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VIRUS AND VIRUS-LIKE DISEASES

OF ROSES IN NEW ZEALAND

A thesis on studies conducted in the Department of Horticulture and Plant Health in partial fulfilment of the requirement for a Doctor of Philosophy Degree at Massey University.

by

P.C. Gardner June 1983

ERRATA

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p 1	Para. 4,	line 4:	Rosa <u>not</u> R.
p 1	Para. 6,	line 2:	diseases not disorders
p 2	Para. 2,	line 10:	'Iowa State Univ - 60 (ISU-60)
p15	Para. 1,	line 2:	thought not though
p15	Para. 8,	line 2:	viruliferous <u>not</u> viuliferous
p20	Para. 5,	line 3:	for <u>not</u> in
p31	Para. 2,	line l:	diseases not disorders
p40	Para. 4,	line 2:	amaranticolor <u>not</u> amaranthicolor
p54	Para. 7:		Delete this paragraph
p57	Para. 1,	line l:	electrophoresis not electorphoresis
p70	Para. 1,	line 3:	TobRSV <u>not</u> TSV
p91	Para. 5,	line l:	condition not conditions
p92	Para. 1,	line 2:	indicator not test

ABSTRACT

i

Roses in New Zealand were surveyed for the presence of symptoms which might be caused by virus or virus-like graft transmissible agents. Representative samples (221) of all symptom types and many apparently symptomless plants were indexed by a number of methods. Prunus necrotic ringspot virus was found to be widespread, occuring in plants both with and without symptoms. Apple mosaic virus was detected in one plant and arabis mosaic virus was detected in some plants of one cultivar clone.

Prune dwarf virus, tobacco streak virus, tobacco ringspot virus, and strawberry latent ringspot virus were not detected.

Two graft transmissible virus-like diseases of rose flowers are described. One of them, rose petal fleck, was widespread in both obviously affected and symptomless plants. The other, rose colour break, was largely confined to some glasshouse cutflower cultivars and a few garden cultivars.

SUMMARY

Two hundred and twenty one rose samples showing a wide range of symptoms which might be caused by virus or virus-like graft transmissible agents were indexed for transmission and perpetuation on *Rosa multiflora* 'Iowa State University 60' understocks.

All samples were indexed on herbaceous hosts and by the enzyme-linked immunosorbent assay (ELISA) serological technique for prunus necrotic ringspot virus (PNRSV), apple mosaic virus (ApMV), prune dwarf virus (PDV), danish plum line pattern virus (DPLPV), tobacco streak virus (TSV), tobacco ringspot virus (TobRSV), strawberry latent ringspot virus (SLRSV) and arabis mosaic virus (ArMV).

A range of selected samples were also indexed on 'Golden Queen' peach seedlings, apple understocks, 'Shirofugen' cherry and *Rosa multiflora* 'Burr'.

The ELISA technique was modified to detect heterologous strains of PNRSV and a method was developed to detect the presence of any one or more of three viruses in any one or more of ten plants simultaneously.

One plant only, with heavy gold leaf blotching, was found to be infected with ApMV. All other plants tested with mosaic type leaf symptoms or rose wilt type decline and dieback of mature plants were found to be infected with PNRSV as were a number of apparently symptomless plants. Symptomless ArMV was found on some plants of one cultivar clone only. No other viruses were detected.

The symptoms in mature plants attributed to 'rose wilt virus' could invariably be associated with the presence of PNRSV but the proliferation symptom in maiden plants could not.

In infected plants PNRSV reached a higher titre in more metabolically active and younger tissue than in older tissue but virus could not be detected in embryos excised from seeds.

Four symptomless plants of supposedly 'high health' status of each of 274 rose cultivars were indexed. Fourteen cultivars were positive for PNRSV and one cultivar positive for ArMV.

ii

Approximately 200 cultivars of so-called "old and species type" roses were indexed. Seventeen cultivars were positive for PNRSV and all tests for other viruses were negative.

Six clones of *R. multiflora* understock, in commercial use for some years, were all at least in part infected with PNRSV but were negative for other viruses.

A polyacrylamide gel electrophoresis separation of ribonucleic acid (RNA) extracted from PNRSV showed a multipartite genome with RNA with molecular weights of about 1.4, 1.0, 0.7 and $0.3 + 10^{6}$.

Two virus-like diseases of flowers were described. Rose petal fleck (RPF) and rose colour break (RCB) were transmitted by grafting both separately and together to healthy rose plants. No causal agent was detected for these disorders.

Rose petal fleck was widespread occuring in both obviously infected and apparently symptomless cultivars. A number of so-called "old and species type" roses consistently had flecked petals which is considered normal for those cultivars. On indexing they were found to have RPF.

Rose colour break was found only occasionally, mainly in glasshouse cutflower varieties.

iii

PREFACE

With increased understanding and recognition of the effects of virus infection, transmission and perpetuation in vegetively propagated woody plants it has become essential for the horticultural industry to have access to propagative material as free of viral pathogens as possible.

To achieve this objective a knowledge of the virus and virus-like pathogens infecting a crop and rapid and reliable methods for their detection are essential.

Crops considered to be of significance to the New Zealand economy have been given high priority by the Department of Scientific and Industrial Research for such investigations.

Although roses are an important nursery and cut flower crop within New Zealand they have a relatively low export potential and therefore despite an obvious need for investigation of their viral status the same priority could not be given to roses as to export fruit crops.

However, in recognition of the urgent need for research in rose viruses the DSIR made funds available to Massey University in 1978 for a Rose Research Contract.

The New Zealand rose industry and the rose growing public at large are indebted to DSIR for providing the funds which made this investigation possible. The continuing work by the New Zealand Nursery Research Centre, at Massey University, is now enabling the practical application of these results.

I wish to express my thanks to Professor K.S. Milne and the Department of Horticulture and Plant Health at Massey University for guidance, support and patience, and to Mr H.F. Neilson for invaluable technical assistance. The opportunity to work with Dr J.B. Sweet (Massey Post-doctoral Fellow), who had considerable experience with viruses of rosaceous plants was also most helpful.

For the supply of materials, antisera and advice, thanks are due to Dr P.F. Fry, Plant Diseases Division, DSIR, Auckland, Professor R.W. Fulton, University of Wisconson, U.S.A. and Dr B.D. Harrison, Scottish Horticultural Research Insititute, U.K.

Members of the nursery industry throughout New Zealand have always been supportive, allowing access to their crops and the opportunity to collect material for investigation. I trust these results will be of benefit to them in the future.

Last but not least, my thanks to my wife and family for their patience and long-suffering understanding.

P.C. Gardner June 1983

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION	1			
Source of Rose Material	2			
Rose Symptoms	5			
Rose Mosaic - Review	9			
Rose Wilt and Proliferation - Review	19			
Association of <i>Prunus</i> Necrotic Ringspot Virus with Rose Mosaic and Rose Wilt Symptoms	24			
CHAPTER 2. INDEXING				
Bud Grafting				
Rosa multiflora 'ISU-60'	28			
Rosa multiflora 'Burr'	31			
Prunus serrulata 'Shirofugen'	33			
Apple Rootstocks	36			
Peach 'Golden Queen' Seedlings	37			
Mechanical Transmission	38			
Electron Microscopy	43			
Serological Methods	45			
CHAPTER 3. PRUNUS NECROTIC RINGSPOT VIRUS - Purification	46			
Characteristics of Particles	50			
Antiserum Production and Testing	60			
CHAPTER 4. ENZYME-LINKED IMMUNOSORBENT ASSAY	63			
Methods and Results of ELISA Indexing	67			
"Diseases Collection"	71			
"High Health Mother Plants"	78			
Simultaneously for a Range of Viruses	81			
"Old Rose Collection"	83			
Non Rose Hosts and Rose Rootstocks	84			
Distribution of Virus Within the Rose Plant	86			
CHAPTER 5. VIRUS-LIKE FLOWER DISORDERS	90			
Rose Colour Break	93			
Rose Petal Fleck	94			
CHAPTER 6. CONCLUSIONS	102			
BIBLIOGRAPHY 10				
APPENDIX I Back	cover			

vi

FIGURES

1.	Optical density profiles of five isolates of	
	prunus necrotic ringspot virus nucleoprotein	53
2.	Optical density profile of prunus necrotic ringspot virus, rose isolate '154' after polyacrylamide gel electrophoresis	58
3.	ELISA plate showing reactions, against PNRSV-RA (Fulton) antiserum, of isolates from the rose "disease collection", PNRSV-G (Fulton), ApMV (Fultons's serotype from rose) all ex cucumber	77a
4.	ELISA plate showing reactions, against ApMV-C (Fulton) antiserum, of young leaf samples from 46 roses in the "disease collection" (samples '45' to '90' inclusive), healthy rose, <i>C. quinoa</i> infected with ApMV-C (Fulton), and cucumber infected with PNRSV-G (Fulton)	77ь
5.	ELISA plate showing PNRSV-RA (Fulton), ApMV-C (Fulton), and the combination of these two antisera with ArMV (Harrison) against isolates of rose '21' infected with PNRSV, <i>Rosa multiflora</i> grafted with rose '72' (ApMV), rose '101' in cucumber, and ApMV (Fulton) in <i>C. quinoa</i>	77c
6.	Two flowers of the rose cultivar 'Bridal Pink' showing severe symptoms of rose colour break	93a
7.	Two fully open flowers of the rose cultivar 'Fragrant Cloud' used as an indicator showing symptoms of rose petal fleck induced by an infected test bud and a healthy negative test	94a

CHAPTER 1 INTRODUCTION

Over the past 10 to 15 years, increasing concern has been expressed by nurserymen and the rose growing public at the apparent increase in the incidence of virus and virus-like diseases in roses in New Zealand. In 1978 DSIR made funds available for a three year Rose Research Contract UV/4/31 specifically for an investigation of viruses affecting roses in New Zealand. This investigation is a direct result of that grant.

As has been the case in the past with many vegetatively propagated horticultural subjects the occasional virus infection of roses has been perpetuated by the use of infected material for propagation. Until recently it has been standard nursery practice in New Zealand to bud-graft rose cultivars onto rooted cuttings of *Rosa multiflora* Thunb. taken during winter from the stock tops of plants budded the previous season. Budwood for bud-grafting during summer being also taken from the same plants.

