36 'Mistletoe' plants inoculated with chrysanthemum stunt, developed faint symptoms, the experiment was repeated using leaf tissue from 'Mistletoe' scions showing leaf fleck symptoms. In this experiment, 15 out of 36 'Mistletoe' plants developed symptoms. This low incidence of symptom development in sap-inoculated 'Mistletoe' leaves could be attributed to the unsatisfactory growth conditions under which the indicator plants were grown.

4.2 HOST RANGE AND SYMPTOMS

The long latent period shown by chrysanthemum stunt in grafted 'Mistletoe' has led to attempts at discovering a satisfactory local lesion host. Host range studies have been reported by several workers (9,10,79).

In this study, sap from stunt-infected 'Fred Shoesmith' and 'Shantung' was triturated with 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, buffer. Inoculations were then made to carborundum dusted leaves of three plants of the following:

Antirrhinum majus L. 'Tom Thumb' (snapdragon).

Callistephus chinensis Nees. 'Mixed Colours' (china aster).

Capsicum frutescens L. 'Yolo Wonder' (sweet pepper).

Celosia argentea L. 'Painted Ostrich' (celosia).

Cheiranthus cheiri 'Paris Market' (wallflower).

Chenopodium amaranticolor Coste. & Reyn.

Chenopodium quinoa Willd.

Cucumis sativus L. 'Moneymaker' (cucumber).

Dahlia 'Unwins Ideal' (dahlia).

Gomphrena globosa L.

Helianthus annuus L. (sunflower).

Helichrysum bracteatum Andr. (strawflower).

Lactuca sativa L. 'Great Lakes'
'Imperial Triumph'
'Yatesdale' (lettuce).

Lupinus polyphyllus Lindl. 'Russell Mixed' (lupin).

Lycopersicon esculentum Mill. 'Potentate' (tomato).

Nicotiana clevelandii Gray.

Nicotiana glutinosa L.

Nicotiana glutinosa L. x N.clevelandii Gray.

Nicotiana tabacum L. 'Samsun' (tobacco).

Petunia hybrida Vilm. var. nana compacta 'Rose of Heaven' (petunia).

Pisum sativus L. 'Green Feast' (green pea).

Primula obconica Hance.

Primula malacoides Franch.

Senecio cruentis D.C. 'Yates Large Flowered' (cineraria).

Tagetes erecta L. 'Orange Mum' (african marigold).

Tetragonia expansa Murr. 'Everlasting' (New Zealand spinach).

Vicia faba L. 'Coles Early'
'Exhibition Longpod'
'Atlas Early' (broad bean).

Zinnia elegans Jacq. var. dahliiflora (zinnia).

Keller (79) reported S.cruentis and Dahlia as giving symptoms when inoculated with stunt but in this study only S.cruentis developed symptoms (faint chlorotic-white lesions) after 5 weeks in 1 of the 4 plants inoculated. The failure of Dahlia to develop symptoms could be due to the differences in the Dahlia cultivars used or the unsuitable environmental conditions.

Further inoculations to S.cruentis showed that 4 plants out of 10 developed symptoms of chrysanthemum stunt.

4.3 STARCH LOCAL LESION TEST

Lawson (85) used S.cruentis (cineraria) to test the starch local lesion method for detecting chrysanthemum stunt. Such a technique has the advantage of reducing the long delay involved in graft indexing to a systemic host and could provide a valuable assay method.

Inoculation was made to 12 S.cruentis ('Yates Large Flowered') when the leaves were 5-6 cm in width, with sap from each of 5 chrysanthemum cultivars known to be infected with stunt.

The inoculated plants were exposed to 24 hours darkness after 11 days and the leaves harvested. Leaves were boiled in ethyl alcohol for 2-3 minutes until bleached and then stained with a solution of 0.5% iodine and 2% potassium iodide. After washing and drying, the leaves were examined using back-illumination to highlight stained starch deposits.

The experiment was repeated using half-leaf inoculations and the leaves were harvested at different intervals in an attempt to reduce the amount of non-specific starch which obscured the local lesions in the first experiment. The results are shown in table 24.

TABLE 24. Chrysanthemum stunt infectivity in S.cruentis as determined by starch local lesions.

Source	Number of half-leaves inoculated	Number of half-leaves showing local lesion symptoms		
		11 days	15 days	total
'Mauve Dawn'	30	4	5	9
'Sussex Pride'	30	3	2	5
'Madame E. Rogers'	30	5	6	11
'Sussex Pink'	30	4	3	7
'Sussex Amber'	30	5	5	10
Total	150	21	21	42

These results showed that the presence of non-specific starch deposits obscured the determination of local lesions caused by chrysanthemum stunt. No difference in the amount of non-specific starch accumulation was observed between the leaves harvested 11 days after inoculation and those harvested after 15 days.

Of the indexing methods for chrysanthemum stunt examined in this study the most reliable still appears to be graft inoculation using 'Mistletoe', although this is far from ideal.

4.4 PROPERTIES IN VITRO

The unreliability of 'Mistletoe' as an indicator for stunt when sap inoculation is used and the glasshouse space required to keep sufficient 'Mistletoe' plants to make any results significant, precluded any attempt at determining the physical properties of chrysanthemum stunt in New Zealand chrysanthemums.

Overseas reports indicate the following properties *in vitro* for chrysanthemum stunt.

TABLE 25. Properties *in vitro* of chrysanthemum stunt in chrysanthemum sap.

	Keller 1953 (79)	Brierley 1953 (13)
Thermal inactivation point	96-100 C	96-98 C
Dilution end point	10^{-2}-10^{-3}	10^{-5}
Longevity *in vitro* at 21 C	55 days	49-56 days
at 3 C	100+ days	
at 0 C	1 year +	
Longevity in dired tissue	2 years +	

CHAPTER 5.

VIRUSES OF THE LEAF MOTTLE GROUP

5.1 SYMPTOMS ON CHRYSANTHEMUMS

5.1.1 Natural Infection

Visual observation of 20 chrysanthemum cultivars revealed isolated instances of leaf mottling symptoms in 7 cultivars (chapter 2.3.1). Electron microscopic examination of samples from these plants showed the presence of rod-shaped particles of the leaf mottle type (fig.13). Symptomless infection was also observed in 22 cultivars through the electron microscopic examination of leaf-dips.

5.1.2 Sap Inoculation

Sap inoculations were made to 'Good News' from 'Shantung' showing symptoms of vein mottling, and from 'Rivalry' showing symptoms of chlorotic mottling. Symptoms characteristic of the leaf mottle group (yellow vein banding, mottling and distortion (11)) failed to develop although rod-shaped particles of the leaf mottle type were observed in all cases. The lack of symptom expression could be due to the unsatisfactory conditions under which the indicator plants were grown, for inoculations made in the winter also failed to produce symptoms.

5.1.3 Graft Transmission

Grafting plants of 'Shantung' and 'Regalia' with 'Good News' resulted in the production of diffuse chlorotic leaf mottle symptoms after 2 months (fig 11). No observable differences in symptom expression were observed between the 'Shantung' and 'Regalia' plants with leaf mottle symptoms, and those infected with rod-shaped particles but not showing symptoms. However, when these two plants - 'Shantung' and 'Regalia' - were indexed for TAV infection by sap inoculation to N.glutinosa and C.amaranticolor, symptoms of TAV developed, making further screening necessary in order to obtain plants for determination of the characteristics of the leaf mottle group of viruses.

5.2 SEPARATION

After screening 26 chrysanthemums for stunt by grafting with 'Mistletoe' and for TAV by inoculation to N.glutinosa and C.amaranticolor, two plants were obtained showing the presence of rod-shaped particles of the leaf mottle-type, but free from both TAV and chrysanthemum stunt. These plants - 'Shantung' showing faint vein mottle symptoms and 'Ethyl Edmonds' showing no symptoms - were used as sources of leaf mottle virus.

Grafting with 'Good News' gave symptoms similar to those described in the previous section (5.1.3) showing that the presence of TAV did not affect the development of leaf mottle symptoms in 'Good News' grafted to stocks infected with viruses of the leaf mottle group.

5.3 HOST RANGE AND SYMPTOMS

'Shantung' chrysanthemum was used as a source of the leaf mottle virus (chapter 5.2). Infected sap was triturated with 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, buffer and inoculated to carborundum dusted leaves of the test plants.

The following results were obtained when 3 plants of each test species were inoculated. Leaf-dip examinations confirmed the presence of rod-shaped particles of the leaf mottle group.

Nomenclature based on: Bailey, L.H. Manual of Cultivated Plants (1949).

Antirrhinum majus L. 'Tom Thumb' (snapdragon).

Yellow-brown semi-necrotic lesions after 2 weeks. No systemic infection was observed by leaf-dip tests.

Helichrysum bracteatum Andr. (strawflower).

Black necrotic spots after 2 weeks in 1 plant of the 3 inoculated. No systemic infection observed by leaf-dip tests.

Nicotiana glutinosa L.

No symptoms but rod-shaped particles observed in leaf-dip tests.

Petunia hybrida var. nana compacta Vilm 'Rose of Heaven' (petunia).

Chlorotic local lesions after 2-5 weeks in 2 of 6 plants inoculated and lesions spreading along midrib in one leaf.

Senecio cruentis D.C. 'Yates Large Flowered' (cineraria).

No symptoms observed but rod-shaped particles observed in leaf-dip tests.

Tetragonia expansa Murr. 'Everlasting' (N.Z. spinach).

Few chlorotic lesions after 2 weeks. No systemic infection was observed by leaf-dip tests.

No symptoms were observed in each of 3 plants of the following species when inoculations were made with infected 'Shantung' sap.

Brassica oleracea var. capitata L. 'Succession' (cabbage).

Brassica oleracea var. botrytis L. 'Deep Heart' (cauliflower).

Brassica pekinensis Rupr. 'Chi Hi Li' (chinese cabbage).

Callistephus chinensis Nees. 'Mixed Colours' (aster).

Capsicum frutescens L. 'Yolo Wonder' (sweet pepper).

Cheiranthus cheiri L. 'Paris Market' (wallflower).

Celosia argentea L. 'Painted Ostrich' (celosia).

Chenopodium amaranticolor Coste. & Reyn.

Chenopodium quinoa Willd.

Cucumis sativus L. 'Moneymaker' (cucumber).

Dahlia pinnata Cav. 'Unwins Ideal' (dahlia).

Dianthus barbatus L.

Gomphrena globosa L.

Helianthus annuus L. (sunflower).

Lupinus polyphyllis Lindl. 'Russell Mixed' (lupin).

Lactuca sativa L. 'Great Lakes' (lettuce).

Lycopersicon esculentum Mill. 'Potentate' (tomato).

Nicotiana tabacum L. 'Samsun' (tobacco).

Pisum sativum L. 'Green Feast' (pea).

Primula malacoides Franch.

Tagetes erecta L. 'Orange Mun' (marigold).

Vicia faba L. 'Atlas Early'
'Coles Early'
'Exhibition Longpod' (broad bean).

Vigna sinensis Endl. 'Black-eye' (cowpea).

Zinnia elegans var. dahliiflora Jacq. (zinnia).

The symptoms developed in Antirrhinum majus and Helichrysum bracteatum agree with Noordam's results for chrysanthemum virus B (97). Hollings (48) however, attributes these symptoms to chrysanthemum latent virus, which also gives symptoms on Chenopodium amaranticolor, Gomphrena globosa, Celosia argentea, Nicotiana glutinosa and Nicotiana tabacum, none of which gave symptoms in this study. No symptoms developed on Callistephus chinensis (nor were rod-shaped particles observed in leaf-dip tests) which has been recorded by Noordam (97) as a host for chrysanthemum virus B. Hollings (48) records Vicia faba as a host for chrysanthemum virus B but not for chrysanthemum vein mottle, and no infection was observed on Vicia faba in this study. These results suggest that the host range of the leaf mottle virus present in the two cultivars examined is comparable to that reported for chrysanthemum virus B, but the lack of symptom expression in V.faba suggests a relationship with Hollings' chrysanthemum vein mottle.

Results of the host range study indicated a low incidence of symptoms developing in P.hybrida, which has been used overseas as a local lesion host (19,40,48,97,99) and several P.hybrida cultivars were tested for their susceptibility to viruses of the leaf mottle group.

5.4 SYMPTOMS ON P.hybrida

Initial sap inoculation tests from 'Shantung' to the P.hybrida cultivar 'Fire Chief' showed that symptom expression of chlorotic local lesions characteristic of the leaf mottle viruses was erratic in development, particularly during summer. Consequently several other petunia cultivars were tested for their reaction to leaf mottle infection (table 26).

TABLE 26. Symptom development on several cultivars of P.hybrida inoculated with leaf mottle virus.

Cultivar	Symptom development	
	Summer	Winter
'Rose of Heaven'	1*/10	3/10
'Fire Chief'		1/10
'Colorama'	0/10	1/10
'Rosy Morn' (P.multiflora)		0/10

* Number of plants developing lesions/total number inoculated.