Not only may budwood from an infected plant produce a number of infected plants of that variety the following season, but also infected stock cuttings from that plant may be budded with buds of previously uninfected varieties. Over a number of years this practice may result in widespread infection in the whole crop.

In many other rose-producing countries, where commercial crops are propagated on seedling understocks the spread of infection appears to be less rapid. The possibility of seedborne infection with *Prunus* necrotic ringspot virus (PNRSV) in *R.multiflora* seedlings of the order of one percent has been reported (238, 243).

The aim of this investigation has been to survey the virus and viruslike graft transmissible disorders of roses in New Zealand; isolate, characterise and identify the causal agents; examine various methods of indexing using herbaceous and woody indicator hosts; purify, prepare antisera to viruses isolated and examine various serological methods for rapid detection of these viruses in roses.

Source of Rose Material

Initially a general survey of roses in the North Island was carried out. Visits were made to public rose gardens and public rose plantings in Wellington, Lower Hutt, Levin, Palmerston North, Hastings, Napier, Wanganui, Taihape, Hawera, New Plymouth, Rotorua, Te Kuiti, Hamilton and Auckland. A number of private rose gardens in these towns were also inspected. All major rose producing nurseries in the North Island were inspected as well as three major growers of glasshouse roses for cut flowers.

During these inspections samples of budwood were collected from a selection of plants to give a range of all symptom types which may possibly have been attributable to virus or virus-like pathogens. This selection included all growth, leaf or flower abnormalities observed which had no obvious other pathogenic cause. They were selected so as to not give too many samples of the same or similar symptoms and therefore cannot be considered as a statistical sample of the occurrence of symptoms. Two hundred and twenty one samples were collected at various times and each sample was initially budded to five *R. multiflora* 'ISU-60' cutting grown understocks. After the buds had taken, the stock tops of three of each five understocks were removed and the grafts grown on as rose bushes. The stock tops of the other two were left *in situ*.

The symptoms observed on each source plant at the time of collecting samples were recorded. The plants to which the samples had been grafted were observed over two to three growing seasons. The two plants of each sample with the stock top intact were examined for transmission of symptoms and the rose bushes were examined for perpetuation of symptoms and any progress or development of further symptoms.

This collection of plants produced from the samples was used as source material for all subsequent investigations including transmission to herbaceous hosts, virus purification and antiserum production,

-2-

serological testing, electron microscope examination and further indexing by budding to Prunus serrulata L indl. 'Shirofugen', R. multiflora 'Burr', virus free seedlings of P. persica 'Golden Queen', virus free apple understocks, and double budding with susceptible rose cultivars as indicators for rose petal fleck (RPF) and rose colour break (RCB).

This collection of plants will be referred to hereafter as the "disease collection".

A second collection of rose plants which had been commenced late in 1970, built up in the main during 1971-72 and subsequently added to from time to time, will be referred to as "high health mother plants".

This collection consisted of 4 plants per cultivar of 274 cultivars obtained from sources as likely to be free of known viruses as possible. The majority of cultivars were obtained direct from the breeder of each cultivar and the budwood obtained from plants as close as possible at the time to the original hybrid seedling, from which the clone originated.

Other additions to the collection were made from amateur and commercial sources known to be endeavouring to maintain plants free of known viruses.

The high health mother plants were regularly checked over subsequent years by observation and by observation of their progeny in a nursery situation for any sign of virus like disorders. In the case of two cultivars approximately 25% of the progeny showed the vein-banding symptom usually associated with PNRSV and/or ArMV. The offending one of the four mother plants in both cases was identified and destroyed. Subsequent progeny appeared healthy.

A third collection involved in this investigation consisted of one plant each of approximately 200 "old and species" type roses situated at a nursery involved in the production of this type of rose.

-3-

In addition 15 cultivar clones and six rootstock clones of varieties, not known to be available in New Zealand as high health material, were imported from the University of California, USA, foundation stock of heat treated and indexed roses.

Rose Symptoms

In the course of this study a wide range of symptoms were observed in roses. Some symptoms were consistently associated with viruses, some symptoms were regularly graft-transmitted but no pathogen was found that could be associated with them, some symptoms were able to be perpetuated by grafting but were not transmitted to healthy roses and other symptoms were only characteristic of the source plant and were not perpetuated by grafting.

Expression of symptoms is often variable and they do not always fall clearly into any one category. They can however be approximately defined and classified as follows:

A. Symptoms found to be associated with virus

- Vein banding, referred to in the USA and Italy as yellow vein or yellow mosaic, is described as broad chlorotic yellow bands about the midrib and as a chevron pattern about the main lateral veins.
- 2. Vein netting involving a finely netted chlorosis of all minor veins.
- 3. Chlorotic line patterns may take the form of either an oakleaf shaped pattern or random lines and rings. Symptoms were recorded as either heavy or light depending on colour intensity and either broad or fine depending on width.
- 4. Large, 5mm or more, chlorotic yellow blotches.
- 5. Chlorotic mottle, expressed as 2-4mm chlorotic spots, which may be dense or diffuse.
- Light chlorotic stipple a very fine stippling or frosting effect with the 'stipples less than lmm in diameter (plants with this symptom subsequently showed rose wilt type symptoms).

<u>NOTE</u>: The symptoms (1-6 inclusive) just described can be grouped as rose mosaic type symptoms.

- General decline and reduction in size of the plant usually accompanied by the following three symptoms (8 to 10 inclusive) which also may occur separately on other plants.
- Rosetting. Little leaves becoming recurved and bunched together on short shoots with no internodal elongation, arising from lateral buds on previous seasons canes on mature bushes.

- 9. Dieback and necrosis of old canes.
- 10. Thin, twiggy, abortive lateral shoots with normal internodal elongation but of less than normal diameter with somewhat smaller than normal leaves with epinastic leaflets recurved about the rachis and cupped abaxially.

NOTE: The symptoms (7-10 inclusive) just described occurring on mature bushes can be referred to collectively as rose wilt type symptoms.

B. Virus-like symptoms

Symptoms which could be regularly graft-transmitted but no pathogen could be associated with them.

- 1. Rose petal fleck (RPF) is expressed in the flowers of rose varieties, with flowers of orange, red or purple shades, as small, irregular, elongated flecks of deeper colour. The flecks are elongated in the direction of the veins at the outer edge of the petal and are generally between 5 and 15mm in length. Where flecks are adjacent they are joined by lateral bands giving patches of flecking with an appearance resembling 'chinese characters'. Mild symptoms are more apparent towards the edges on the inner surface of the petals. The flecked tissue appears shrunken with resultant loss of petal texture giving a crepe-paper appearance and consequent loss of cut-life. In those rose varieties with flowers of white, yellow and some pink shades some loss of petal texture may be apparent or they may be virtually symptomless.
- 2. Rose colour break (RCB), as described by Hunter (153, 154) in 'Queen Elizabeth' is expressed in its severest form as a distinct greening of the petals with the main veins thickened. The outer petals are reduced in size, green with thickened veins, crimped and distorted appearing almost fleshy and lettuce-like. The inner petals may be papery with blotched areas of colour variation. In milder forms it may be expressed only on the outer petals as a slight crimping with the main veins appearing more prominent on the reverse of the petals and tinged with red or green

-6-

C. Non-transmissible symptoms

Various symptoms observed and recorded of uncertain etiology. Some were perpetuated by grafting and were probably chimeral. Others were not and were probably due to physiological conditions, mineral deficiencies, spray damage and other than viral pathogens.

None of these symptoms were consistently associated with any of the viruses studied.

1. Proliferation. The first shoot from a grafted bud is short (one to 20cm), tapers quickly to a more or less necrotic tip and bears leaves with normal sized stipules but grossly reduced leaflets. This is followed by multiple shoots with similar symptoms arising from the grafted bud and/or the base of the first shoot. More normal shoots may arise and if the graft does not break at the union these will produce a normal plant if no other unassociated pathogens are present.

The proliferation symptom in New Zealand is generally associated with excessive callus tissue at the graft union and may also be associated with excessive callus and galling of the understock at the site of other wounds (111, 112).

- Failure of the grafted bud to grow either may be an extreme expression of the proliferation symptom or may be due to other causes such as faulty technique.
- 3. Stem splitting and corkiness
- 4. Stem purple blotching and/or necrotic patches
- Pellucid dots on stems due to raised, watersoaked spots in the vicinity of lenticels.
- A general reduction in size with all the plant parts reduced to 50 to 75% of normal size.
- An extreme bushy stunt with all the growth thin and twiggy and the mature plant reduced to less than quarter normal size.
- Wilting of soft growth on young canes which either may or may not be followed by dieback and necrosis of young canes. (This symptom is typical of infection by *Verticillium* wilt fungi).
- 9. Bullate leaflets giving a blistered effect with the margins recurved and the interveinal areas arched upwards (adaxially)
- 10. Splitting and corkiness of the rachis.

-7-

- 11. Vein clearing and flecking involving small irregular translucent areas on and about veins.
- 12. Narrow strap-shaped leaflets.
- 13. Leafy enations on the midrib and veins.
- 14. General yellowing and thin, unthrifty appearance of the leaves.
- 15. Pale green between the veins of the leaflets.
- 16. Premature senescence of the leaves.
- 17. Reduction in size of petals may occur in flowers of a variety, which normally has flowers of the overlapping classical Hybrid Tea form, resulting in a mass of small, narrow, rather strap shaped petals.
- 18. Cupping of petals with some reduction in number and size usually associated with a slight shift in colour away from that normal for the variety.
- 19. Phyllody of the carpels which then protrude from the centre of the flower.

Rose Mosaic - Review

The term "rose mosaic virus" has been used loosely by most workers in this field to apply to any graft transmissible pathogen associated with mosaic type symptoms in rose. As will be subsequently described these symptoms have been associated with a number of different virus entities. In this report the term "rose mosaic" will be used to describe symptoms of this type and where possible the virus associated with the symptoms will be quoted.

Fulton's (92) isolate from *Rosa setigera* Michx. with mosaic type symptoms was subsequently shown to be serologically identical with apple mosaic virus (ApMV) (100, 101). Many other isolates from roses showing various mosaic type symptoms have been shown by various authors to be PNRSV.

A number of other viruses have also been associated with mosaic type symptoms in rose.

An isolate of tobacco streak virus (TSV) was obtained from a rose with chlorotic patches and chlorosis of the veins (103).

Roses with mosaic symptoms in Iowa, USA, yielded tobacco ringspot virus (Tob RSV) (184).

The isolation by Halliwell & Milbrath (130) of tomato ringspot virus (Tom RSV) from roses exhibiting rose mosaic has not been confirmed and may be suspect in the light of the serological reactions they reported.

Arabis mosaic virus (ArMV) and occasionally strawberry latent ringspot virus (SLRSV) may also be associated with symptoms which can be confused with rose mosaic (157, 158, 253).

Rose ring pattern, a graft transmissible virus-like disease also has symptoms of the rose mosaic type

One of the symptoms attributed to rose streak, namely yellowish-green veinbanding, resembles veinbanding associated with the rose mosaic syndrome but with rose streak this is accompanied by other symptoms not of the rose mosaic type (37).

The following review of the literature outlines the progress in elucidating the viruses associated with the rose mosaic complex.

The first evidence of the existence of a graft transmissible chlorosis of the rose was recorded in France by Vibert in 1863 when he described the effect of grafting chlorotic material onto rootstocks.

It was not until some 65 years later that White published a series of papers on an infectious chlorosis of roses in USA (280, 281, 282) and further reported (283) the effect of mosaic on flower production of the rose cultivar 'Talisman'.

The importance of *Rosa canina* L. rootstock in the dissemination of rose mosaic in Czechoslovakia was recognised by Blattny (22).

During the 1930's there were numerous reports in the USA of rose mosaic type symptoms (35, 187, 193, 199, 256, 279) and also in Italy (115).

Subsequently rose mosaic has been recorded in many countries including New Zealand (90), Denmark (172), South Africa (192), Britain (84, 143), India (20, 226), Germany (229, 230), Holland (27, 28), Australia (19), Cuba (160), Crimea and Southern Ukraine (291) and France (44).

In USA three distinct mosaic types were recognised;- "Rose Mosaic 1" chlorotic spots and flecking with adjacent distortion; "Rose Mosaic 2" chlorotic lines bands and broad blotches; "Rose Mosaic 3" - broad chlorotic blotches, oak-leaf pattern, vein clearing and a few lines and rings (256).

By 1956 rose mosaic virus in two distinct symptom forms - vein banding and line pattern - was widespread in New Zealand (90). Subsequently a further symptom form, chlorotic mottle, was described and a comparison made of the effect of these three forms on growth and flowering (151, 152).

-10-

Grafting to rose of pip and stone fruit with virus symptoms, including apple mosaic (256), Winters peach mosaic (257), and peach yellow bud (257), did produce mosaic symptoms in rose, although not identical to the three types just described.

Brierley & Smith (37) found that the various types of mosaic symptoms were not constant, but rather varied when transmitted to different rose varieties and tended to change from one type to another in the one variety. These researchers failed to reproduce rose mosaic symptoms with any of the 31 species of insect tested for transmission.

A popular summary of the knowledge to date of the virus diseases of roses appeared in the American Rose Annual 1944 (191).

Roses with mosaic symptoms, budded onto peach, produced typical ringspot symptoms in peach (61).

Based on similarities of symptoms in cucumber, infectivity, and some physical properties, Gilmer (116, 117) suggested that two virus isolates obtained from *R.multiflora* were identical to those from several *Prunus* L. spp. and were probably PNRSV.

In Italy a rose with yellow mosaic symptoms gave typical PNRSV symptoms and killed 'Kwanzan' cherry when chip budded into it (124) and further transmission tests supported this view (125).

Thirty eight percent of roses tested in Washington were infected with PNRSV (163).

The first report of mechanical transmission from a rose to herbaceous hosts - cucumber (*Cucumus sativus L*), and cowpea (*Vigna sinesis* Savi.) - was from *Rosa setigera* showing mosaic type symptoms (92).

The reports of Halliwell & Milbrath (130) and Halliwell (1931), on the isolation and identification of tomato ringspot virus (Tom RSV) from rose plants exhibiting rose mosaic, appears contradictory after considering subsequent work. These workers claimed a serological relationship between three of their isolates of rose mosaic, Fulton's (92) isolate of rose mosaic, and Tom RSV. Fulton (99)

-11-

subsequently showed that his isolate of rose mosaic was not serologically related to Tom RSV. Although his isolate of rose mosaic had some antigens in common with PNRSV it was serologically identical with ApMV (100, 101). As Tom RSV is now known to be a nepovirus and not related to the ilarvirus group, Halliwell's(131) findings must be suspect without subsequent confirmation. He did however, find that PNRSV occurred in 45% of the roses tested in Oregon.

The relationship between some of the viruses occurring in pip and stone fruit and rose was further elucidated when Fulton (100, 101) reported that Danish plum line pattern virus (DPLPV) and PNRSV were two strains of a single serotype.

While endeavouring to sort out the various viruses and virus strains causing mosaics and ringspots in apple, rose and stone fruits, Traylor Williams and Nyland (263) using various *Prunus* ringspot virus cultures from stone fruits, Fulton's ringspot virus cultures A,E,G, & H and rose virus cultures from California typical of rose mosaic or rose yellow mosaic, found that most stone fruit isolates caused typical rose mosaic symptoms on rose and most rose isolates caused typical *Prunus* ringspot symptoms on stone fruit indicators.

In Oregon, 40 isolates from 60 roses gave typical non-yellows *Prunus* ringspot virus symptoms on Buttercup squash - but 28 of these also infected Bountiful bean. Roses indexing positive on Shirofugen infected both squash and bean. Six rose isolates infected bean but return inoculations to squash were negative. As no stone fruit *Prunus* ringspot isolates from Oregon had infected beans it appeared that these rose isolates were similar to those in stone fruits in the midwest and eastern USA (137).

Testing of roses from various parts of USA with antiserum to Fulton's (92) isolate APMV and PNRSV antisera resulted in all;eight isolates obtained from California reacting with PNRSV antiserum; of three from Kansas one reacted with Fulton's (92) isolate ApMV antiserum and two with PNRSV antiserum; and of five from Wisconsin three reacted with Fulton's (92) isolate $A_{\rm D}MV$ antiserum, one with PNRSV antiserum and one failed to react with either (99). This isolate which showed symptoms of

-12-

chlorotic patches and chlorosis of the veins was characterized and found to be typical of TSV (103).

Wild roses (*Rosa* L. spp) from 4 of 17 collecting sites in Oregon were found to be infected with TSV (66).

In Iowa a further virus (Tob RSV), was isolated from roses with mosaic symptoms (184).

Rose streak which has some symptoms similar to rose mosaic was first reported by Brierley in 1935 (34) and later described in detail (37). Rose streak has also been reported once in Europe (231), in South Africa (166), and in USA (232). It does not appear to have become a major problem anywhere as there is little else in the literature. Ikin and Frost (157) concluded there was no evidence of this disease in commercial crops in Great Britain.

From California there has been a recent report of a virus-like disease of roses with fine line patterns, rings and chlorotic flecking of the leaves similar to rose mosaic; this was first tentatively called "rose X" disease (232). A further more detailed report of the characteristics of this disease (233) named it rose ring pattern and considered it a further component of the rose mosaic complex. No causal agent was identified.

In Australia a virus was isolated from roses showing line pattern symptoms (19). The isolates reaced in gel-diffusion tests against antisera to both PNRSV and ApMV serotypes. It was reported that the Australian isolates only infected *C. sativus* and *Chemopodium quinoa* Willd. as herbaceous hosts although a bacterium, *Pseudomonas* Migula. sp. transmitted simultaneously caused virus-like symptoms on *Momordica balsamina* L., *V. sinensis* and guar (*Cyamopsis tetragonoloba* L.) (18), all of which had previously been reported as hosts of virus from rose mosaic plants (92, 99).

The great variation in symptom severity and expression, depending on virus isolate and cultivars infected, was examined by budding various isolates to a range of cultivars (30). Mild isolates in some varieties produced severe symptoms in others and *vice versa*. Both ApMV and PNRSV

-13-

types were studied and although ApMV was generally more severe the symptoms produced were often indistinguishable from those produced by PNRSV.

A virus transmitted from rose with yellow net symptoms was shown, by symptoms on herbaceous hosts and serological reaction, to be PNRSV (107, 108). This isolate however could only be transmitted from rose using polyvinylpyrrolidone and unlike other isolates of PNRSV could not be transmitted to *Prunus mahaleb* L. *Prunus pensylvanica* L.f. or *Prunus persica* Batsch.

There are few reports in the literature of viruses associated with rose mosaic being successfully transmitted from rose to herbaceous hosts and back to rose.

Fulton's original isolate of rose mosaic (92), subsequently shown to be an ApMV serotype (100, 101), was transmitted from rose to cowpea and back to rose by rubbing an infectious extract of cowpea leaves on the roots of a *R. setigera* seedling. The rose showed characteristic symptoms and the virus was mechanically transmitted from it back to herbaceous hosts and by grafting to other roses (92).

A virus, indexed as PNRSV, was selected from a single local lesion on *C. sativus*, multiplied in *Chenopodium*, graft transmitted to peach seedlings and then graft transmitted from peach to *Rosa indica* Hort. seedlings. Typical ringspot symptoms were observed on the *R. indica* (44).

Although it is probably more analagous to graft transmission from rose - to other rosaceous fruit trees as already described, Frazier (85) reported a successful attempt to transmit rose mosaic by grafting from rose to strawberry (*Fragaria vesca* L.) and back to rose where the original symptoms were reproduced.

The technique of using strawberry as an indicator host for rose viruses — as described by Frazier (85) is one which probably should have been used more widely by subsequent investigators. He not only transmitted the three types of rose mosaic, as described by Thomas and Massey (256), but also showed positive transmissions with ten out of eleven roses showing a variety of virus symptoms other than typical mosaics. Also six out of seventeen symptomless roses, though, to be virus-free, indexed positive on strawberry.

In England the presence of both arabis mosaic virus (ArMV) and strawberry latent ringspot virus (SLRSV) in field grown roses was confirmed although it appeared likely that in some cases infection by SLRSV may have been introduced in imported rootstocks rather than acquired locally (43).

Harrison (138) showed that viruliferous nematodes, *Xiphinema diversicaudatum* Micol., from soil in a glasshouse growing SLRSV infected roses, transmitted the virus to cucumber seedlings. Also some stunted standard roses from Northern Ireland were infected with SLRSV. One also contained ArMV.

Ikin (156) confirmed that SLRSV was being disseminated in Great Britain by infected imported *Rosa rugosa* standard rootstocks.