Hollings (48) has reported better symptom development in the winter than in the summer and this was confirmed in these studies, when a total of 5 out of 40 plants developed symptoms compared with 1 out of 20 in the summer. The petunia cultivar 'Rose of Heaven' gave the best symptom development although only 3 plants out of 10 developed symptoms. Leaf-dip examinations of inoculated leaves from these 10 plants showed the presence of rod-shaped particles in 7 plants, suggesting a variation in the susceptibility of P.hybrida to viruses of the leaf mottle group. This variation in symptom expression and the difficulty in transmission during the summer have been reported overseas (48) but not to such an extent.

5.5 APHID TRANSMISSION

Several species of aphid have been reported as vectors for leaf mottle viruses, including Myzus persicae, Myzus solani Kltb. Macrosiphum euphorbiae Ths., Rhopalosiphum rufomaculatum and Macrosiphoniella sanborni, although the efficiency of transmission is generally below 30% (19,48,89).

In this study, 2 experiments were undertaken at different times of the year to determine the transmission characteristics of the leaf mottle virus infecting 'Shantung' and 'Ethyl Edmonds' chrysanthemums.

After starvation for 2 hours, M.persicae were given an acquisition-access period of 5 minutes on the infected plants before being transferred (10/plant) to 'Good News' and P.hybrida test plants for an inoculation-access period of 30 minutes (table 27).

TABLE 27. Transmission of the leaf mottle virus from 'Shantung' and 'Ethyl Edmonds' by M.persicae.

Source	Test plant			
	'Good News'		P.hybrida	
	Winter	Summer	Winter	Summer
'Ethyl Edmonds'	1*	0	1	0
'Shantung'	1	0	2	0

* Figures are number of plants developing symptoms on P.hybrida/27 plants exposed.

No symptoms were observed in any of the 'Good News' plants inoculated although sap inoculation to P.hybrida from 'Good News' inoculated in the winter, gave symptoms diagnostic of leaf mottle viruses from 2 of the chrysanthemum plants.

Three of the P.hybrida test plants developed chlorotic local lesions when aphid transmission tests were carried out in the winter.

5.6 PROPERTIES IN VITRO

The low incidence of symptom expression in P.hybrida made the determination of the physical properties of the leaf mottle virus from 'Shantung' solely on the development of lesion production a difficult matter. Some of these properties were determined by sap inoculation to P.hybrida and leaf-dip examination of the inoculated leaves to confirm the presence of rod-shaped particles of the leaf mottle type. This method, although not as accurate as lesion assessment, in that the failure to observe rod-shaped particles may not mean the plant is free from infection (40), gave an indication of the dilution end point (table 28) and the longevity in vitro (table 29) of the leaf mottle virus.

5.6.1 Dilution End Point

Using young 'Shantung' leaves as a source of the leaf mottle virus, 5 P.hybrida plants were inoculated with sap diluted with 0.033M Na_2HPO_4, pH 7.3, buffer. After 5 weeks, 2 leaf-dips were made from the inoculated leaves of each plant and these were examined for rod-shaped particles (table 28).

TABLE 28. Dilution end point of the leaf mottle virus from 'Shantung'.

P.hybrida	Dilution			
	1	10^{-1}	10^{-2}	10^{-3}
Lesion development	$1^{*}/_{5}$	$0/_{5}$	$0/_{5}$	$0/_{5}$
Presence of rod-particles			$1/_{2}$	$0/_{5}$

* Figures are the number of plants developing lesions or showing the presence of rod-shaped particles/number of plants inoculated or observed.

These results suggests a dilution end point of between 10^{-2} and 10^{-3}.

5.6.2 Longevity *in vitro*

Young 'Shantung' leaves were triturated with 0.033M Na_2HPO_4, pH 7.3, and stored at room temperature. Inoculations to P.hybrida leaves were made at various intervals. After 5 weeks these inoculated leaves were examined for rod-shaped particles (table 29).

TABLE 29. Longevity *in vitro* of the leaf mottle virus from 'Shantung'.

P.hybrida	Time (hours)					
	0	1	6	12	24	36
Lesion development	$0^{*}/_{5}$	$1/_{5}$	$0/_{5}$	$0/_{5}$	$0/_{5}$	$0/_{5}$
Presence of rod-particles				$1/_{1}$	$0/_{5}$	

* Figures are the number of plants infected/number inoculated or tested.

These results suggest an infective life *in vitro* of between 12 and 24 hours for the leaf mottle virus infecting 'Shantung'.

5.7 PARTICLE MORPHOLOGY

The dimensions of the rod-shaped particles found in chrysanthemum sap were measured from photographs taken of leaf-dips from several chrysanthemum cultivars infected with viruses of the leaf mottle group, after negative staining with PTA (fig. 13).

The dimensions of these rod-shaped particles were compared with those of tobacco mosaic virus (TMV) observed in leaf-dips from infected tomato plants. The TMV particles were assumed to ca. 280 nm in length (109).

TABLE 30. Dimensions of rod-shaped particles of the leaf mottle type from infected chrysanthemum cultivars.

Cultivar	Number of particles measured	mean length (nm)	mean diameter (nm)
'Golden Favorite'	25	700 (670-740)	15
'Yellow Madonna'	18	690 (670-710)	15
'Madame R. Rogers'	25	690 (680-700)	15
'Shantung'	25	700 (680-740)	15

Results indicate that the rod-shaped particles observed in this study (ca. 700 x 15 nm) agree with overseas reports for viruses of the leaf mottle group (40,48,61). Noordam's report (97) of particles ca. 600 x 30 nm appear to differ from later reports, particularly in the particle diameter measurement but Hollings (48) considered the difference insignificant.

CHAPTER 6.

METHODS FOR CONTROLLING VIRUS DISEASES OF CHRYSANTHEMUMS

6.1 INTRODUCTION

The intensive culture of many horticultural crops, and the rapid vegetative propagation methods used today, have resulted in the widespread dissemination of a large number of pests and diseases. Chemical methods of control have been used successfully to combat many of these insect pests, fungal and bacterial diseases. Virus diseases however have proved more difficult to control. This is because virus particles have an intimate association with plant cells, and any chemicals which inactivated plant viruses have had a detrimental effect on the host. Because of this, most of the control measures used at present involve some form of prevention, designed to reduce the sources of infection, and to limit the introduction and spread of the virus (fig. 1).

6.2 REMOVAL OF INFECTION SOURCES

Theoretically, if a crop is virus-free when initiated and there is no source of infection in or near the crop, there should be no virus problem. Consequently, by removing any sources of infection it is possible to minimise the incidence of virus diseases.

Sources of virus infection include:

perennial weed hosts;
perennial ornamental plants or unrelated crops;
volunteer plants remaining from a previous crop;
infected plants in a crop.

In chrysanthemum crops, the effectiveness of removing sources of infection varies with the virus concerned. Elimination of TAV by this means, for example, is almost impossible because of the wide host range of the virus. The major problem with most chrysanthemum virus diseases is that they are present to a greater or lesser extent in the chrysanthemum stocks of most cultivars grown commercially, and often symptoms are expressed too late to enable roguing to be an effective means of control. Chrysanthemum stunt, for example, can remain symptomless for up to 15 months, and during this time propagation and other cultural activities can spread the virus. Consequently, removal of infection sources is relatively ineffective and uneconomic until the stock plants used are known to be free from virus infection.

6.3 CONTROL OF VIRUS VECTORS

If there is a known vector for a particular virus disease, elimination of this vector can reduce the incidence of the virus in the crop. Aphids, particularly M.persicae and M.euphorbiae, are vectors of TAV and several viruses of the leaf mottle group. Other insect vectors include leafhoppers and thrips which are vectors for aster yellows and tomato spotted wilt respectively (46,48,69,109).

For effective control of insect-borne viruses however, the elimination of the vector should be complete, since a relatively few individuals can result in rapid spread of the virus.

Insecticides are regularly used in commercial chrysanthemum crops for aphid control, but these materials usually kill the aphids after they have succeeded in transmitting the virus, since the aphid-borne chrysanthemum viruses are stylet-borne. As a result the use of insecticides does not prevent infection with these viruses but may reduce the incidence of the disease within the crop.

Oil sprays have been shown by Bradley (8) to reduce aphid transmission of the stylet-borne potato virus Y, but these have not been tested on chrysanthemums, possibly because of phytotoxicity problems.

Non chemical methods of preventing or minimising aphid movement have been investigated:

Barrier crops of barley for example, have been shown by Broadbent to reduce the incidence of aphid-borne viruses in cauliflower seedlings (87). There is the possibility that maize shelter-belts, which are often planted in outdoor chrysanthemum crops, could reduce aphid spread, but this would need to be investigated before any conclusions could be made.

Aluminium foil has been shown to repel aphids in several crops, with varying degrees of success. The incidence of cucumber mosaic virus in gladiolus crops was reduced by two-thirds when aluminium foil was placed between the rows (107). In chrysanthemums, however, Hakkaart (39) demonstrated that although aphid populations were reduced, the reduction was not sufficient to give effective virus control.

Parasites and predators of aphids could become more important in the future if more specific chemical materials are introduced for controlling aphid populations. However, the nature of this biological control is such that the aphids can never be entirely eliminated and consequently the use of parasites and predators for aphid control does not appear to be compatible with the high level of aphid control required to eliminate virus infection.

6.4 VIRUS-TESTED PLANTING MATERIAL

Vegetative propagation of many horticultural plants has resulted in some cultivars becoming totally infected with virus. In such cases a method of treatment is required whereby healthy plants can be obtained, the number of which need only be minimal. In other cases however, it is possible to index sufficient plants until one or two uninfected plants are discovered and these are then used to propagate further healthy plants.

Indexing methods for the major virus diseases infecting chrysanthemums are discussed in chapter 2.

Two main approaches have been utilised for obtaining virus-free planting material.

6.4.1 Erratic Virus Distribution

a) Utilisation of the erratic distribution of viruses WITHIN A CROP

This approach is the basis for the detailed indexing of sufficient plants, in order to obtain one or two that are virus-free. In this context the term 'virus-free' means free from the known and specified viruses for which tests have been made. An equivalent term is 'virus-tested'.

b) Utilisation of the erratic distribution of viruses WITHIN A PLANT

It has been found with some viruses that the final distribution of virus particles in the plant is not always uniform and in some cases, particularly in woody perennials, propagation of non-infected parts of the plant can result in virus-free material which can then be used as mother plants (36). The use of "mini-cuttings" (1-2 cm) for example, has resulted in the production of chrysanthemums free from TAV (45).

Meristem-tip culture is a specialised application of the principle of erratic virus distribution. The term meristem-tip has been used rather indiscriminantly for various pieces of tissue ranging from 0.1-10 mm in size. The most commonly used tissue unit consists of the meristem dome, plus one or two leaf primordia, and this 0.1-0.5 mm section is referred to by Hollings and Stone (59) as the meristem-tip. A longer, but morphologically more correct term, used by Jacobs, Bornman and Allan (72), is 'shoot apical meristem'. This term includes the tissue piece from immediately below the last node upwards, thus consisting of the apical initials, their derivatives, and one or two leaf primordia. This eliminates any confusion between shoot or root meristem-tips. Jacobs et al also reserved the term shoot tip (which was used by Parke (100) to describe the shoot apical meristem), for the shoot apical meristem plus variable lengths of shoot proximal to the apex.

The technique for obtaining virus-free propagative material was pioneered by Morel (92), when he demonstrated that virus-free dahlias and potatoes could be obtained by the aseptic culture of small pieces of meristematic tissue cut from shoot tips of infected plants. The success of this technique is attributed to the inability of some viruses to infect or survive in the actively dividing meristematic tissue of the host plant (53).

Several hypotheses have been proposed to explain the drop in virus concentration shown to exist towards the meristematic zone, including the following:

i) the rate of cell division is greater than the rate of virus multiplication and therefore the most recently formed cells are free from viral particles;

ii) there is a mechanical blockage to virus invasion;

iii) the biochemical state of the dividing cells inhibits virus replication.

Until more is known about the host/virus relationship, these hypotheses must remain as such.

Although meristem-tip culture has been used to eliminate viruses from many infected host species (68), the application of this technique, on its own, to chrysanthemum virus elimination has only been used occasionally (53).

In 1956, Holmes (45) used a method similar to meristem-tip culture to eliminate TAV from infected chrysanthemums. He succeeded in obtaining 13 plants free from TAV, by grafting scions (4-8 mm in length) from infected plants to 244 healthy stock plants. Since then Hollings (66) has reported the elimination of several viruses of the leaf mottle group (chrysanthemum virus B and chrysanthemum vein mottle), and Monsion, Huchet and Dunez (91) have succeeded in eliminating TAV from infected chrysanthemums.

6.4.2 Heat Treatment

The other approach to the problem of obtaining virus-free planting material involves the use of heat treatment.

Early workers showed that heat treatment, using hot water in the case of sugar cane setts (Kobus 1889), and hot air in the case of peach trees (Kunkel 1936), was successful in controlling diseases now known to be caused by viruses (sugar cane sereh) or virus-like organisms (peach yellows) (53,75,84,98).

In 1957, Kassanis (74) concluded that over half the viruses infecting horticultural plants could be eliminated by heat treatment while in 1965, Hollings (53) listed over 90 viruses that have been eliminated from at least one host plant by heat treatment. Four years later this number had risen to 120 (98) and crops as diverse as apples, chrysanthemums, peaches, mushrooms, potatoes and strawberries have now been successfully heat treated.

Heat treatment of plants to obtain healthy propagating material can be considered in two categories, depending on the temperature used and the exposure time of the plant to this temperature.

(a) High temperature-short time

This type of treatment generally involves the use of temperatures ranging from 35-55 C, for relatively short periods of time (6 minutes to 36 hours). Hot water is recommended because it seems to give a more even heat distribution during the short times involved, than does hot air (98).

The successful use of hot water is dependent on the ability of the plant material, usually dormant, to withstand temperatures high enough to inactivate the virus. Hot water treatment has been used successfully for over 25 viruses infecting more than 16 different plant species, including peach, sugarcane, potato, strawberry, cherry and hops (53,74,98).

(b) Low temperature-long time

Exposure of infected plants to lower temperatures (35-38 C) but for considerably longer periods of time (1-8 months) has also shown to be effective in eliminating viruses from many plants. In this more commonly used method of heat treatment, air is the heating medium and in general, plant survival and the chances of virus-free propagative material being obtained are better than with hot water. Some of the crops for which this method has been used successfully include carnation, chrysanthemum, strawberry, mushroom, apple, potato, lemon and lucerne (53,74,98).

It is generally believed that heat treatment is successful when the rate of virus degradation within the plant, exceeds the rate of virus multiplication (76).

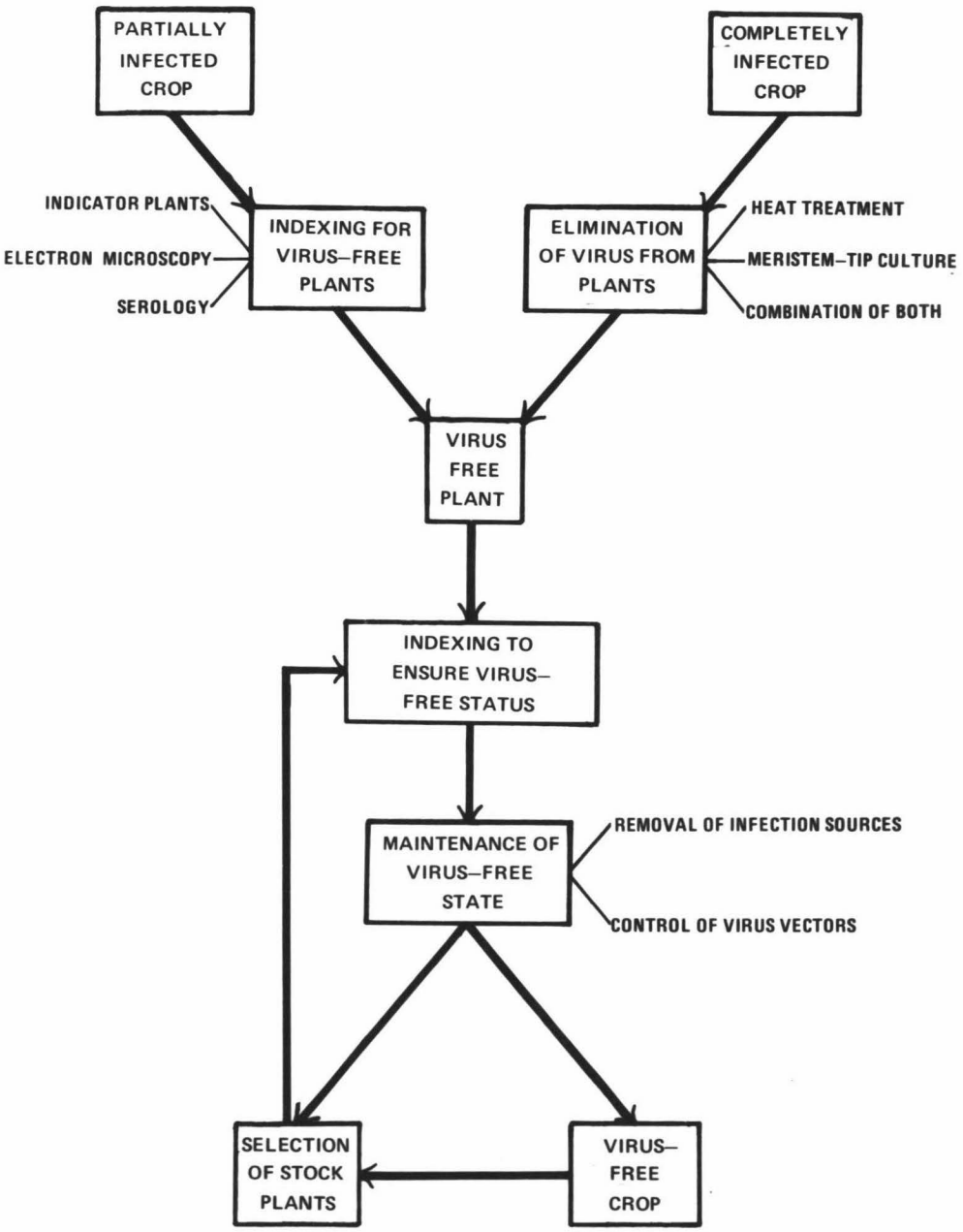
The time-temperature regime chosen varies depending on the heat tolerance of the plant and the heat susceptibility of the virus. By subjecting plants to a gradual rise in temperature, the heat tolerance of the plant can be increased (53). There are also reports of plant survival being increased by the use of intermittent temperatures (98).

Following the heat treatment of the non-dormant plants, portions of the plant, usually tip cuttings, are propagated. However, because heat treatment is not infallible as a method of obtaining virus-free material, it is essential that the material chosen for propagation is indexed to ensure virus elimination has occurred. Hollings (53) stresses the importance of adequate indexing of treated material in order to eliminate the possibility of an "attenuated" form of the virus being present, such as that seen in heat-treated carnations, and to identify any delayed resurgence of virus infection which has been reported in heat-treated plants such as carnations (56).

Heat treatment alone has enabled virus-free clones of many horticultural crops to be produced, and similar successes have been reported using meristem-tip culture.

However, there are some viruses that have so far resisted all attempts to eliminate them either by heat treatment or meristem-tip culture. Several of these have now been successfully eliminated by a combination of heat treatment followed by meristem-tip culture of the resultant shoot tip. Chrysanthemum stunt, for example, has been successfully eliminated from a few cultivars by such a procedure (62). This combination of heat treatment and meristem-tip culture also increased the efficiency of virus B elimination from infected chrysanthemums, from 5% when meristem-tip cultured and 40% when heat treated, to 95% when a combination of heat treatment and meristem-tip culture was used (59).

FIG. 1. POSSIBLE PROCEDURE FOR OBTAINING AND MAINTAINING VIRUS–FREE CHRYSANTHEMUMS.



CHAPTER 7.

MERISTEM-TIP CULTURE FOR THE ELIMINATION OF TAV AND LEAF MOTTLE VIRUSES FROM CHRYSANTHEMUMS

7.1 THE USE OF MERISTEM-TIP CULTURE

The incidence and properties of the viruses examined in this study influence the choice of methods available for the control of virus diseases of chrysanthemums. The presence of symptomless infection of the three viruses observed in chrysanthemums in the Manawatu, makes their control by the roguing of plants showing symptoms a relatively inefficient process. Further, their ability to be transmitted by aphid vectors - with the exception of chrysanthemum stunt which has been shown to be transmitted during cultural operations such as disbudding and picking (79) - enables the spread of the viruses through the crop from infected plants.

The most satisfactory method of control is the propagation from 'virus-free'* stock plants, and the prevention of re-infection from outside sources or from infected volunteer plants by aphid vectors.

'Virus-free' stock plants can be identified by sap inoculation to N.glutinosa, C.amaranticolor and P.hybrida to index for TAV and viruses of the leaf mottle group, and by

---

* In this context, 'virus-free' means free from TAV, chrysanthemum stunt and viruses of the leaf mottle group.

grafting with 'Mistletoe' to index for chrysanthemum stunt (chapter 2.2.2). The availability of an electron microscope would facilitate the indexing procedure in that the lack of symptoms on C.amaranticolor and the failure to observe rod-shaped particles of the leaf mottle group could indicate, within 1 week, the absence of TAV and leaf mottle viruses. Such plants could then be indexed for chrysanthemum stunt, while further indexing for TAV could be undertaken. This would reduce the number of graft-indexing tests required, and results could be obtained within 3 months.

However, if no 'virus-free' plants are found because of the complete infection of the cultivar, a situation which was not observed in this study, virus elimination by heat treatment, meristem-tip culture, or a combination of both, can be used. Overseas reports indicate that both TAV and some of the leaf mottle group have been eliminated by heat treatment and by meristem-tip culture, with more satisfactory results being obtained with the culture of meristem-tips from heat treated plants (table 31). This method also shows promise for the elimination of chrysanthemum stunt which has not been eliminated from chrysanthemums by meristem-tip culture alone (62).

In this study, the feasibility of meristem-tip culture to eliminate TAV and viruses of the leaf mottle group was determined.

TABLE 31. Elimination of viruses from infected chrysanthemums.

Virus	Heat Treatment	Meristem-tip culture	Heat + Meristem-tip culture
Flower distortion	(21)*		
Green flower	(66)		(66)
Ringspot	(55)		
Rosette	(22)		
Leaf mottle group	(14,41)	(53,66)	(41,103)
Tomato Asperny	(55)	(91)	
Stunt			(62)

* References reporting successful virus elimination.

7.2 VIRUS STATUS OF SELECTED CHRYSANTHEMUMS

Stunt-free chrysanthemums were selected on the basis of graft indexing results with 'Mistletoe'. After 3 months no symptoms of stunt infection were observed in 19 of the 50 chrysanthemums tested, and these were assessed for TAV infection by sap inoculation to N.glutinosa and C.amaranticolor, and for viruses of the leaf mottle group by electron microscopic examination (table 32). Shoot tips were then harvested from the stunt-free chrysanthemums and the meristem-tips cultured (Appendix I).

TABLE 32. Results of indexing for TAV and leaf mottle viruses in chrysanthemums selected for meristem-tip culture.

Source	Number of plants free from stunt	Number of meristem-tips cultured		
		TAV	Leaf mottle	TAV + leaf mottle
'Shantung'	$^{7}/_{20}$	8	10	7
'Madame E. Rogers'	$^{8}/_{20}$	4	4	3
'Ethyl Edmonds'	$^{2}/_{5}$	3	0	7
'Regalia'	$^{2}/_{5}$	3	0	4
Total		18	14	21

7.3 SURVIVAL OF MERISTEM-TIPS

After 13 weeks, of the 8 surviving plantlets, 1 was large enough to be transplanted to a mini-pot (Appendix 3); 2 had developed stems and were ca. 1 cm high; the remaining 5 plantlets had developed roots but had not initiated stem growth (table 33).

TABLE 33. Survival of meristem-tips from chrysanthemums infected with TAV and leaf mottle viruses.

Source	Meristem-tip survival (after 13 weeks)			
	TAV	Leaf mottle	TAV + leaf mottle	Total
'Shantung'	1*/8	2**/10	2/7	5/25
'Madame E. Rogers'	1/4	1/4	0/3	2/11
'Ethyl Edmonds'	0/3		1/7	1/10
'Regalia'	0/3		0/4	0/7
Total	2/18	3/14	3/21	8/53

* Surviving meristem-tips/total number cultured.

7.4 INDEXING FOR 'VIRUS-FREE' STATE

Indexing the 'Shantung' plant which was large enough to transplant** (table 33) failed to reveal the presence of rod-shaped leaf mottle particles in 4 leaf-dip examinations, and tentatively the plant was assumed to be free from viruses of the leaf mottle group.

Both the surviving plantlets cultured from TAV-infected plants were triturated with 0.033M Na_2HPO_4 + 0.05M DIECA, pH 7.4, and inoculated to N.glutinosa and C.amaranticolor. In neither case did symptoms of TAV develop. The slower development of the remaining 4 plantlets, 3 of which were from chrysanthemums infected with both TAV and viruses of the leaf mottle group, precluded any determination of their virus content.

These results indicate that using the methods outlined in this study (Appendix I), the elimination of TAV and viruses of the leaf mottle group from infected chrysanthemums is feasible, although further indexing to confirm the virus-free state of the plantlets is required before definite conclusions can be reached.