Strawberry latent ringspot virus was reported for the first time in glasshouse roses in the Netherlands (29).

A survey of virus diseases of roses in the United Kingdom revealed that in some nurseries SLRSV occurred very frequently in standard roses budded on *Rosa rugosa* Thunb. rootstocks and in one nursery most of the cultivars budded on *R. rugosa* standards were infected with ArMV (157).

Following a meeting with the British research workers in this field the Dutch instituted a survey of the areas being cropped for *R. rugosa* standard canes. They found that the distribution and percentage of infection closely approximated that found on *R. rugosa* standards in Great Britain. Three out of twenty five Dutch nurseries had about 20% infection, two of which had SLRSV only and one ArMV (268).

In glasshouse roses infections of SLRSV and ArMV were found to be associated with the presence of viuliferous nematode vectors which appeared to be responsible for the spread of infection as no virus was found infecting any of the rootstocks or young nursery plants (238, 239, 240). Eight batches of imported *R*. *rugosa* rootstocks from different sources in continental Europe were grown and budded and assessments of the incidence of SLRSV were made on 1000 plants in each batch (241). The distribution and percentages of infection was similar to that reported by earlier workers (157, 268).

In a summary of virus disease of roses in the U.K. Ikin and Frost (157) reported not only that both PNRSV and ApMV were present but also that they occurred as mixtures of these two viruses with either ArMV or TSV.

Thomas (249) on the contrary reported PNRSV to be the only ilarvirus in roses in the U.K.

A study of the incidence, distribution and effects of SLRSV in the U.K. was made using *Chenopodum amaranticolor* Coste & Reyn. *C. quinoa* and *C. sativus* as herbaceous indicators with serological gel-diffusion tests of the herbaceous hosts for confirmation (158).

An extensive survey of the rose mosaic complex in the U.K. recorded mosaic type symptoms associated with ArMV and PNRSV either separately or together. Although ApMV was graft-inoculated from apple to rose it was not found occurring naturally in the U.K. (253). Chlorotic vein banding was generally associated with infections of ArMV plus PNRSV although during prolonged periods of high temperatures the symptom occurred in some roses infected only with PNRSV. Thomas (253) suggested that as rose mosaic is a complex disease, the various symptoms being induced by more than one virus, the virus(es) should, where possible, be named when using the term.

The major factor involved in the spread of rose mosaic in rose appears to be the use of infected propagative material.

In Virginia, USA, growing mosaic-infected roses next to heat cured plants failed to provide any evidence of natural transmission (149).

The possibility of seed transmission of a rose mosaic type symptom was raised in a report from Italy in 1963 of seed from a plant showing symptoms of rose yellow mosaic producing seedlings with similar symptoms,

-16-

although the symptoms did not appear until the second year of growth (126). It was not known however, which virus was associated with the symptoms transmitted.

The term "rose yellow mosaic" refers to the mosaic symptom now generally known as vein banding which is frequently associated with the presence of either ArMV alone or together with PNRSV (253).

Both ArMV (173) and PNRSV (49, 60) are known to be seed transmitted in hosts other than rose.

Seed transmission of PNRSV in *Rosa multiflora* was indicated when three seedlings among plants from two batches of approximately 100 seed showed chlorotic line pattern symptoms (238). PNRSV was subsequently isolated from two of these three indicating approximately 1% level of seed transmission (243). Pollen transmission was unlikely as few of the seedlings had flowered. PNRSV was detected in ELISA tests in the pollen of infected rose cvs 'Peace' and 'Queen Elizabeth' but not in the pollen of infected *R. multiflora* (242).

There does not appear to be any evidence in the literature of natural transmission, other than by grafting, of ilarviruses in rose except the reports from Sweet (238, 242, 243). The report of seed transmission from Italy (126) would seem from the symptoms transmitted, to be more likely transmission of the nepovirus ArMV.

Vector transmssion of the nepoviruses ArMV and SLRSV in rose by nematodes would appear likely as viruliferous nematodes are frequently associated with roses infected with these viruses (138, 238, 239, 240).

The first use of heat treatment on mosaic infected roses to produce apparently healthy growth was reported by Holmes (146, 147, 148).

Although no details are given Nyland & Goheen (1964) reported inactivating at least one virus in rose by heat treatment.

Heat treatment for rose mosaic resulted in six clonal lines of rose rootstocks and 25 commercial rose varieties being indexed free of virus (261).

-17-

Heat treated (virus free) material of 'Chrysler Imperial' produced a higher proportion of top grade plants than material infected with PNRSV (181, 182).

Similarly in a commercial glasshouse heat treated virus tested 'Baccara' yielded 13.5% more blooms than commercial stock (215, 216).

To summarise, the various chlorotic patterns, flecking, vein banding, vein netting, mosaic and yellow mosaic occurring in the leaves of roses and loosely termed rose mosaic may be caused by the same virus in different cultivars, different isolates of the same virus or by different viruses either singly or in mixed infections.

The viruses which to date, have been associated with some or all of the symptoms are: PNRSV (99) ApMV (100, 101) ArMV (157, 158) Tob RSV (184) TSV (103, Farrar & Frost cited Ikin & Frost (157) SLRSV, yellow flecking (158) and one unconfirmed report of Tom RSV (130).

The virus-like diseases, rose ring pattern (233) and rose streak (37) also exhibit some symptoms of the rose mosaic type.

Numerous brief reports of research work in progress at various research establishments appear in various research station reports. Also informative and instructional articles in more popular publications have helped keep horticulturists and specialist gardeners informed on current knowledge (2,3,4,5, 42, 63, 86, 87, 194, 203, 228, 237, 266).

-18-

Rose Wilt and Proliferation - Review

A graft-transmissible wilt of roses distinct from the wilt caused by *Verticillum* Nees ex Wallr. fungi has, in this investigation, been found to be associated with a strain of PNRSV. The proliferation symptom on maiden bushes was not found to be associated with PNRSV.

However as the viral wilt symptoms in mature plants has been associated in the literature with the proliferation symptom in maiden plants, both are reviewed here.

A wilt and dieback in roses was described in 1929 by Brundrett (40) in the Australian Rose Annual. This disease and its transmission was further described, in detail, by Grieve (121, 122, 123) and its cause attributed to a virus.

In Italy a new virus was reported which in some respects resembled rose wilt (115) and a further similar virosis was reported on roses in Czechoslovakia (164, 165).

Several Australian reports record rose wilt as being epidemic in specific years (200, 201), a further description of the symptoms (247) and research on transmission (202).

In 1960 rose wilt was recorded for the first time in South Africa (192) and in the same paper separate mention made of a condition described as "little leaf". From the description "little leaf" appears to be similar to the rosetting described by Fry & Hammett (89) as part of the rose wilt syndrome.

A symptom referred to as 'pinch off' in Oregon USA, which is manifest as a failure of the first shoot arising from a grafted bud, was investigated by Roberts (223). This condition is similar to but not identical with the condition which has since become known as 'proliferation'.

The proliferation symptom was subsequently attributed to the wilt syndrome (89) although no experimental evidence of this association has been produced.

-19-

The experimental transmission of rose wilt using the common rose aphid, *Macrosiphum rosae* L. was reported by Anderson (7) although there do not seem to be any subsequent confirmatory reports in the literature.

Unpublished work on proliferation in New Zealand (219, 220) was unable to demonstrate any evidence of graft transmission of this syndrome.

Proliferation was known to occur in California, USA in 1966 but only in relatively low percentages (10-15%) of plants budded (Morey, pers. comm.).

Rose rosette described by Thomas & Scott (258) appears to be distinct from the rosetting associated with rose wilt. Rose rosette was reported to be transmitted by an eriophid mite (6) and appears to have been confined to wild roses and occasional cultivated plants in adjacent rural areas in parts of USA (6). Experimental graft transmission to *R. multiflora* 'Burr' serves as a useful indicator (232).

In Great Britain a disorder of roses, known there as stunt disease, which closely resembles proliferation, was becoming serious with records in some areas of over 80% infection (155). Efforts at Shardlow Hall to transmit the disease by budding failed to show any evidence of graft transmission (155). In 1973 Rose Wilt was reported as widespread in the south east of England but the symptoms were not described (145). The association of excessive callus and galling with the proliferation symptom as it occurred in New Zealand was noted and that high auxin levels are a characteristic both of uncontrolled cell division and reduction of leaflet size in roses (110).

An extensive investigation of rose wilt, including the proliferation symptom, in New Zealand by Fry & Hammett (89) showed that buds from established plants showing typical symptoms attributed to rose wilt in established plants when budded on an understock together with an indicator bud of 'Super Star' induced shoot epinasty in the indicator. Although Fry & Hammett (89) assumed, on the basis of similarity of symptoms, that the proliferation symptom in young plants and the dieback, loss of apical dominance and recurved leaflets in established plants were due to the same causal agent not all investigators agree on this point (111). Similarly Bos & Perquin (31, 32) noted that

-20-

the proliferation symptom on maiden plants in the Netherlands did not result in rose wilt type symptoms in established plants. Their investigation led them to suppose that proliferation was due to either a hormonal imbalance brought about by the act of budding, or by a pathogenic micro-organism occurring in low concentration in affected tissue or disappearing from it after the onset of the pathological process.

The similarity between the symptoms of rose wilt as originally described by Grieve (121) and the symptoms of *Verticillium* wilt was pointed out by Dimock (74). A comparison between the symptoms of these two diseases was made by Hammett (132) although at this stage a wider range of symptoms were attributed to rose wilt than originally described by Grieve (121).

A report of the apparent spread of rose wilt in an established bed of roses in Christchurch, New Zealand (190) would seem to indicate the presence of a vector. Although soil samples were tested for possible nematode vectors no mention is made of checking for *Verticillium* wilt fungi which would spread in the manner described and produce barely distinguishable symptoms.

Rose stunt in Great Britain was transmitted by double budding to 'Elizabeth of Glamis' indicator buds but not to 'Super Star' (238, 244). Nursery plants affected with rose stunt were transplanted and observed for some years but those that survived produced normal shoots and did not subsequently show any obvious symptoms (157).

A brief report that mycoplasma-like bodies had been observed in sections from proliferated bushes (8) was subsequently advised by one of the authors as not confirmable (Thomas, pers. comm.)