These results also indicate that the development of the meristem-tips could be influenced by the presence of one or more viruses in the source plants, in that those meristem-tips taken from plants infected with both TAV and leaf mottle viruses developed at a slower rate than those infected with either virus. However, the effect of virus presence and the effect of different cultivars on the development of the meristem-tips cannot be determined with any accuracy until further experiments are undertaken.

Meristem-tip culture, either alone or in conjunction with heat treatment, can be used to the best advantage where indexing of existing chrysanthemums has failed to show the presence of naturally occurring virus-free plants which can be used for propagation purposes. Results of the survey undertaken in this study (chapter 2) indicate that this is not yet the case in New Zealand.

CHAPTER 8.

CONCLUSIONS

Results of surveys based on visual examination and indexing tests confirm that TAV, chrysanthemum stunt and leaf mottle group viruses are widespread in chrysanthemums grown in the Manawatu. Reliance on symptom expression is not a satisfactory method for determining the virus-infected state of chrysanthemums. Many plants which were free of obvious symptoms were subsequently demonstrated to contain one or more of the above viruses. No other viruses reported overseas as infecting chrysanthemums were observed in this study.

Characterisation of TAV on the basis of symptoms in chrysanthemums, host range, physical properties and stylet-borne aphid transmission indicate the isolate tested conforms in general to overseas isolates of TAV. The type of buffer solution used with tissue triturates did influence physical properties of TAV, with DIECA causing a marked increase in longevity *in vitro* and the infectivity of TAV from chrysanthemum sap. The beneficial effects of DIECA indicate the possibility that this substance prevented virus inactivation during sap inoculations.

The lack of a suitable local lesion host for chrysanthemum stunt hampered characterisation and sap inoculation to 'Mistletoe' gave erratic symptom development. Graft transmission to 'Mistletoe' resulted in chlorotic-white leaf spots ('measles') within 2 months if leaves above the graft were removed.

The cineraria starch local lesion test for detection of chrysanthemum stunt proved unsatisfactory.

Viruses of the leaf mottle group were identified on the basis of their rod-shaped particles (ca. 700 x 15 nm), and the local lesion reaction of P.hybrida when inoculated with sap containing particles of this morphology. The erratic reaction of P.hybrida, however, necessitated electron microscopic examinations of negatively stained leaf-dip preparations. This was found to be the most reliable indexing method for leaf mottle group viruses. Graft transmission to 'Good News' resulted in symptoms similar to those attributed to the mosaic group of viruses in the U.S.A. Host range was similar to chrysanthemum virus B of Noordam but differed from that described by Hollings for virus B in that he attributed symptoms on antirrhinum (Antirrhinum majus) and strawflower (Helichrysum bracteatum) to chrysanthemum latent virus which was not recognised in this study. Aphid transmission of the leaf mottle virus was shown to be of the stylet-borne type, but symptom development in 'Good News' and P.hybrida was erratic.

None of the cultivars examined were found to be completely infected with either TAV, chrysanthemum stunt, or viruses of the leaf mottle group. Consequently, the most satisfactory method for controlling these viruses is the propagation of virus-tested plants, obtained by using the indexing procedures outlined in this study, combined with measures to prevent re-infection from outside sources.

If no such virus-free plants can be obtained using this method, meristem-tip culture, heat treatment or preferably a combination of both must be resorted to. Preliminary work in this study demonstrated that elimination of both TAV and viruses of the leaf mottle group is feasible in New Zealand but the treatment of the meristem-tips during culture and over the "hardening off" process, is critical for the survival of the plantlets.

APPENDIX I.

MERISTEM-TIP CULTURE TECHNIQUES

Until recently most work on the aseptic culture of plant tissue had been performed with callus tissue and undifferentiated tissues. Little of the information obtained was directly applicable to meristem-tip culture, where root and shoot development is more important than growth responses of tissue blocks. In the last 10 to 15 years however, considerable advances have been made in this field and several factors have been shown to influence the success of meristem-tip culture. These factors can be summarised as follows:

(a) Aseptic conditions to prevent contamination of the sensitive meristem-tips.

(b) Adequate culture media to provide the necessary minerals and growth substances for meristem-tip growth.

(c) Physical growth factors such as light and temperature to ensure optimum growth of the plantlets and to facilitate their transfer from aseptic to more normal growing conditions.

During this study the following procedure, based on that used by Hollings and Stone (59), was used to determine the feasibility of meristem-tip culture as a method of eliminating TAV and viruses of the leaf mottle group from cultivars of Chrysanthemum morifolium grown commercially in New Zealand.

Details of equipment, media, and techniques are presented in the following sections (1 to 10).

1. CULTURE CABINET

To prevent contamination of the meristem-tips during dissection and transfer to the culture bottles, a wooden cabinet (4' x 3' x 2'6") with a sloping perspex front and sliding glass doors was used (fig. 14). The sloping front had a six-inch hole cut in it and this was sealed with a sponge-rubber gasket through which the microscope eyepieces protruded.

The cabinet was placed in the laboratory where air movement was minimal and the surrounding air was sprayed with alcohol before the cabinet was used. This removed the necessity for a complete airtight seal and obviated the necessity for surgical gloves which would hamper the operator's dexterity. Aseptic conditions were achieved within the cabinet by using an ultra-violet germicidal lamp (Hytex T.U.V. 15 watt*) permanently installed inside the cabinet. Twenty-four hours exposure to the ultra-violet light, plus the thorough spraying of the cabinet and the operator's hands with alcohol kept contamination of the meristem-tip cultures to a low level.

2. MICROSCOPE

A binocular dissecting microscope (Olympus Zoom Stereoscopic, SZIII*) with a magnifying power ranging from 7 x to 40 x, was used inside the cabinet.

The initial exposure of the meristem-tips by the removal of the young leaves was made under low power (10 x), while the final dissections were made at higher magnifications of 25-30 x.

3. DISSECTING INSTRUMENTS

A fine tipped drawing pen proved satisfactory for the initial removal of young leaves and exposure of meristem-tips, since it

---

* Trade names.

caused a minimum of damage. The removal of the meristem-tip was done with triangular section of razor blades (0.5 to 1.0 cm long), mounted in Ash No. 4* needle holders. 7-o-clock* blades proved to be satisfactory for 8 to 10 dissections (Fig. 15). Instruments were sterilised under ultra-violet light before use and when not in use they were kept in alcohol.

4. CULTURE TUBES

Various types of tubes and bottles have been used for culturing meristem-tips, the most common being pyrex tubes (ca. 7.5 x 2.0 cm) with aluminum caps to prevent contamination (110). In this study, McCartney bottles (8.0 x 2.5 cm) with metal screw-tops proved both convenient and satisfactory. Bottles were thoroughly washed and boiled in distilled water before use.

5. CULTURE MEDIA

The composition of media used in meristem-tip culture varies in complexity from the elaborate used by Kassanis (73) and Quak (103), to the much simpler media preferred by Baker and Philips (2) and Stone (110).

Although the media may differ considerably they all contain the standard major and minor elements, glucose or sucrose as a carbon source, and a number of vitamins and growth substances.

A comparison of several media showed that under the conditions described here, Murashige and Skoog's medium (94) gave the best results (Appendix II).

Various stock solutions were prepared with double-distilled water. These were then diluted as required and combined to give the final culture medium.

---

* Trade names.

"Major Element" stock solution (g/litre)

NH_4NO_3	16.5
KNO_3	19.0
$CaCl_2.2H_2O$	4.4
$MgSO_4.7H_2O$	3.7
KH_2PO_4	1.7
$MnSO_4.4H_2O$	2.23
$ZnSO_4.7H_2O$	0.86
H_3BO_3	0.62
KI	0.083

"Minor Element" stock solution (g/litre)

$Na_2MoO_4.2H_2O$	0.25
$CoCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.02

"Iron Chelate" solution

Na_2EDTA*	15.0 g	boiled in 200 ml distilled water
$FeSO_4$	12.5 g	boiled in 200 ml distilled water

These two ingredients were mixed together while boiling and made up to 500 ml with double distilled water.

* disodium ethylenediaminetetraacetate.

"Growth Factor" stock solution (g/100 ml)

Nicotinic acid	0.05
Pyridoxin HCl	0.05
Thiamine HCl	0.01

The culture medium was made up by:

1) Dissolving 30 g sucrose in 600 ml of double distilled water.

2) Adding: 100 ml of the "Major Element" stock solution
1 ml of the "Minor Element" stock solution
1 ml of the "Iron Chelate" solution
1 ml of the "Growth Factor" stock solution.

3) Adding: 0.1 g meso-inositol
0.002 g glycine.

4) Adding, if required, 0.005 g indoleacetic acid (IAA).

5) Adjusting the solution to a pH of 5.5 - 6.0.

6) Making the solution up to 1 litre with double-distilled water.

Five mls of this solution was then placed in each culture bottle, together with a filter-paper bridge, and autoclaved at 15 psi for 10 minutes.

The meso-inositol and glycine were added to the solution separately because when stored at 5 C in stock solutions they did not remain in solution.

6. SUPPORT MEDIUM

Most of the early culture work was done with agar supplemented by the various nutrient solutions, but in a comparison with filter-paper bridges Heller (43) found that agar gave less root formation, was more difficult to remove from the plantlet roots without damage, and was prone to dessication (34). Stone (110) reported a four-fold increase in carnation meristem-tip growth with filter-paper bridges compared with growth on agar.

7. CONTAMINATION

Most meristem-tips, particularly those of chrysanthemum, were found to be well protected by a wrapping of "sterile" leaves and leaf hairs, which protected the meristem-tips from infection. Provided the dissecting tools were kept sterile by the use of an alcohol bath, it was possible to remove the meristem-tips and transfer them aseptically to the culture bottles with little or no contamination.

Some workers (24,96) have suggested the use of a mercuric chloride or silver nitrate rinse to surface sterilise plant tips before dissection but this was not found to be necessary with chrysanthemums. Elliott (34) found that exposure of sweet potato Ipomoea batatas L. Poir. to temperatures of approximately 38 C for 2 weeks before dissection, reduced contamination in meristem-tip cultures, but again in this study this was unnecessary.

8. DISSECTION

Hollings and Stone (59) showed with chrysanthemums that apical meristem-tips gave better results in terms of virus-free plants than lateral buds (32% success as opposed to 18% from lateral buds).

Shoot tips approximately 5 cm in length were taken, the larger leaves removed by hand, and the tips then placed in sterile petri dishes containing moist filter-paper pads to prevent dessication (fig. 16).

Each dissection was performed on separate sterile rubber pads inside the sterile cabinet. The remaining protective leaves were peeled back, using the fine-tipped drawing pen, to expose the shiny meristem dome and leaf primordia (fig. 16). Care was taken to ensure that the dissection tools were free from alcohol before use. The meristem dome - including the first two leaf primordia - was then rapidly dissected out with a

razor-blade knife and transferred to the filter-paper bridge inside an autoclaved culture bottle. The screw top was then replaced, the bottle labelled, and placed under a light-bank.

9. MERISTEM-TIP GROWTH

Tubes containing the meristem-tips were placed 15-20 cm below a light-bank consisting of two 40 watt 'Grolux'* fluorescent tubes and one 40 watt 'Deluxe warm white'* tube, and given 22 hours illumination per day (112).

An average temperature of 24 C was maintained by placing the culture tubes in a small room within the laboratory, which reduced the temperature fluctuations to ± 3 C. Hollings and Stone (59) recommend 22-24 C as the optimum temperature for chrysanthemum meristem-tip growth.

Once green leaves developed the meristem-tips were transferred to fresh culture tubes, identical to the initial ones except for the omission of IAA, and returned to the growth room for a further 6-8 weeks to allow the already initiated roots to develop (fig. 17). During the transfer operations aseptic conditions were maintained, and care was taken not to damage the root initials.

10. "HARDENING OFF"

Once plantlets were 1 to 2 cm high, they were transferred to minipots and gradually exposed to more 'normal' conditions (fig. 19).

Difficulties were experienced in preventing contamination during the hardening off process, and finally a method based on a combination of that used by Hollings and Stone (59) and the procedure recommended by Elliott (34) proved satisfactory (Appendix III).

---

* Trade names.

In this method, plantlets were transferred to mini-pots containing peat/perlite and 20 mls of Murashige and Skoog's nutrient solution (less IAA) which had been autoclaved for 10 minutes at 15 psi inside pint preserving jars covered with petri-dish lids (fig. 18). After 2-3 days the lids were replaced by plastic bags and these were gradually cut open to "harden off" the plantlets.

The plantlets were then indexed for TAV and viruses of the leaf mottle group to determine the success of the meristem-tip culture in eliminating these two viruses.

APPENDIX II.

COMPARISON OF FIVE TISSUE CULTURE MEDIA FOR GROWTH OF CHRYSANTHEMUM MERISTEM-TIPS

1. MEDIA TESTED

On the basis of composition the five media selected for testing can be grouped into two sections:

1.1 High Salt Media

a) Murashige and Skoog's medium which is a modification of White's medium (24) and has been used with success in the bioassay of tobacco cultures (94).

b) Elliott's modification of White's medium which has been successfully used for the culture of kumara (New Zealand sweet potato) meristem-tips (34).

c) Miller's medium, used in the cultivation of soybean (Glycine max. 'Acme') for the assay of zeatin (90) and also used in the study of soybean protoplast growth and development (35).