A review by Thomas (251) of five years observations and experiments on rose stunt at the Glasshouse Crops Research Institute in the U.K. reports that they have been unable to consistently isolate a pathogen or induce typical symptoms on the growth from a rose indicator bud.

-21-

In California investigation of a disease, similar to rose wilt, was reported (50, 162) and described in more detail by Gumpf & Weathers (128). It was subsequently shown that the disease was a complex involving two separate graft transmissible disorders, rose leaf curl and rose spring dwarf (234, 235).

A number of the symptoms in established plants, attributed in California to rose leaf curl, namely, clearing or flecking of leaflet veins, splitting and corkiness of rachis and leaflet veins, splitting of mature canes, necrosis in the bark and pitting in the wood of mature canes (235, 232, 170) were not observed as being characteristic of rose wilt in New Zealand (89).

Transmission of rose leaf curl to nursery plants resulted in proliferation of shoots in a significant proportion of infected plants but not in healthy controls, although it is noted that proliferation alone should not be considered diagnostic for rose leaf curl in nursery plants, because any treatment which breaks apical dominance will initiate numerous lateral shoots (235).

This is the only report in the literature of graft transmission of the proliferation symptom and the above comment would appear significant in that it indicates the probability that not all occurrences of proliferation are due to the same causal agent.

If the rose wilt symptoms in mature plants are due to a different causal agent to the proliferation symptom then many of the currently conflicting reports could be explained.

A viral etiology for rose wilt symptoms in mature plants would support Fry & Hammett's (89) transmission experiments. The assumption of soilborne causal agent, probably a bacterium occurring in low concentration in affected tissue or disappearing from it after the onset of the pathological process, for proliferation would be in agreement with the investigations of Gardner (111) and Bos & Perquin (31, 32).

-22-

This would account also for the failure to obtain graft transmission of the proliferation symptom (111, 155, 219, 220, 251) and the lack of rose wilt symptoms on mature plants which have exhibited proliferation symptoms as maiden plants (31, 32, 111, 157, 219, 220).

Association of PNRSV with Rose Mosaic and Rose Wilt Symptoms

All rose samples in the disease collection were indexed, as described later, by enzyme-linked immunosorbent assay (ELISA) at least once using gamma-globulin from isolate '154'; at least once using gamma-globulin from Fulton's(100) isolate (PNRSV-RA) and at least once using gamma-globulin from Fulton's(100) isolate of DPLPV a serotype of PNRSV. Tests on any one rose sample were consistently either all positive or all negative for that rose.

The presence of PNRSV in those samples which tested positive by ELISA was confirmed by mechanical transmission to *C. quinoa* and/or *C. sativus* although several attempts were necessary with some samples before symptoms could be induced in these hosts. Any herbaceous hosts showing symptoms which were considered not quite typical of PNRSV were tested by ELISA and the presence of PNRSV was confirmed.

On this basis 120 of the 221 samples in the disease collection were considered to be infected with PNRSV.

Many but not all of these infected samples were also detected by transmission of symptoms to *R. multiflora* and by indexing on *P. serrulata* 'Shirofugen'.

Prunus necrotic ringspot virus was invariably present in those roses which showed mosaic type symptoms including all symptoms of the fine to broad chlorotic line pattern types, chlorotic rings, blotches, mottle, stipple, vein banding and vein netting with the exception of the one rose found to be infected with ApMV which also showed distinctive mosaic symptoms but consisting of larger bright gold blotches than any of the other specimens.

However PNRSV was found to be also present in some but not all of the specimens showing a wide range of other symptoms including 10 out of 14 apparently symptomless specimens. Such other symptoms would appear to occur independently of the presence of PNRSV and be due to some other cause not necessarily of viral etiology. Some virus-like symptoms on roses grown in partial shade can be caused by the phytotoxicity of certain spray materials (167). Specimens showing symptoms of either RPF and/or RCB or other flower abnormalities were in some cases infected with PNRSV but not invariably so.

Similarly types of leaf symptoms generally attributable to nutritional disorders such as pale colour, thin texture, general yellowing interveinal yellowing, premature senescence, and reduction in size occurred independently of PNRSV infection as also did other leaf symptoms such as enations, distortion, epinasty, vein clearing and the gross reduction of the lamina characteristic of proliferated shoots.

The symptoms on established plants attributed to rose wilt virus by Hammett (132) i.e. rosetting associated with short shoots with no internodal elongation arising from lateral buds on old canes, dieback of old canes and general decline of the plant with the formation of numerous abortive side twigs did however appear to be associated with infection by PNRSV. Of nineteen samples recorded at the time of collection as showing these rose wilt type symptoms, all were subsequently shown to be infected with PNRSV. These samples included five taken from plants in the collection gathered by Fry & Hammett for their investigation on rose wilt (89).

Forty eight of the samples not originally recorded as showing symptoms of the rose wilt type developed these symptoms during the period they were subsequently observed.

All of these 48 indexed positive for PNRSV. Twelve of the 48 had been originally recorded as showing a light chlorotic stipple whereas the other 36 had been originally recorded either as symptomless or showing some other symptom not associated with PNRSV.

Of the 51 samples orignally recorded as showing symptoms of the various rose mosaic types only the 12 which had been recorded as showing a light chlorotic stipple subsequently showed symptoms of die-back and decline of the rose wilt type. The other 39 with rose mosaic symptoms developed a slight reduction of growth in some samples but not typical rose wilt symptoms.

-25-

Because of variability of symptom expression and type it is difficult to be empirical but based on independently recorded observations it would appear as if there is more than one strain of PNRSV associated with different symptoms on host plants.

The strain or strains associated with typical rose mosaic symptoms other than the light chlorotic stipple do not appear to be associated with rose wilt type symptoms in mature plants.

The strain or strains associated with rose wilt type symptoms in mature plants do not consistently produce symptoms other than those associated with rose wilt decline in mature plants.

The proliferation symptom which has been associated with the rose wilt syndrome (89) has of recent years virtually disappeared from rose nurseries in New Zealand and in the U.K. (251, Sweet pers. comm.). No proliferation was observed on any of the 6 to 8 bushes budded of each of the 221 samples in the disease collection. Samples of proliferation were not included in the disease collection as it is virtually impossible to propagate from proliferated material and propagation from apparently normal shoots which may arise from proliferated plants does not result in the transmission of proliferation. (31, 32, 155, 219, 220, 251).

However, a few specimens of proliferated plants were obtained from an Auckland and a Hamilton nursery and tested for PNRSV using the ELISA technique. The presence of PNRSV appeared to be at random with 6 of the 15 plants indexing positive and 9 negative.

From these observations it was concluded that although PNRSV could be associated with rose wilt symptoms in mature plants it could not be associated with the proliferation symptom.

Some growth abnormalities, such as bushy stunt, were recorded in some of the samples which indexed negative for PNRSV but in all cases these

-26-
symptoms were quite distinct from those associated with rose wilt.

The occurrence of rose wilt type symptoms in the 120 samples which indexed positive for PNRSV can be summarised as follows:

Symptoms recorded at time of collection of samples which indexed positive for PNRSV		Occurrence of Rose Wilt symptoms during subsequent growth	
		Rose Wilt	No Rose Wilt
Rose mosaic type symptom:	s (51)	12*	39
Various other symptoms of symptomless	r (50)	36	14
Rose wilt type decline	(14)	14	-
P.D.D. rose wilt collection (5)		5	-
	120	67	53

*The 12 which developed rose wilt symptoms were recorded as light chlorotic stipple

-28-

CHAPTER 2

INDEXING

Indexing on Rosa multiflora 'ISU-60'

Since 1928 when White (1928) reported that rose mosaic type symptoms could be experimentally transmitted by grafting to *R. multiflora* this method has been commonly used to demonstrate the graft transmission of rose mosaic symptoms (90, 92, 151, 152, 192).

Rosa multiflora 'Clarke' is used in South Africa as an indicator for indexing for rose mosaic (Klesser pers. comm.). The symptoms produced in *R. multiflora* by grafting with ApMV infected roses have been described as severe chlorosis (253).

However, there does not appear to be any record in the literature as to the reliability of *R. multiflora* as an indicator to index for PNRSV and ApMV in rose.

Method

The only strain of *R. multiflora* available at the time, which appeared to be free of known viruses, was one developed at Iowa State University known as 'ISU-60'.

Cuttings approximately 25cm long were taken in winter, the proximal and distal ends were trimmed with a sharp knife and all the shoot buds except the two at the distal end were excised. They were planted immediately in rows in the open ground.

The following summer as scions became available from the various samples selected for the disease collection five plants were bud-grafted from each sample.

Bud-grafting was carried out using a conventional T-incision and the bud shield inserted with a thin sliver of wood left intact behind the bud. The following winter three of the stock tops were removed from each sample and two were left intact in order to observe transmission of symptoms to *R*. *multiflora* 'ISU-60'.

The plants were observed over three growing seasons and any symptoms occurring were noted.

Results

Symptoms transmitted to the stock tops were generally different to those in the original sample and were not constant for any particular symptom type. For example the vein banding symptom, which is quite distinctive, usually occurs as the only symptom on an infected cultivar. However, the plants of *R. multiflora* to which five samples showing this symptom had been budded each developed a different symptom namely, leaf yellowing, yellow flecking, chlorotic blotch, chlorotic line pattern and vein netting.

Similarly other symptom types in the samples could not be associated with any consistent reaction in the *R. multiflora*.

The occurrence of symptoms on *R*. *multiflora* budded with samples found to be infected with PNRSV by indexing with herbaceous hosts and ELISA was not sufficiently reliable to serve as an indexing method.

31 of the 120 samples considered to be infected with PNRSV, by indexing with ELISA and herbaceous hosts, produced no observable symptoms in *R. multiflora* when budded to it. These 31 samples were all originally recorded as either symptomless, or as having light chlorotic stipple or decline and die-back of the rose wilt type or as having some other symptom not associated with PNRSV.

All samples originally recorded as having either chlorotic line pattern or large chlorotic blotches or vein banding produced at least some symptoms of the rose mosaic type in *R. multiflora* when budded to it.

These symptoms were not necessarily either obvious or consistently produced and in some instances were only observed on a few leaves on one or two occasions during the three years of observations.

The transmission tests to *R*. *multiflora* indicate that there is more than one strain of PNRSV occurring either separately or as mixtures in roses. Those strains which produced rose mosaic type symptoms in the original sample induced rose mosaic type symptoms, but not necessarily of the same type, in *R*. *multiflora* when budded to it.