1.2 Low Salt Media

a) Gautheret's medium, which was devised by one of the earlier workers in this field and since then has been used successfully in the culture of several different plant meristem-tips, notably grapes, sequoias and Perilla (24).

b) Hollings' modification of Neergaard's medium which has been used successfully in England for growth of carnation and chrysanthemum meristem-tips (110).

TABLE 34. Components of nutrient media tested for meristem-tip culture of chrysanthemums (table 35).

Component	Murashige & Skoog (94) g/litre	Elliott (76) g/litre	Giles (35) g/litre	Gautheret (24) g/litre	Hollings (110) g/litre
$NH_4Mo_7O_{24}.4H_2O$			$1.00x10^{-4}$		
NH_4NO_3	1.65	1.65	1.00		
KNO_3	1.90	1.90	1.00	$1.25x10^{-1}$	$1.25x10^{-1}$
$CaCl_2.2H_2O$	$4.40x10^{-1}$	$4.40x10^{-1}$			
$MgSo_4.7H_2O$	$3.70x10^{-1}$	$3.70x10^{-1}$	$7.15x10^{-2}$	$1.25x10^{-1}$	$1.25x10^{-1}$
KH_2PO_4	$1.70x10^{-1}$	$1.70x10^{-1}$	$3.00x10^{-1}$	$1.25x10^{-1}$	$1.25x10^{-1}$
FeNa EDTA	$2.00x10^{-2}$	$2.00x10^{-2}$	$1.32x10^{-2}$		
H_3BO_3	$6.20x10^{-3}$	$6.20x10^{-3}$	$1.60x10^{-3}$	$2.50x10^{-5}$	$2.50x10^{-5}$
$MnSO_4.4H_2O$	$2.23x10^{-2}$	$2.23x10^{-2}$	$1.40x10^{-2}$	$1.00x10^{-3}$	$1.00x10^{-3}$
KI	$8.30x10^{-4}$	$8.30x10^{-4}$	$8.00x10^{-4}$	$2.50x10^{-4}$	$2.50x10^{-4}$
$ZnSO_4.7H_2O$	$8.60x10^{-3}$	$8.60x10^{-3}$	$3.80x10^{-3}$	$5.00x10^{-4}$	$5.00x10^{-4}$
KCl			$6.50x10^{-2}$		
$Na_2MoO_4.2H_2O$	$2.50x10^{-4}$				
$CuSO_4.5H_2O$	$2.50x10^{-5}$	$2.50x10^{-5}$		$2.50x10^{-5}$	$2.50x10^{-5}$
$CoCl_2.6H_2O$	$2.50x10^{-5}$	$2.50x10^{-5}$		$2.50x10^{-5}$	$2.50x10^{-5}$
$NiCl_2.6H_2O$				$2.50x10^{-5}$	$2.50x10^{-5}$
$Fe_2(SO_4)_3$				$2.50x10^{-2}$	$2.50x10^{-2}$
H_2SO_4				1.0 ml	1.0 ml
$Ca(NO_3)_2.4H_2O$			$5.00x10^{-1}$	$5.00x10^{-1}$	$5.00x10^{-1}$
$Cu(NO_3)_2.3H_2O$			$3.50x10^{-4}$		
Sucrose	30	30	30		
Glucose				40	40
Adenine				$8.00x10^{-3}$	
IAA	$5.00x10^{-3}$				
NAA		$1.00x10^{-3}$	$2.00x10^{-3}$	$1.00x10^{-3}$	$1.00x10^{-3}$
Meso-inositol	$1.00x10^{-1}$	$1.00x10^{-1}$	$1.00x10^{-1}$		$1.00x10^{-3}$
Nicotinic acid	$5.00x10^{-4}$	$5.00x10^{-4}$	$5.00x10^{-4}$		
Pyridoxin HCl	$5.00x10^{-4}$	$1.00x10^{-4}$	$1.00x10^{-4}$		
Thiamine HCl	$1.00x10^{-4}$		$1.00x10^{-4}$		
Glycine	$2.00x10^{-3}$	$2.00x10^{-3}$		$1.00x10^{-3}$	$1.00x10^{-3}$

2. SOURCE PLANTS

The chrysanthemum cultivars used were 'Mistletoe' and 'Good News', both of which were virus-tested for TAV, stunt and the leaf mottle group. These cultivars were used to minimise the possible effect of virus infection on the growth of the meristem-tips as Hollings and Stone (62) have reported that chrysanthemum stunt can increase the development time of the meristem-tips. They also report that the size of the meristem-tip can influence not only the development time, but also the percentage of virus-free plantlets obtained. For these reasons, efforts were made to select meristem-tips of approximately the same size (0.25 - 0.5 mm) and at the same stage of development, i.e. vegetative tips, not flower bud primordia.

TABLE 35. Effect of media composition on chrysanthemum meristem-tip growth and development.

Medium	Number of meristems used	Shoot Growth after 6 weeks** (with IAA)	Root & Shoot Growth (12 weeks)** (without IAA)
Murashige & Skoog	50	15*	6
Elliott	50	6	1
Giles	50	3	0
Gautheret	50	10	2
Hollings	20	10	1

* Number of meristem-tips.

** Fig. 17.

After 12 weeks the plantlets were transferred to mini-pots for "hardening off" (Appendix III).

3. DISCUSSION

Examination of the meristem-tips after 6 weeks showed that shoot development occurred most rapidly in Murashige and Skoog's high-salt medium, while those grown in Elliott's medium, differing only in the type of hormone used, developed at a considerably slower rate. Hollings' medium, shown in England to be adequate for chrysanthemum and carnation meristem-tip growth, was found in this study to be inferior for shoot development to Murashige and Skoog's medium.

Later assessment of meristem-tip growth was made prior to transferring the plantlets to mini-pots, and it was apparent that the high-salt medium of Murashige and Skoog gave the best results in terms of root and shoot growth, while Hollings' low-salt medium also showed significant improvements over the other media tested.

It therefore seems that under the conditions described in this study, Murashige and Skoog's modification of White's medium, using IAA as a growth hormone gave better results in terms of root and shoot growth and development than did the other media tested.

There was no significant difference in meristem-tip growth and development between the two chrysanthemum cultivars 'Mistletoe' and 'Good News'.

APPENDIX III.

COMPARISON OF SEVERAL METHODS OF "HARDENING OFF" PLANTS DERIVED FROM CHRYSANTHEMUM MERISTEM-TIPS

1. INTRODUCTION

The treatment of chrysanthemum plantlets derived from meristem-tip culture, during the transition from aseptic conditions in the culture bottles to the more "normal" growth conditions in the glasshouse, proved to be crucial.

The "hardening off" or gradual exposure of the plantlets to normal glasshouse conditions was studied and several methods tested to determine the most suitable.

Three factors were considered:

2. SUPPORT MEDIUM

Various supporting media have been used by different workers in the growth of chrysanthemum and carnation plantlets, including peat (59), soil (112) and a mixture of peat/sand/soil (110). Vermiculite has been shown to be satisfactory for the growth of sweet potato (Ipomoea batatus) plantlets (34).

The media tested in this study were perlite, vermiculite, and a mixture of peat and perlite.

3. NUTRIENT STATUS

Since the three media selected for testing contained little or no nutrient, a nutrient solution was also required to ensure adequate plantlet growth.

Several nutrient combinations were tested:

Hoagland's nutrient solution which Elliott (34) found satisfactory in combination with vermiculite for the growth of sweet potato plantlets (see Appendix V).

North Carolina nutrient solution which is used as a general nutrient for plants grown in the controlled climate laboratory of the Plant Physiology Division of the D.S.I.R. (see Appendix V).

Murashige and Skoog's meristem-tip culture nutrient solution which was used to culture the meristem-tips up to the "hardening off" stage (see Appendix I).

"Chrysanthemum" potting mix used in this study for general plant growth (see Appendix IV).

4. CONTAMINATION

Two approaches to this problem were studied to determine the most effective procedure in terms of plantlet growth (table 36).

The first approach, used by Sutton and Taylor (112) in the cultivation of carnation meristem-tips, was to transfer the plantlets to mini-pots, hold them under mist for 18-24 hours, then move the pots to a shaded area in the glasshouse.

The other, more gradual approach, was based on the "mini-cloche" principle used by Elliott (34) in the culture of sweet potato plantlets. With this technique plantlets were transferred to mini-pots which had been autoclaved inside pint preserving jars covered with petri-dish lids (fig. 18).

After 2-3 days the lids were replaced by plastic bags and the bags were gradually cut open to "harden off" the plantlets. The pots were then removed from under the light-bank and grown in the glasshouse.

TABLE 36. Treatment used to "harden off" plantlets derived from chrysanthemum meristem-tip culture.

Support medium	Nutrient status	Plantlet environment
Vermiculite	Hoaglands solution	mist
Vermiculite	North Carolina solution	'mini-cloche'
Vermiculite	North Carolina solution	mist
Perlite	Hoaglands solution	'mini-cloche'
Perlite	North Carolina solution	'mini-cloche'
Perlite	Murashige & Skoog's solution	mist
*Peat/perlite	Murashige & Skoog's solution	'mini-cloche'
Peat/perlite	"Chrysanthemum" potting mix	mist
Peat/perlite	Murashige & Skoog's solution	mist

5. RESULTS AND CONCLUSIONS

Success in plantlet survival was achieved in the *treatment including the peat/perlite medium with Murashige and Skoog's nutrient solution where the plantlet was initially protected by a 'mini-cloche'.

This success may be attributed to the gradual transition from aseptic to normal growth conditions, combined with an initial nutrient status approximating that under which the meristem-tips had been grown.

In all treatments involving the use of mist, contamination by saprophytic fungi caused the death of the plantlets, even when a 0.25 g/litre phenyl-mercuric-chloride drench was applied at the onset of the fungal invasion.

The plantlets grown in perlite failed to develop, although no saprophytic fungi could be observed. Since the perlite particles were rather coarse it is probable that root dessication or root damage during transfer was at fault.

The lack of growth observed in those plantlets grown in the North Carolina nutrient solution, in both vermiculite and perlite, could be attributed to the relatively low soluble salt level (in comparison with Murashige and Skoog's nutrient solution) because in neither case could saprophytic fungi be observed.

These results show that to obtain plants from meristem-tip culture the initial procedures of dissection and culturing of the meristem-tips are important but also the procedures used in "hardening off" the plantlets are critical.

APPENDIX IV.

GROWTH REGIME

1. INTRODUCTION

All the plants grown for use as indicator hosts, either for indexing purposes or for host range studies, were grown from seed - with the exception of the virus-tested 'Mistletoe' and 'Good News' chrysanthemums which were taken as cuttings from stock plants - and were isolated from virus infected plants to prevent contamination.

The seeds were either sown in seedling trays (6" x 4") containing grade # 2 vermiculite, and pricked out into 4" plastic pots, or direct seeded into the 4" pots containing a layer of vermiculite over the potting mix.

The plants were hose-watered up to twice a day during the hot weather and on demand during the winter.

2. POTTING MIX

A 1 : 1 ratio of peat/perlite was used as an inert support medium throughout this study, and several fertiliser combinations were tested to obtain the best results in terms of soft, rapidly growing plants.

Several of the fertiliser combinations gave relatively high soluble salt readings and unsatisfactory pH readings. The fertiliser combination used for most of this study, was based on that used in the commercial production of pot chrysanthemums in the Manawatu.

TABLE 37. Fertiliser combination used in conjunction with peat/perlite potting mix.

Material	Quantity g/bushel
Osmocote (18.6.12.)	165.0
Dolomite	82.5
Superphosphate	41.2
Agricultural lime	41.2
Dried blood	31.0
Sulphate of potash	10.3
Urea	6.4
Iron chelates	0.45
Borax	0.45

3. TEMPERATURE

Initial problems were encountered with the cooling system in the glasshouse, and during the summer, temperatures of up to 36 C were recorded. These high temperatures, combined with unsuitable fertilisers, resulted in the production of "hard" growth in many plants, particularly P.hybrida seedlings and 'Mistletoe' cuttings.

4. PEST AND DISEASE CONTROL

Insect control was achieved by the application of Lannate L.* (1.1 g/500 ml) sprays when required, to kill aphids, and Kelthane** (1.1 g/500 ml) for mite control.

---

* Trade name for methomyl.

** Trade name for dicopol.

APPENDIX V.

SUPPLEMENTARY NUTRIENT SOLUTIONS

1. HOAGLANDS NUTRIENT SOLUTION (34)

	(g/litre)
$Ca(NO_3)_2$	4.10×10^{-1}
KNO_3	2.52×10^{-1}
$MgSO_4$	1.20×10^{-1}
KH_2PO_4	1.36×10^{-1}
H_3BO_3	6.20×10^{-3}
$MnSO_4.4H_2O$	2.23×10^{-2}
FeNa EDTA	2.00×10^{-2}
$ZnSO_4.7H_2O$	8.60×10^{-3}
KI.	8.30×10^{-4}
$Na_2Mo_7O_{24}.4H_2O$	2.50×10^{-4}
$CuSO_4.5H_2O$	2.50×10^{-5}
$CoCl_2$	2.50×10^{-5}

2. NORTH CAROLINA NUTRIENT SOLUTION (pers. comm.)

	(g/litre)
$Ca(NO_3)_2 \cdot 4H_2O$	3.18×10^{-1}
NH_4NO_3	1.60×10^{-1}
KNO_3	1.27×10^{-1}
Na_2SO_4	7.10×10^{-2}
$MgSO_4 \cdot 7H_2O$	6.16×10^{-2}
NaFe chelate*	5.96×10^{-2}
KH_2PO_4	2.50×10^{-2}
K_2PO_4	1.10×10^{-2}
H_3BO_3	7.00×10^{-4}
$MnCl_2$	5.20×10^{-4}
$ZnSO_4 \cdot 7H_2O$	5.00×10^{-5}
$CuSO_4 \cdot 5H_2O$	2.00×10^{-5}
(pH = 6.5)	

---

* Geigy 330 Fe chelate; Na/Fe^{3+} diethylenetriamine pentaacetate.

FIG. 2. Flower distortion symptoms on TAV-infected 'Madame E. Rogers'. Top. Non-infected flower; Lower. Obverse and reverse aspects of distorted flowers. Note smaller size.

FIG. 3. Leaf distortion and 'bootlace' symptoms on TAV-infected Nicotiana glutinosa, 4 months after inoculation.

FIG. 4. Enations on the undersurface of TAV-infected Nicotiana glutinosa (see Fig. 3).

FIG. 5. Leaf mottle and distortion in TAV-infected Petunia hybrida 'Rose of Heaven' 2 months after inoculation.

FIG. 6. Leaf mottle symptoms on TAV-infected Nicotiana tabacum 'Samsun'. Left. Healthy control plant.

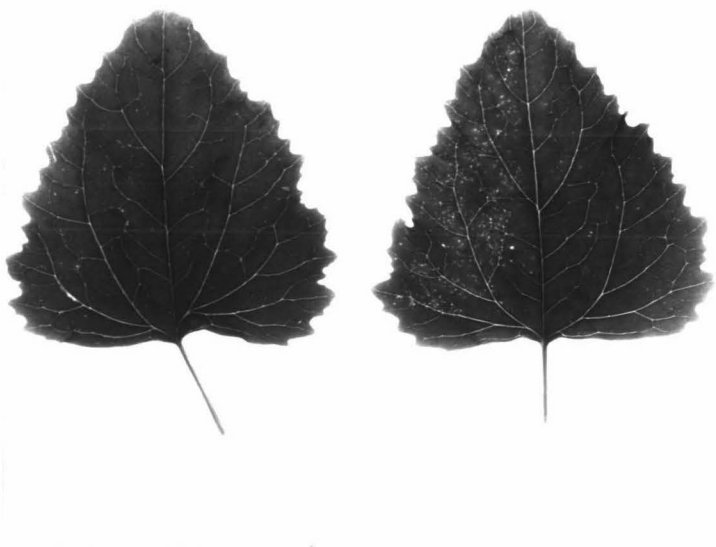


FIG. 7. Chlorotic local lesions on TAV-infected Chenopodium amaranticolor. L. to R. Water and Na_2HPO_4 (pH 7.3) controls; infected chrysanthemum sap diluted with phosphate buffer to 1 : 1 and 1 : 100.

FIG. 8. Top. Flower from chrysanthemum stunt-infected 'Shantung', note small size. Lower. Flower from healthy control plant free of stunt symptoms.

FIG. 9. Indexing for chrysanthemum stunt - 'Mistletoe' scion grafted to 'Madame E. Rogers'.

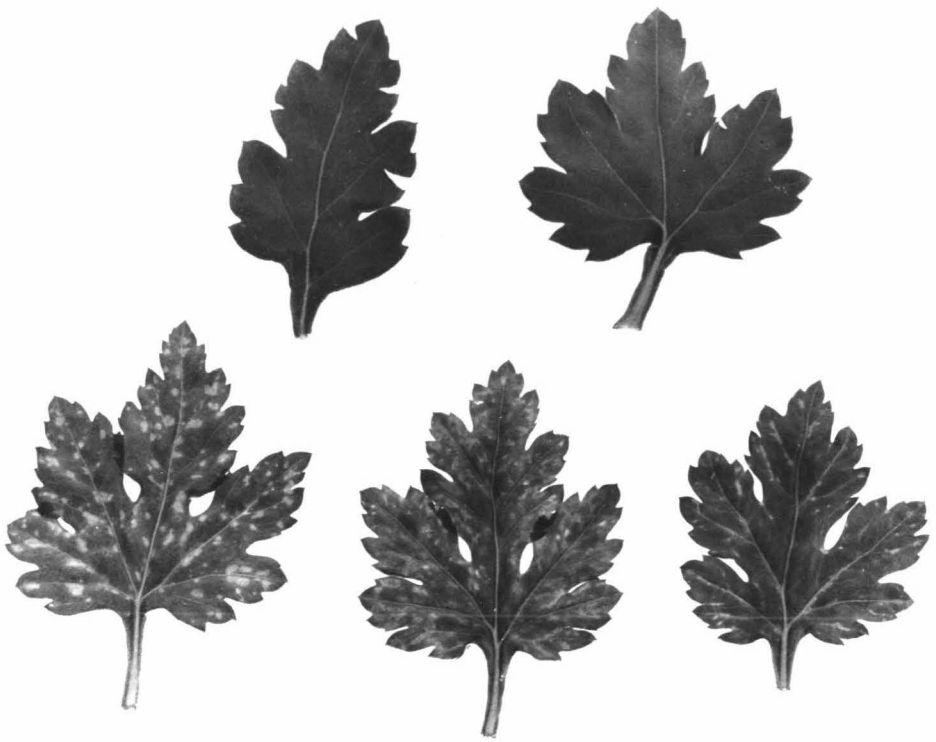


FIG. 10. Leaf fleck symptoms of chrysanthemum stunt on 'Mistletoe' scion leaves. Top. Healthy control leaves.

FIG. 11. Symptoms of leaf mottle virus infection. L. to R. Control, 'Rivalry', 'Waltz Time', and 'Good News' scion on 'Rivalry'.

FIG. 12. Chlorotic leaf mottling on 'Rivalry' leaf infected with virus of the leaf mottle group.

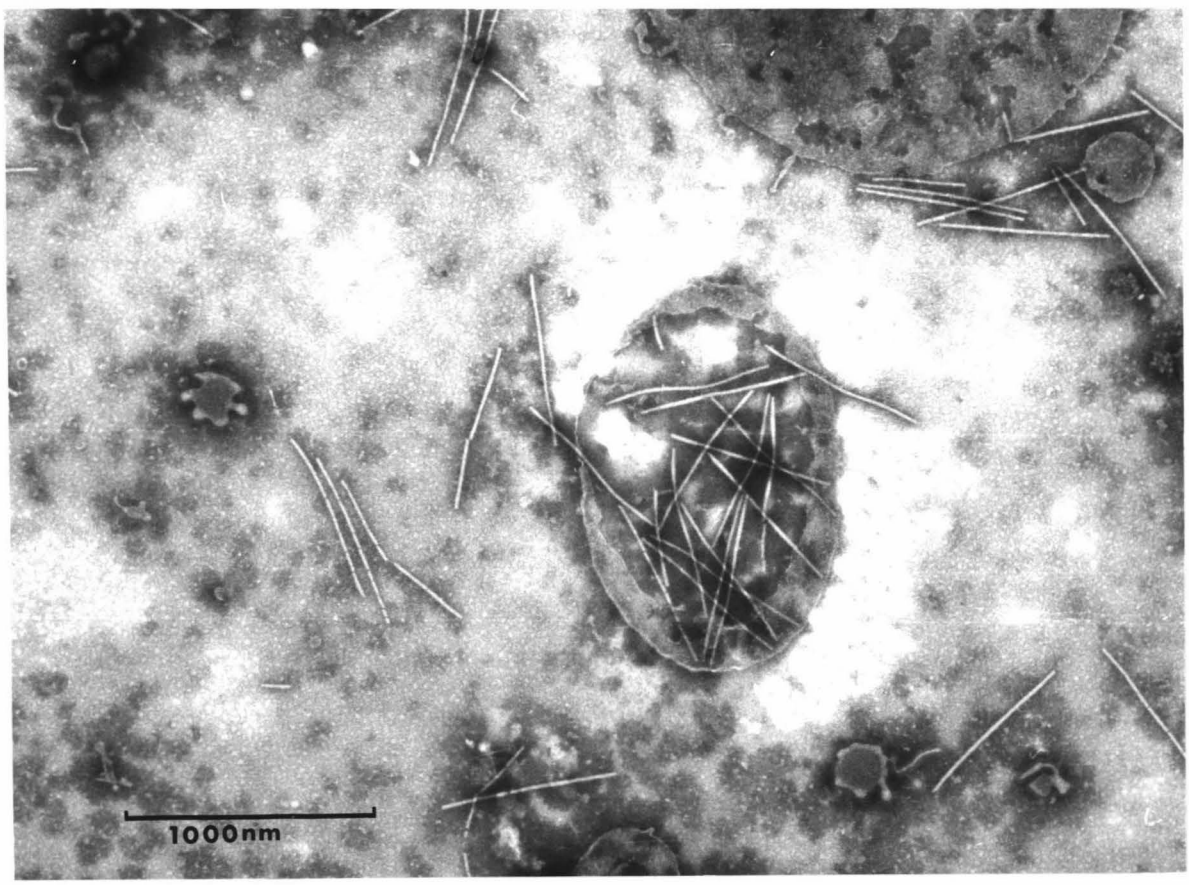


FIG. 13. Electron micrograph of rod-shaped particles of the leaf mottle group from 'Shantung'.

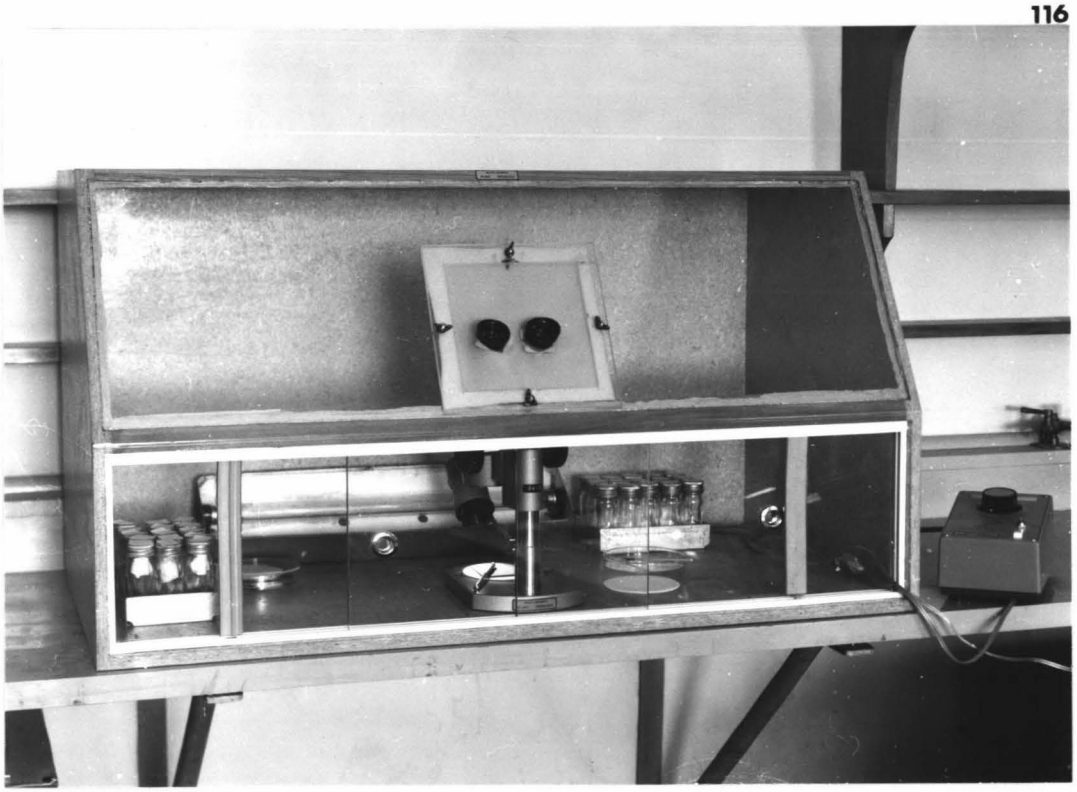


FIG. 14. Meristem-tip culture cabinet.