Those strains associated with rose wilt type symptoms may also either produce a light chlorotic stipple or be apparently symptomless.

One sample, '101', a symptomless 'Diamond Jubilee' neither produced symptoms on *R. multiflora* nor on any of its progeny over a number of years yet there was no doubt it was infected with PNRSV as it not only indexed positive with ELISA, on herbaceous hosts and on 'Shirofugen' cherry but also purified virus from it produced an antiserum which reacted with other PNRSV isolates.

Rosa multiflora 'ISU-60' did not reliably detect all strains of PNRSV in rose and cannot be recommended as an indicator for detecting this virus.

Indexing on Rosa multiflora 'Burr'

In California U.S.A., reports of two graft transmissible diseases of roses, rose spring dwarf (234) and rose ring pattern (233) both describe the use of *R. multiflora* 'Burr' as an indicator for these diseases.

In order to check for the presence of these disorders in New Zealand, 'high health' 'Burr' multiflora was imported from the University of California, Davis.

Method

Cuttings of *R. multiflora* 'Burr' were prepared, planted and bud-grafted in the same manner as already described for *R. multiflora* 'ISU-60' except three understocks only were used for each sample in the disease collection.

The stock tops were observed over the latter two years of this investigation and any symptoms occurring were noted.

Results

In October 1979 symptoms similar to those attributed to rose spring dwarf by Slack *et al* (234) appeared on 'Burr' multiflora budded with three of the disease collection samples. The symptoms only occurred on one or two branches of one plant in each test.

Further symptoms could not be induced by stripping the foliage and pruning back as described by Slack *et al* (234). Symptoms did not reoccurr the following spring.

While investigating the incidence and cause of proliferation in roses in New Zealand (219, 220) a symptom distinct from proliferation was noted on the initial growth from the buds of budded roses and described as stunted. This symptom resembles that described (234) as characteristic of rose spring dwarf on budded roses.

-31-

This stunting symptom in New Zealand appeared to correlate with the use of infected budsticks (111).

Symptoms similar to rose spring dwarf were noted on *R. multiflora* some years ago (Gardner pers. observ.) but have not been observed recently.

Although results in California suggest that rose spring dwarf is unevenly distributed within infected rose plants and may not induce symptoms in rootstocks or varieties (234) the current evidence is insufficient to assume the disease occurs in New Zealand.

However, although there is no conclusive evidence at this stage rose spring dwarf could well be found to be present in New Zealand.

'Burr' multiflora is also considered by Secor & Nyland (233) to be a reliable indicator for rose ring pattern, but no symptoms resembling this disease were observed on the 'Burr' multiflora budded with disease collection samples.

Symptoms associated with PNRSV were apparent in some 'Burr' multiflora budded with PNRSV infected samples but these were not expressed as frequently or as distinctively as the symptoms on 'ISU-60' multiflora. This effect could have been due to the shorter period during which the 'Burr' was observed.

Indexing on Prunus serrulata 'Shirofugen'

Although the technique of indexing for PNRSV and prune dwarf virus (PDV) by budding test buds onto *P. serrulata* 'Shirofugen' is widely used and documented, it is not entirely reliable as the local necrotic reaction does not invariably occur with all buds from an infected plant. Using 3 buds per test from cherry trees infected with PDV Hampton (133) found that in up to 24% of the tests on one variety of cherry all 3 buds indexed negative when budded in mid-summer and two months later in late summer 87% of the tests failed to detect virus in any of the three buds.

Helton & Bolwyn (141) obtained more reliable results with 'Italian' prune infected with PNRSV, with up to 12% of the test buds failing to detect the presence of virus.

An evaluation (83) of 'Shirofugen' as a reliable indicator for PNRSV in rose showed that the reliability of detection not only varied widely between rose cultivars but also varied with the position of the bud on the infected rose plant. Indexing infected 'Super Star' roses failed to detect the virus in 28 to 50% of the buds whereas indexing infected 'Baccara' and 'Dr A.A. Verhage' roses failed to detect the virus in 59 to 93% of the buds depending on the position of the test bud on the infected plant.

The necrotic response on 'Shirofugen' is initially localized, thus enabling a large number of samples to be indexed on the different branches of one tree (83, Sweet pers. comm.).

Method

Fourteen, 7-year old trees of *P. serrulata* 'Shirofugen' growing in the open ground, where they had been budded on "virus-free" seedling understock with buds from plants supplied by Plant Diseases Division, DSIR, Auckland, for indexing purposes, were used as indicators.

Indexing was done on the current season's growth during late January early February 1980. Five buds from each rose sample to be tested were inserted in T-cuts on one strong growth for each sample and tied in place with rubber budding ties. All tests were read 5 to 6 weeks after grafting by examining for gumming and necrosis under the bud.

It had been noted during previous indexing by this method that infection frequently became systemic throughout the length of the growth in which infected buds had been inserted resulting in collapse of that growth during the following spring. This symptom was also recorded in spring 1980.

Results

One hundred and sixty six of the samples in the disease collection were indexed by this method. The balance of the samples did not have suitable material available at the time.

Ninety three of the samples indexed on 'Shirofugen' were shown to be infected with PNRSV by indexing on herbaceous hosts and ELISA.

The 'Shirofugen' indexing did not give such a clear determination.

Some gummosis frequently occurred around the buds of samples that indexed negative by all other methods used. Although the amount of gummosis was generally greater around infected buds it was very much a matter of opinion rather than an empirical indication as to how much gummosis determined a positive test.

Similarly the degree of necrosis varied from barely discernable in one out of five infected buds to severe necrosis in all five infected buds. In some samples that indexed negative by all other methods used, damage to the cambium at time of budding could not be distinguished from the slight necrosis occurring with some infected buds.

Although collapse and death of the shoot the following spring occurred only with infected buds it only gave a positive reaction with 47 of the 93 infected samples.

The degree of symptom expression in the 'Shirofugen' test could not be correlated with the type of symptom expressed by the rose from which the buds were taken.

-34-

Although the presence of severe necrosis behind the test buds proved a positive indication of infection with PNRSV this method would have failed to detect the presence of PNRSV in 24% of the samples found by other means to be infected.

Death of the shoots the following spring was also a positive indication of the presence of PNRSV but would have failed to detect the virus in 50% of the infected samples.

The use of 'Shirofugen' cannot be recommended as a means of detecting roses free of PNRSV not only because it is a time consuming and lengthy process, but also because of its limited reliability.

Indexing on Apple (Malus sylvestris Mill.)

Apple mosaic virus has been artificially transmitted from apple to rose by budding (30, 253, 255) although with some difficulty, but once in rose the virus can be readily transferred from rose to rose (30, 253).

A 'Queen Elizabeth' rose showing mosaic symptoms when budded to apple indicators induced a similar mosaic on the indicators but many of the rose buds failed to form a union with the apple rootstocks (245).

Thomas (253) found that bud survival was not necessary for transmission of PNRSV to occur from rose to 'Mazzard' cherry but eleven attempts to graft-inoculate to apple were unsuccessful. However, one of ten roses became infected when he budded them with buds from ApMV - infected apple.

Method

Fifty rooted canes from stools of an apple understock free of known virus were obtained from the N.Z. Fruitgrowers Federation Ltd Nursery in Levin. These were budded with a selection of 50 samples from the disease collection using the conventional T-budding method. It was not known at the time that rose sample '72' was infected with ApMV and this sample was not included in those tested.

Results

All the rose buds failed within two weeks of budding. The apple rootstocks were inspected over the following 15 months but no symptoms which might indicate viral transmission were observed.

The apple rootstocks indexed negative for ApMV when tested by ELISA.

-36-

Indexing on peach (Prunus persica) 'Golden Queen' seedlings

Peach has been used for indexing for PNRSV in rose (61) and also as an intermediate host in the transmission of PNRSV from rose to herbaceous hosts (18, 19).

In order to test the reliability of peach as an indicator for PNRSV a selection of rose samples from the disease collection were budded onto 'Golden Queen' peach grown from seed from virus indexed trees shown to be free of PNRSV.

Transmission occurred from PNRSV infected rose samples except when the rose bud died shortly after grafting.

Although PNRSV could be detected in the infected peach seedlings by ELISA, the expression of symptoms on 'Golden Queen' indicators was obscure and erratic.

Because of the unreliability of bud survival and the difficulty of observing symptoms, budding rose to peach could not be considered a satisfactory technique for indexing for PNRSV.

-37-

Indexing by Mechanical Transmission to Herbaceous Hosts

Mechanical transmission of a virus from rose plants showing mosaic type symptoms was first achieved by Fulton (92). The virus transmitted was subsequently shown to be ApMV (100).

As early as 1948 cucumber had been shown to be a useful host for viruses from sour cherry (196).

In 1956 Gilmer (116, 117) transmitted two virus isolates from
R. multiflora to cucumber, compared them with a number of stone fruit
isolates and concluded they were probably PNRSV.

Fulton (93) carried out an extensive investigation of the herbaceous host ranges of four virus isolates from cherry including his virus 'G' subsequently found to be PNRSV. This isolate infected 48 out of 150 herbaceous host species tested by mechanical inoculation.

In 1962 twelve of fourteen isolates mechanically transferred from rose to herbaceous hosts were indexed as PNRSV (163).

In a survey of virus diseases of roses in the U.K., Ikin and Frost (157) reported that with the use of a 0.06M phosphate buffer, pH 7.8 containing 10% polyvinyl pyrrolidone (PVP) they sap transmitted SLRSV, ArMV, PNRSV, ApMV and TSV from rose to herbaceous hosts.

In France, indexing of roses with mosaic, using *C. quinoa* and *C. sativus* as herbaceous indicators and peach 'GF 305' as a woody indicator gave widely varying results. The average percentage transmission to *C. quinoa* varied from 70% to 23% depending on the species or cultivar being indexed. Cucumber was even more unreliable with average percentage transmissions of 23% down to 2% (44).

Method

As the buffer used by Ikin & Frost (157) had successfully transmitted a wide range of viruses from rose to herbaceous hosts this buffer was initially used for transmission from rose samples.

Young rose leaves or flower petals were ground in buffer with the addition of celite as an abrasive and inoculated to the cotyledons of *C. sativus* 'Crystal Apple' and the leaves of *C. quinoa*. The most consistent results were obtained with cucumber when the cotyledons were almost fully expanded but before the appearance of true leaves and with *C. quinoa* when the plants had six to ten true leaves. Although the plants were grown in a heated glasshouse time to reach optimum stage for inoculation varied with the time of the year.