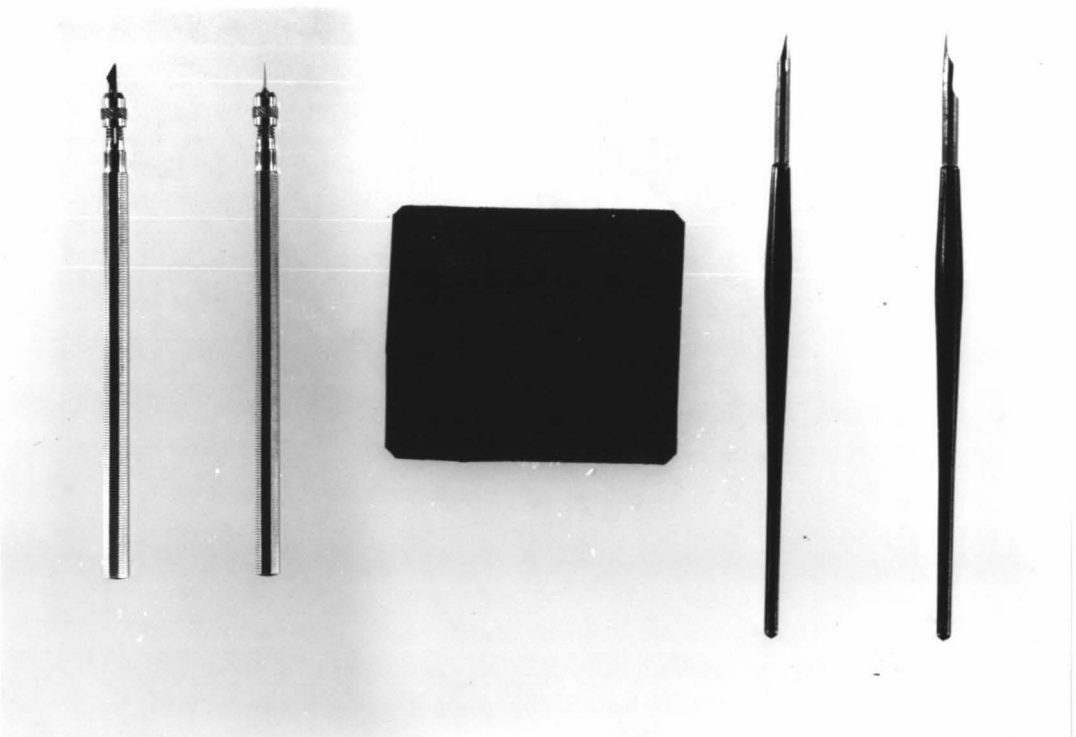


FIG. 15. Dissection equipment for meristem-tip culture. L. to R. Dissecting knives, dissecting pad, drawing pens used for leaf removal.

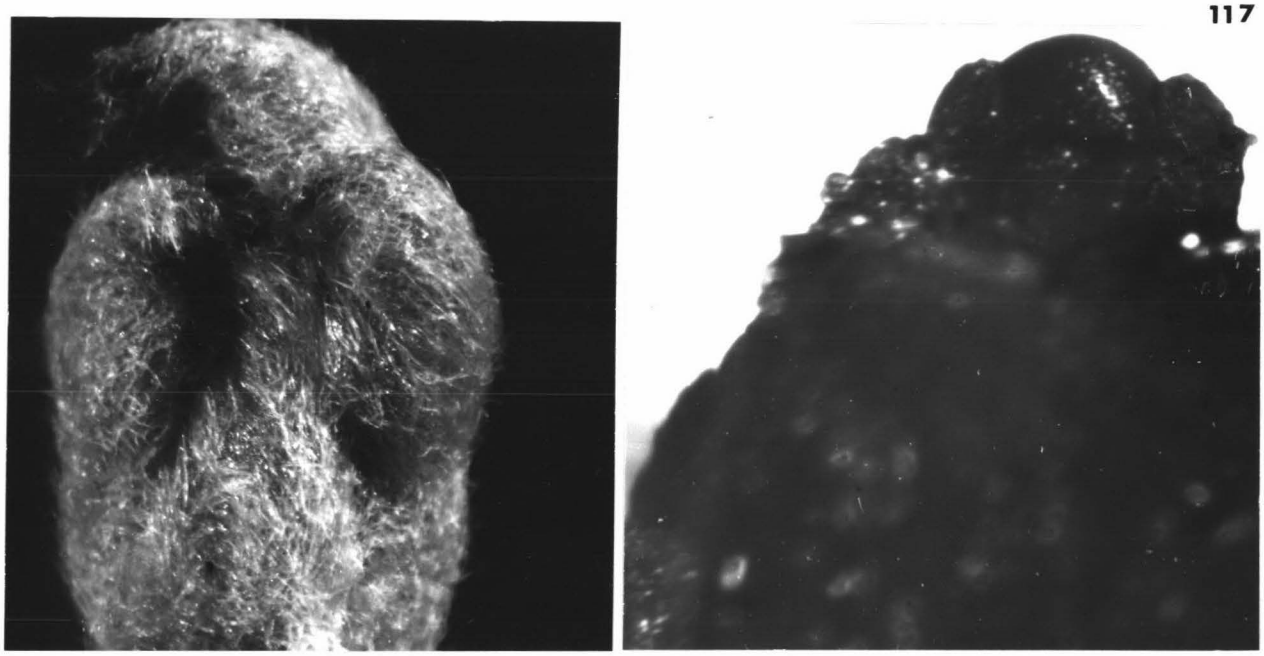


FIG. 16. Chrysanthemum shoot tip (15x) and meristem-tip (20x).

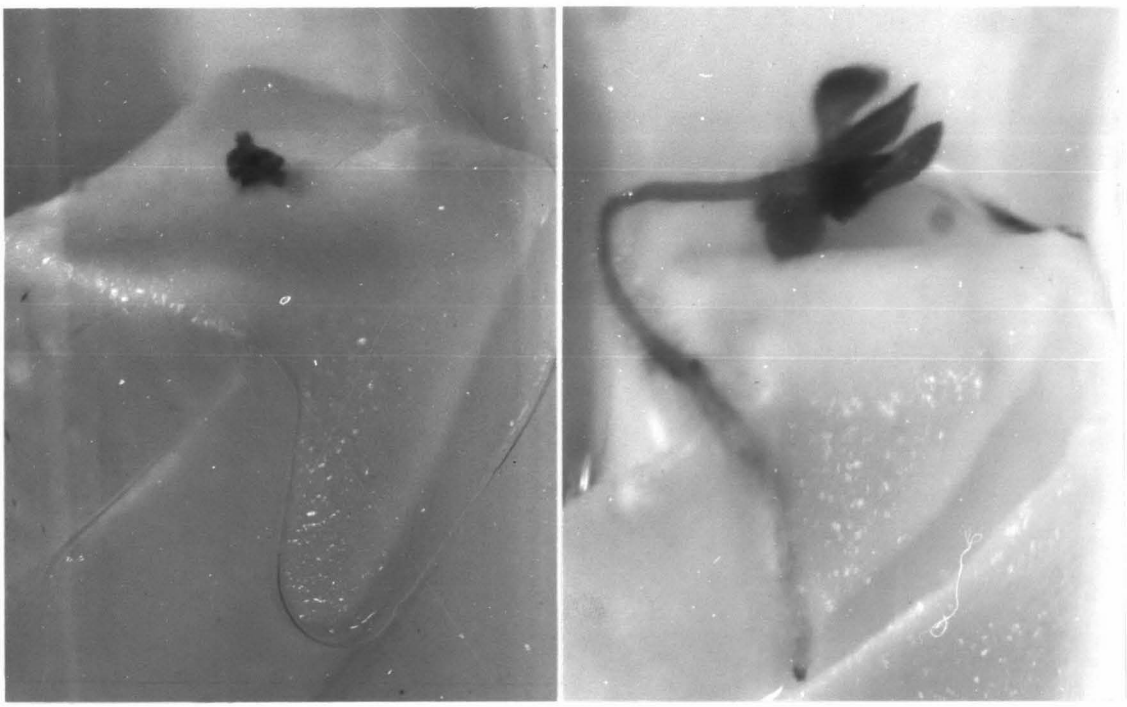


FIG. 17. Chrysanthemum meristem-tip after 6 and 12 weeks growth in culture bottle (5x).

FIG. 18. Chrysanthemum plantlet after transfer from culture bottle, prior to 'hardening off'.

BIBLIOGRAPHY

1. Ainsworth, G.C. (1936). Detection of spotted wilt virus in chrysanthemums. Nature, Lond. 137 : 868.

2. Baker, R. & D.J. Phillips (1962). Obtaining pathogen-free stock by shoot tip culture. Phytopathology 52 : 1242-1244.

3. Bawden, F.C. & F.M. Roberts (1948). Photosynthesis and predisposition of plants to infection with certain viruses. Ann. appl. Biol. 35 : 418-428.

4. Bewley, W.F. & A.A. Richards (1951). A mosaic disease of chrysanthemum. Gdnr's. chron. (Lond.) 129 (3341) : 20.

5. Black, L.M. (1943). Some properties of aster-yellows virus. Phytopathology 33 : 2.

6. Blencowe, J.W. & J. Caldwell (1949). Asperny - a new virus disease of the tomato. Ann. appl. Biol. 36 : 320-326.

7. Bos, L., D.J. Hagedorn & L. Quantz (1960). Suggested procedures for international identification of legume viruses. Tijdschr. Pl Ziekt. 66 : 328-343.

8. Bradley, R.H.E. (1963). Some ways in which a paraffin oil impedes aphid transmission of potato virus Y. Can. Jour. Micro. 9 : 369-380.

9. Brierley, P. (1950). Host plants of chrysanthemum stunt virus. Phytopathology 40 : 869.

10. _____ (1953). Some experimental hosts of the chrysanthemum stunt virus. Pl. Dis. Reptr. 37 (6) : 343-345.

11. _____ (1955). Symptoms induced in chrysanthemums on inoculation with the viruses of mosaics, asperny and flower distortion. Phytopathology 45 : 2-7.

12. _____ (1955). 'Blazing Gold' as a test variety for the chrysanthemum flower distortion virus. Pl. Dis. Reptr. 39 : 899-901.

13. _____ (1963). Revision of some plant virus property values. Pl. Dis. Reptr. 47 (5) : 445.

14. _____ & P. Lorentz (1960). Healthy tip cuttings from some mosaic-diseased Asiatic chrysanthemums; some benefits and other effects of heat treatment. Phytopathology 50 : 404-408.

15. _____ & C. Olsen (1956). Development and production of virus-free chrysanthemum propagative material. Pl. Dis. Reptr. Suppl. 238 : 63-68.

16. _____ & F.F. Smith (1949). Evidence points to virus as cause of chrysanthemum stunt. Flor. Rev. 103 (2676) : 36-37.

17. _____ (1949). Chrysanthemum stunt. Phytopathology 39 : 501 (Abstr.).

18. _____ (1951). Survey of virus diseases of chrysanthemums. Pl. Dis. Reptr. 35 (12) : 524-526.

19. _____ (1953). Noordam's B virus of chrysanthemum - detected in the U.S. Pl. Dis. Reptr. 37 (5) : 280-283.

20. _____ (1955). Chrysanthemum ringspot, a newly recognised pathogen found in combination with chrysanthemum stunt virus. Pl. Dis. Reptr. 39 : 33-34.

21. _____ (1957). Symptoms of chrysanthemum flower distortion, dodder transmission of the virus and heat cure of infected plants. Phytopathology 47 : 448-450.

22. _____ (1958). Some characteristic of 8 mosaic and 2 rosette viruses of chrysanthemum. Pl. Dis. Reptr. 42 (6) : 752-763.

23. _____ & S.P. Doolittle (1955). Some hosts and vectors of tomato asperny virus. Pl. Dis. Reptr. 39 : 152-156.

24. Butenko, R.G. (1968). In 'Plant tissue culture and plant morphogenesis'. Translated by M. Artmann, Israel Program for Sci. Trans. Ltd., (291p.) 20-53.

25. Chamberlain, E.E. (1946). A list of plant virus diseases in N.Z. N.Z. Jl. Sci. Technol. 28 (4) : 234-237.

26. Cooke, A.A. (1967). Isolation and maintainance of potato virus Y strains virulent on selected pepper varieties. Phytopathology 57 : 385-388.

27. Dahl, D. & C.A. Knight (1963). Some nitrous acid-induced mutants of tomato atypical mosaic virus. Virology 21 : 580-586.

28. Diener, T.O. & W.B. Raymer (1969). Potato spindle tuber virus: a plant virus with properties of a free nucleic acid. II Characterisation and partial purification. Virology 37 : 351-366.