In order to assess the most suitable extractant for routine indexing a wide range of alternative buffers and stabilizing buffer-additives were tested. Of those tested 2 - mercapto-ethanol (2-ME), sodium diethyldithiocarbamate (NaDIECA), cystein hydrochloride and ascorbic acid each at 0.02M in 0.02M Na/K phosphate buffer pH 8.0 all gave many local lesions on *C. quinoa* even after five hours storage of the extract at room temperature, but transmission to cucumber was unreliable. Buffer plus 10% polyvinyl pyrolidone (PVP)/(W/V) also gave many local lesions on *C. quinoa* as well as consistent transmission to cucumber.

In subsequent tests for optimum pH and concentrations pH 7.5 and pH 8.0, 0.02M and 0.06M Na/K phosphate buffer and 5% and 10% PVP (W/V) showed no apparent difference and gave more consistent transmission than lower or higher pH and concentration.

For subsequent indexing of roses on herbaceous hosts, 5% PVP (W/V) in 0.02M Na/K phosphate buffer pH 8.0 was used.

Results

The variation between isolates from different sources was very apparent in the range of herbaceous hosts infected. Of 120 different samples from the disease collection found, by subsequent indexing, to be infected with PNRSV, 89 infected both cucumber and *C. quinoa*, 27 infected cucumber only and 4 infected *C. quinoa* only.

-39-

Those isolates which only infected one of these two herbaceous hosts generally required 2 or 3 attempts before transmission occurred and the orginal samples did not show typical mosaic type symptoms but rather were variously recorded as either vein clearing, distortion, pale green between leaf veins, general decline or die back of the rose wilt type. All source plants of these isolates and the infected herbaceous hosts indexed positive for PNRSV using ELISA.

Wide variation was also apparent in the host range of those isolates which infected both cucumber and *C. quinoa*.

Isolates from all of the 120 samples infected with PNRSV were transmitted by mechanical inoculation with varying degrees of difficulty to one or more of the following herbaceous hosts:

CHENOPODIACEAE

Chenopodium amaranthicolor Coste & Reyn. Chenopodium quinoa Willd.

CUCUBITACEAE

Cucumis sativus L. 'Crystal Apple' Cucurbita maxima Duchesne Cucurbita pepo L. Momordica balsamina L.

EUPHORBIACEAE

Ricinus communis L.

LEGUMINOSAE

Phaseolus aureus Roxb. Phaseolus vulgaris L. 'Pinto' Vigna sinensis Savi.

SCROPHULARIACEAE

Torenia fournieri Lind.

SOLANACEAE

Nicotiana clevlandii Gray Nicotiana tabacum L. 'White Burley' Nicotiana rustica L.

-40-

No one isolate was able to be transmitted to all of these hosts. No definite correlation was able to be made between symptoms in rose and the host range of the isolate.

There was however a trend, with some exceptions, for isolates from roses with mosaic type symptoms to infect a wider host range than those from roses with rose wilt type symptoms.

Rose samples '79' and '80' from two plants in a bed of 'Silver Lining' in the Dougal McKenzie rose garden, Palmerston North, both exhibited chlorotic mottle and chlorotic blotches in different degrees of severity.

Both isolates from these samples infected *C. sativus*, *C. quinoa*, *C. amaranticolor*, and *N. clevlandii*. In addition however, only isolate '79' infected *N. tabacum* 'White Burley', *N. rustica*, *P. vulgaris*'Pinto' and *T. fournieri* and only isolate '80' infected *C. maxima*, *C. pepo* and *R. communis*.

Sample '154' from a plant of 'Chrysler Imperial' in the Parnell Rose Gardens, Auckland showed severe decline and leaf bronzing. Similar symptoms occurred in the roses budded from it in the disease collection except they also exhibited thin growth, recurving and bunching of the leaves, which were brittle and dropped easily, followed by severe dieback. Plants were almost dead after two years.

Similar, but less severe symptoms were induced in the *R*. *multiflora* 'ISU-60' understock, which also showed a light chlorotic mottle.

Initial transmission to cucumber 'Crystal Apple' and *C. quinoa* was achieved with some difficulty but further transmissions between these hosts and to *C. amaranticolor* was no problem. Local lesions were produced on all three of these hosts followed by systemic mottle and stunting on cucumber and systemic line pattern on both *C. amaranticolor* and *C. quinoa*.

-41-

The isolate produced no symptoms on a range of other herbaceous hosts except when a partially purified and concentrated preparation of virus was used for inoculation. Symptoms were then produced as follows: *P. aureus* and *P. vulgaris* 'Pinto' - local purple veining, chlorosis and loss of apical dominance; *M. balsamina* - local lesions and systemic distortion; Pinto bean - local purple veining and systemic mottle and *N. clevlandii* - large, local chlorotic spots on senescing leaves, and symptomless systemic infection.

Inoculation back to cucumber from these hosts showed both local and systemic infection.

This was the widest host range of any of the isolates from roses exhibiting rose wilt type symptoms. By contrast sample '67' from a plant of 'Mischief' also with severe decline of the rose wilt type was inoculated to *C. sativa* and *C. quinoa* on three separate occasions. Two plants of each were used in the first two tests. The first test produced no symptoms on any of the inoculated plants. A second attempt resulted in two local lesions on one of the cucumbers. For the third test eight plants of each of the two herbaceous hosts were inoculated. A total of five local lesions on the eight cucumbers were the only symptoms observed.

This sample indexed positive for PNRSV by ELISA and five out of five buds were positive on 'Shirofugen' cherry with death of the budded shoot the following spring.

The use of transmission to cucumber and *C. quinoa* as a method of indexing for PNRSV would probably have failed to detect the virus in this sample and a number of other samples which reacted similarly unless a considerable number of plants were inoculated and observed carefully.

-42-

Indexing by Electron Microscopy

The use of dip preparations with negative staining is particularly useful for the detection of rod-shaped virus particles (142) in extracts from infected plants. This method can also be applied to spherical particles (142).

Negative staining with potassium phosphotungstate may cause rupture and distortion of the virus particles (82, 99, 161).

Fixation with 1% glutaraldehyde (99) or 10% formaldehyde (82, 161) or the use of 1% uranyl acetate instead of potassium phosphotungstate (59) stabilized the particles and avoided rupture.

Method

Leaf dip preparations both from rose and infected herbaceous hosts were prepared by placing a drop of stain on a coated grid and dipping a freshly cut leaf surface in the stain. After draining they were examined in a Phillips 200 Electron microscope for the presence of virus particles.

Grids were also prepared by crushing tissue in one or two drops of stain and touching the grid to the surface of the liquid.

Ammonium molybdate and sodium phosphotungstate were tested both separately and together, at a range of different pH levels, with and without coating the grid with bovine serum albumin.

Uranyl formate, prepared after the method of Barnett & Murant (17), was used alone and after fixation by making the leaf dip into 0.75% glutaraldehyde, draining and then negative staining with the uranyl formate solution.

Results

Leaf dip preparations, and crushed homogenates both from roses exhibiting a range of symptoms and from infected herbaceous hosts did not consistently reveal virus particles other than an occasional particle of similar size and appearance to PNRSV. Few viral particles were observed in any of the preparations other than those fixed with glutaraldehyde and stained with uranyl formate.

This method was adopted as being the most satisfactory.

In further preparations it was found that viral particles could not be consistently observed even when several grids were prepared from the same leaf.

As this did not appear to be a satisfactory indexing method it was only used on a selection of the rose samples.

Indexing by Serological Methods

As serology particularly by ELISA is a major component of this investigation, it is treated separately after the section on antiserum production.

-46-

CHAPTER 3

PRUNUS NECROTIC RINGSPOT VIRUS - Purification

The first brief report (94) of the use of freshly precipitated calcium phosphate to partially purify unstable viruses from *Prunus* as well as virus from a rose with mosaic was published in 1957.

Fulton (97) gave details of the method of preparation of the hydrated calcium phosphate (HCP) gel and its use to absorb host material in the partial purification of PNRSV.

A virus isolate from rose with mosaic was purified using this method with the addition of 2-ME (0.02M) and Na DIECA (0.02M) to the 0.02M phosphate extraction buffer and an antiserum to it prepared (99).

Of a number of isolates of virus from rose some reacted in gel diffusion only with this antiserum and the others reacted only with PNRSV antiserum prepared against an isolate from sour cherry (99).

The isolate from rose was subsequently shown to be serologically identical to ApMV (100).

The purification methods recommended in the 'CMI/AAB Descriptions of Plant Viruses' for PNRSV (102) and for ApMV (105) are almost identical until the virus is sedimented by the first high speed centrifugation. It is recommended at that point that PNRSV be further purified by precipitation of host protein with citric acid (102) but that ApMV is eliminated by the use of citric acid (105).

Further purification may be by density gradient centrifugation (272) zone electrophoresis (269) or precipitation of host protein by antihost serum (100, 118).

Methods

Initially purification was attempted using the method recommended for PNRSV (102). Yields of virus were negligible.

As the identity of the virus isolates was not certain at this stage the butanol/chloroform method used by Harrison & Nixon (140) for the purification of ArMV was tried again with negative results.

Filtering the homogenate through celite pads followed by polyethylene glycol precipitation (48) was no better.

Fulton's (102) method for PNRSV was again tried and each step in the purification monitored by inoculation to cucumber and by electron microscope examination of samples from the preparation.

It was found that the virus was lost at the stage of citric acid precipitation.

Fulton's (105) method for ApMV was then tried using density gradient centrifugation for further purification rather than precipitation of host protein with the gammaglobulin portion of host-specific antiserum. This method gave highly infective preparations with good yields of the order of one to three mg. per 100g of host tissue.

The method finally adopted for routine purification was as follows: cucumber seedlings, 1 to 2 weeks old depending on the time of the year, were inoculated and harvested 5 to 7 days later. Infected tissue was homogenized in a Waring blender for 5 minutes in a cold solution of 0.02M Na DIECA, 0.02M 2-ME in 0.02M sodium/potassium phosphate buffer pH 8 with 5% cornings antifoam (1.5ml buffer/g tissue) and strained through muslin.