29. Dimock, A.W. (1947). Chrysanthemum stunt. Flor. Rev. 101 (2607) : 37.

30. Dimock, A.W. & C. Geissinger (1969). A newly recognised disease of chrysanthemums caused by a graft-transmissible agent. Phytopathology 59 : 1024 (abstr.).

31. _____ & R.K. Horst (1971). Chlorotic mottle - a newly recognised disease of chrysanthemum. Phytopathology 61 : 415-419.

32. Dingley, J.M. (1969). Records of Plant Diseases in New Zealand Bull. N.Z. Dep. Scient. Ind. Res. 192. (298p.).

33. Doi, Y., M. Teranaka, K. Yora & A. Asuyama (1967). Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows or Paulownia witches broom. Ann. Phytopathol. Soc. Japan. 33 (4) : 259-266.

34. Elliott, R.F. (1969). Growth of excised meristem-tips of Kumara, Ipomoea batatas (Linn.) poir. in axenic culture. N.Z. Jl. Bot. 7 (2) : 158-166.

35. Giles, K.L. (1972). An inter-specific aggregate cell capable of cell wall regeneration. Plant & Cell Physiol. 13 : 207-210.

36. Gilmer, R.M. & K.D. Brase (1963). Nonuniform distribution of prune dwarf virus in sweet and sour cherry trees. Phytopathology 53 : 819-821.

37. Govier, D.A. (1957). The properties of tomato aspermy virus and its relationship with cucumber mosaic virus. Ann. appl. Biol. 45 (1) : 62-73.

38. Grogan, R.G., J.K. Uyemoto & K.A. Kimble (1963). Evidence that tomato aspermy and cucumber mosaic viruses are serologically related. Virology 21 : 36-42.

39. Hakkaart, F.A. (1967). Effect of aluminium strips on the spread of two aphid-borne chrysanthemum viruses. Neth. Jl. Plant Path. 73 : 181-185.

40. _____ (1969). Comparison of electron microscopy and serology with infectivity tests for detection of chrysanthemum virus B. Neth. Jl. Plant Path. 75 : 355-359.

41. _____ & F. Quak (1964). Effect of heat treatment of young plants on freeing chrysanthemums from virus B by means of meristem culture. Neth. Jl. Plant Path. 70 : 154-157.

42. _____ , D.H.M. van Slogteren & N.P. de Vos. (1962). Chrysanthemum virus B, its serological diagnosis in chrysanthemum, and its relationship to the potato viruses S and M and to carnation latent virus. Neth. Jl. Plant Path. 68 : 126-135.

43. Heller, R. (1949). Sur l'emploi de papier filtre sans cendres comme support pour les culture de tissus vegétaux. Compt. Rend. de la Soc. de Biol. 143 : 335-337.

44. Hitchborn, J.H. & G.J. Hills (1965). The use of negative staining in the electron microscopic examination of plant virus in crude extracts. Virology 27 : 528-540.

45. Holmes, F.O. (1956). Elimination of asperny virus from the 'Nightingale' chrysanthemum. Phytopathology 46 : 599-600.

46. Hollings, M. (1955). Investigation of chrysanthemum viruses. I. Asperny flower distortion. Ann. appl. Biol. 43 (1) 86-102.

47. _____ (1956). Chenopodium amaranticolor as a test plant for plant viruses. Plant. Pathol. 5 : 57-60.

48. _____ (1957). Investigation of chrysanthemum viruses. II. Virus B (mild mosaic) and chrysanthemum latent virus. Ann. appl. Biol. 45 (4) : 589-602.

49. _____ (1957). Reactions of some additional plant viruses on Chenopodium amaranticolor. Plant. Pathol. 6 : 133-135.

50. _____ (1959). Host range studies with fifty two plant viruses. Ann. appl. Biol. 47 (1) 98-108.

51. _____ (1960). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1959 : 67-68.

52. _____ (1962). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1961 : 73-74.

53. _____ (1965). Disease control through virus-free stock. Ann. Rev. Phytopath. 3 : 367-396.

54. _____ (1968). The virus problem in flower crops. Scient. Hort. 20 : 47-56.

55. _____ & B. Kassanis (1957). The cure of chrysanthemums from some viruses by heat. Jl. Roy. Hort. Soc. 82 : 339-342.

56. _____ & O.M. Stone (1962). The attenuation of carnation mottle virus in plants. Rep. Glasshouse Crops Res. Inst. 1961 : 100-102.

57. _____ (1967). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1966 : 93.

58. _____ (1968). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1967 : 94-95.

59. _____ (1968). Techniques and problems in the production of virus-tested planting material. Scient. Hort. 20 : 57-72.

60. _____ (1969). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1968 : 103-104.

61. _____ (1970). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1969 : 128-129.

62. _____ (1970). Attempts to eliminate chrysanthemum stunt from chrysanthemum by meristem-tip culture after heat treatment. Ann. appl. Biol. 65 : 311-315.

63. _____ (1971). Tomato aspermy virus. C.M.I./A.A.B. Description of plant viruses. Oct. 1971 No. 79.

64. _____ & G.C. Boutelli (1967). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1966 : 94.

65. _____ & A. Brunt (1968). Cucumber mosaic virus. Rep. Glasshouse Crops Res. Inst. 1967 : 95-98.

66. _____ & M.E. Norrish (1963). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1962 : 86-93.

67. _____ & S.H. Smith (1965). Chrysanthemum viruses. Rep. Glasshouse Crops Res. Inst. 1964 : 89-90.

68. Houten, J.G. ten, F. Quak & F. van der Meer (1968). Heat treatment and meristem culture for the production of virus-free plant material. Neth. Jl. Plant Path. 74 : 17-24.

69. Ie, T.S. (1970). Tomato spotted wilt. C.M.I./A.A.B. Description of Plant Viruses Oct. 1970 No. 39.

70. Inouye, T., M. Asatani & K. Mitsuhata (1968). Studies on the viruses of plants in Compositae in Japan. I. Tomato asperny virus from chrysanthemum. Nogaku Kenkyu 52 (2) : 55-64.

71. Ishiie, T., Y. Doi, K. Yora & H. Asuyama (1967). Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease. Ann. Phytopathol. Soc. Japan. 33 (4) : 267-275.

72. Jacobs, G., C.H. Borman & P. Allan (1968). Tissue culture studies on Rose. Use of pith explants. S. Af. J. agric. Sc. 11 : 673-678.

73. Kassanis, B. (1957). The use of tissue culture to produce virus-free clones from infected potato varieties. Ann. appl. Biol. 45 : 422-427.

74. _____ (1957). Effects of changing temperature on plant virus diseases. Adv. Virus Res. 4 : 221-241.

75. _____ (1965). Therapy of virus-infected plants. Jl. R. agric. Soc. 126 : 105-114.

76. _____ & A.F. Posnette (1961). Thermotherapy of virus-infected plants. Recent Adv. in Bot. 1 : 557-563.

77. Kegler, H. (1961). Untersungen über die Beständigkeit einiger obstviren in vitro. Tidsskr. Planteavl. 65 : 163-171.

78. Keller, J.R. (1951). Report on indicator plants for chrysanthemum stunt virus and on a prieviously unreported chrysanthemum virus. Phytopathology 41 : 947-949.

79. _____ (1953). Investigations of chrysanthemum stunt virus and chrysanthemum virus Q. Cornell Univ. Agric. expt. stn. Ithaca. N.Y. Memoir 324 (40p.).

80. Kemp, W.G. (1961). Investigations of chrysanthemum viruses in Canada. I. Chrysanthemum rosette virus. Can. Pl. Dis. Surv. 41 (4) : 183-190.

81. Kennedy, J.S., M.F. Day & V.F. Eastof. (1962). A conspectus of aphids as vectors of plant viruses. Commonwlth. Inst. Entomol. (114p.).

82. Kosuge, T. (1956). Biochemical aspects of virus inhibitors in vitro and in vitro. Proc. Int. Conf. on Viruses and Vectors (U.C.). (247p.).

83. Kunkel, L.O. (1926). Studies on aster yellows. Am. J. Bot. 13 : 646-705.

84. _____ (1936). Heat treatment for the cure of yellows and other virus diseases of peach. Phytopathology 26 : 809-830.

85. Lawson, R.H. (1968). Cineraria varieties as starch lesion test plants for chrysanthemum stunt virus. Phytopathology 58 : 690-695.

86. Mason, H.S. (1955). Biochemical aspects of phenolase activity. Adv. Enzymol. 16 : 105.

87. Matthews, R.E.F. (1970). In 'Plant Virology'. Academic Press (778p.) Ch. 18 : 593-594.

88. Maramorosch, K., E. Shikata & R.R. Granados (1968). Structures resembling mycoplasma in diseased plants and in insect vectors. Trans. N.Y. Acad. Sci. 30 : 841-855.

89. Miller, P.R. (1954). Detection of virus B of chrysanthemum in the U.S. F.A.O. Plant Protect. Bull. 2 : 56-59.

90. Miller, C.O. (1965). Evidence for the natural occurrence of zeatin and derivatives: Compounds from maize which promote cell division. Proc. Natn. Acad. Sci. 54 : 1052-1058.

91. Monsion, M., T. Huchet & J. Dunez (1969). Dissémination naturelle des virus de la mosaïque et de l'aspermie du chrysanthème. Annls. Phytopathol. 1 (4) : 583-589.

92. Morel, G.M. (1948). Recherches sur la culture associee de parasites obligatoires et de tissus vegetaux. Annls. Epiphyties (N.S.) 14 : 123-124.

93. Mosteller, F., R.E. Rourke & G.B. Thomas (1961). In 'Probability and Statistics'. Addison-Wesley Publishing Co. Inc. (366p.).

94. Murashige, T. & F. Skoog (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiologia Pl. 15 : 473-497.

95. Nelson, R. (1925). Chrysanthemum yellows; a new disease in the green-house. Michigan Agric. exp. stat. quart. Bull. 7 : 157-160.

96. Nielson, L.W. (1960). Elimination of the internal cork virus by culturing apical meristems of infected sweet-potatoes. Phytopathology 50 : 840-841.

97. Noordam, D. (1952). Virusziekten bij chrysanten in Nederland. Tijdschr. Pl ziekten. 58 : 121-189.

98. Nyland, G. & A.C. Goheen (1969). Heat therapy of virus diseases of perennial plants. Ann. Rev. Phytopath. 7 : 331-354.

99. Oertel, C. (1969). Untersuchungen über viruskrankheiten an chrysanthemum indicum L. Nova Acta Leopoldina 34 (189) : 1-92.

100. Parke, R.V. (1959). Growth periodicity and the shoot tip of Abies concolor. Am. J. Bot. 46 : 110-118.

101. Prentice, I.W. (1952). Chrysanthemum flower distortion - survey 1951. Plant Pathol. 1 : 77-80.

102. _____ (1958). Virus disease problems in flower crops. Scient. Hort. 13 : 90-96.

103. Quak, F. (1957). Meristeem-cultuur, gecombineerd met warmtebehandeling var het verkrijgen van virus-vrije anjer planten. Tijdschr. Pl Ziekten 63 : 13-14.

104. Sastry, K.S.M. (1964). A new strain of chrysanthemum asperny virus from India. Indian J. Microbiol. 4 (2) : 37-40.

105. Searle, S.A. & B.J. Machin (1968). In 'Chrysanthemums the Year Round'. Blanford press. 3rd edition (379p.) Ch. 2 : 30-41.

106. Skillman, E.E. (1951). Further observations on chrysanthemum virus disease. Gdners. chron. 129 : 141.

107. Smith, F.F., G.V. Johnson, R.P. Kahn & A. Bing. (1964). Repellancy of reflective aluminium to transient aphid virus-vectors. Phytopathology 54 : 748 (abstr.).

108. Smith, K.M. (1953). Some virus diseases of vegetables and ornamental plants. Rep. Int. Hort. Congress. Lond. 1952 p. 569.

109. _____ (1957). In 'A Textbook of Plant Virus Diseases'. 2nd ed. J.A. Churchill Ltd. (648p.).

110. Stone, O.M. (1963). Factors affecting the growth of carnation plants from shoot apices. Ann. appl. Biol. 52 : 199-209.

111. _____ & M. Hollings (1963). Tip culture and the production of virus-free clones. Rep. Glasshouse Crops Res. Inst. 1962 : 88.

112. Sutton, J. & R.H. Taylor (1971). Research project for diseases of ornamental plants. 2nd Ann. Rep. Victorian Plant Res. Inst. of Dept. Agric. Victoria. pp. 8-14.

113. Thomson, A.D. (1963). Occurrence of tomato asperny virus, carnation mottle virus and a virus from chrysanthemums in New Zealand. N.Z. Jl. agric. Res. 5 : 396-402.

114. Tochihara, H. (1970). Some properties of chrysanthemum mild mottle virus and comparison of this virus with cucumber mosaic virus. Ann. Phytopathol. Soc. Japan. 36 (1) : 1-10.

115. Welsh, M.F. (1948). Stunt-mottle virus disease of chrysanthemums. Scient. Agric. 28 : 422.