After low speed centrifugation (20 min, 3,000 rpm, 1,400g Sorvall RC 5 centrifuge 6 x 250ml GSA rotor) the supernatant was thoroughly mixed with HCP (0.8 mls HCP/g tissue) and centrifuged again for 20 min at 3000 rpm. If the supernatant was still tinged with green it was mixed again with HCP (0.4 mls HCP/g tissue) to absorb any remaining green plant material and centrifuged for a further 20 minutes at 3000 rpm.

-47-

In order to remove any traces of HCP and green plant material from the decanted supernatant a final low speed centrifugation (10 min 9,000 rpm, 13,000g) was given before the resultant supernatant was centrifuged at 39,000 rpm (160,000g) for $1\frac{l_2}{2}$ hr (MSE 65 centrifuge 8 x 50ml fixed angle rotor) to sediment the partially purified virus.

The pellets were resuspended in phosphate buffer (Na/K phosphate 0.02M pH 8.0) and given a further cycle of low speed/high speed centrifugation (10 min, 9,000 rpm/1½ hr 39,000rpm). On a number of occasions the partially purified virus was sedimented through 15% sucrose (6 ml at the bottom of each centrifuge tube) during the second high speed centrifugation. Examination of spectrophotometer scans before and after sedimenting through sucrose indicated that this procedure resulted in loss of total virus without improving the purity of the preparation.

Density gradient centrifugation (10-40% sucrose, 3h, 24,000 rpm, 100,000g, MSE 65 centrifuge, 6 x 16.5 ml swing out rotor) was used as standard practice for further purification. The sample containing the purified virus was located with an ISCO, UA5 flow through absorbance monitor (A 254) with the recorder and the fraction collected in an ISCO density gradient fractionator 640 model manually operated through an ISCO delay timer model 900 to allow for flow-through time. The fractions from each tube were combined and centrifuged for l_2 hr at 39,000 rpm and resuspended in 1 ml of phosphate buffer.

Results

Purifications carried out by this method regularly yielded preparations containing 1 to 3 mg virus per 100g of infected tissue.

Spectrophotometer scans showed some variation in the purity of the preparations. It was observed that the younger, softer, more succulent the infected cucumber tissue used for extraction the more pure the final preparation.

The spectrophotometer scans of the preparations showed absorbance readings within the following ranges: A max 259 - 262 nm, A min 243-246 nm, A max/min 1.1-1.4 and A 260/280 1.4-1.56.

The preparations were used according to their purity for antiserum

-48-

production, serology, electron microscope examination and RNA analysis.

It is of interest to note that those isolates which were denatured by citric acid precipitation reacted serologically as PNRSV. This is contrary to the reports that citric acid precipitation is a useful method for partial purification of PNRSV from stone fruits (100) but that citric acid denatures ApMV serotypes (99).

Thomas (248) reported similar results to this current investigation in that efforts to purify PNRSV from rose by precipitation with citric acid or polyethylene glycol failed.

This may support the finding that strains of PNRSV from rose (45) and hops (23, 24) may differ from PNRSV occurring in stone fruit. Rose and hops are the only natural hosts from which both ApMV and PNRSV have been obtained and some isolates from these hosts may have certain characteristics in common which are not found in either PNRSV from stone fruit or ApMV from apple (45).

A serological investigation of six virus isolates from rose, by agar gel diffusion using three antisera of the ApMV serotype and two antisera of the PNRSV serotype, showed a more or less continuous spectrum of serological reactions among the isolates, ranging from typical ApMV to typical PNRSV with one isolate reacting with both serotypes (45).

In Australia virus isolates from roses showing line pattern symptoms reacted in gel diffusion tests to both PNRSV and ApMV serotypes (19).

The presence in rose of intermediate serotypes may account for the failure of citric acid precipitation to purify the isolates tested.

Intermediate serotypes were not detected by ELISA, a result which was not unexpected in view of an earlier report (169). Some faint reactions were however obtained by gel diffusion with isolate '154' and some other isolates in herbaceous hosts, when reacted with some ApMV type antisera.

-49-

Characteristics of Particles

(a) Morphological

In 1956 it was reported that partially purified virus from two rose sources examined by electron microscopy were both found to have spherical particles with mean diameters of 28nm and 35.4nm (286).

Virus isolates from rose separated into three zones by sucrose density gradient centrifugation. The top and bottom zones were examined separately with an electron microscope. The top component contained uniform spherical particles with a mean diameter of 22.5nm. The bottom component contained both spherical particles with a mean diameter of 28.7nm and bacilliform particles up to 82nm in length (19).

Thomas (248) reported that the shape and size of PNRSV particles from rose varied from isometric (26.7 to 30.9nm) to quasi-spherical and bacilliform up to 62nm long.

Methods

For electron microscope examination partially purified preparations of virus particles were resuspended in either 0.01M magnesium chloride or 0.75% glutaraldehyde.

Fixation with glutaraldehyde resulted in considerably less disruption of the virus particles than resuspension in magnesium chloride.

In accordance with the results obtained with leaf dip and crushed homogenates (see indexing by electron microscopy) it was found that negative staining with uranyl formate, prepared after the method of Barnett & Murant (17), gave the best results and this method was therefore used.

Results

Electron microscope examination of partially purified preparations of several isolates of PNRSV each contained particles which were mostly roughly spherical ranging from 22 to 28nm in diameter. Those approaching the larger diameter tended to be elongated on one axis. A few particles were bacilliform ranging up to 38 to 40nm long by 22 to 28nm wide.

Some apparently longer particles were observed but as these appeared to be considerably disrupted it was considered that the apparent length was an artifact resulting from distortion.

Although no precise separation of virus particles into top, middle and bottom components was attempted it was noted that samples taken from the upper part of the gradient profile contained predominantly spherical particles. Virtually all the larger and bacilliform particles occurred in samples taken from the lower part of the gradient profile.

Characteristics of Particles

(b) Physical properties

In 1967 Fulton (99) noted that two and often three zones were visible in optical density profiles after density gradient centrifugation of virus isolates from rose and that the relative proportion of the zones was similar for the same isolate but different for different isolates. Similar observations of three zones were made in Australia (19). Thomas (248) also found that PNRSV from rose separated into three sedimenting components in sucrose density gradient and in analytical centrifugation. He noted that the ratios of the top middle and bottom components varied between isolates.

Method

During purification of the isolates by centrifugation in 10-40% sucrose density gradient (see section on purification) optical density profiles of the sedimenting virus particles were recorded.

Results

The optical density profiles of sucrose density gradients of preparations of nucleoprotein from different isolates of PNRSV each showed a UV absorption profile characteristic of that isolate (see Fig. 1).

The ratio of 3 major components - top, middle and bottom - using the nomenclature of Lister & Bancroft (176), was characteristic and constant for each of 5 isolates tested.

The number of preparations tested of each isolate varied from 4 of isolate '100' to 38 of isolate '154'. The profile was characteristic and constant for all preparations of any one isolate. Isolate '154' maintained in herbaceous hosts for 18 months produced the same profile at the end of that period as it did at the beginning of the period.

There did not appear to be any correlation between the ratio of components in the profile of an isolate and the type of symptom expression in the orginal rose sample.



FIGURE 1. Optical density profiles of sucrose density gradients of preparations of nucleoprotein obtained from five different isolates of prunus necrotic ringspot virus, each from different rose samples. The ratios of top, middle and bottom components are characteristic of each isolate. Sedimentation from left to right. Relative absorbance at 254nm.

-53-

Isolates '20' and '22' were both from plants of 'Honey Favourite' growing in the same bed in a public rose garden. Both plants showed symptoms within the general category of rose mosaic but differed from one another in that isolate '20' was recorded as "heavy chlorotic blotches" whereas isolate '22' was recorded as "fine chlorotic line pattern".

Despite both being plants of the same variety, probably from the same source, and both showing symptoms of the rose mosaic type the absorption profiles of the isolates from these two roses are quite distinctive.

The profile of isolate '20' showed maximum absorption by the top component and much less in the middle and bottom components whereas isolate '22' consistently showed maximum absorption by the middle component.

The profile of isolate '22' was not greatly different to that of isolates '101' and '154' both of which also showed maximum absorption by the middle component and only differed in the relative degree of absorption of the top and bottom components.

These latter two isolates however, came from rose samples which did not show any rose mosaic type symptoms. Isolate '101' came from a symptomless 'Diamond Jubilee' and isolate '154' came from a 'Chrysler Imperial' showing severe rose wilt type symptoms with decline and die-back.

On the other hand isolate '100' from a plant of 'Super Star' in the P.D.D. collection of plants with rose wilt, which also showed mild rose wilt type symptoms, produced an absorption profile with two almost equal maxima for the top and middle components and relatively little absorbence by the bottom component.

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The PAGE analysis of viral RNA was carried out using virus from purifications of isolate '154' which had showed a maximum absorption by the middle component in density gradient absorption profiles. Plots of the distance migrated in PAGE by the different RNA species gave closely similar values.

Characteristics of Particles

(c) Ribonucleic acid components

A number of workers have analysed the nucleic acid components of various viruses in the ilarvirus group by polyacrylamide-gel electrophoresis (PAGE). A PAGE analysis of nucleic acid from TSV, the type member of the group, resolved several RNA species of differing molecular weights. Both the RNA and the nucleoprotein virus particles were also separated into several components by density gradient centrifugation (58). Infectivity tests supported an assumed homology - using the nomenclature of Lister and Bancroft (176) - between top, middle and bottom nucleic acid components and top, middle and bottom viral components respectively (58).

The molecular weight estimates for the nucleic acid components were: bottom 1.12 x 10^6 ; middle 0.94 x 10^6 ; top 0.7 x 10^6 daltons. In addition a smaller RNA species of molecular weight 0.35 x 10^6 daltons was present (58).

In the ilarvirus group not only TSV but also citrus leaf rugose virus and citrus variegation virus have all been found to have their genetic information in the three heaviest RNA species of molecular weights about 1.1, 1.0 and 0.7 x 10^6 daltons. However, these RNA's are not infectious unless either a smaller fourth RNA (M.W. about 0.3 x 10^6 daltons) or coat protein is added (120).

Ribonucleic acid from ApMV, isolated from rose, and PNRSV, isolated from sour cherry, separated into four similar components by density gradient centrifugation. The three heaviest RNA species of each virus became infective when activated by the smaller fourth homologous RNA (119).

With ApMV from rose the infectivity of the middle and bottom virus particles, but not the top component nucleoprotein alone (99), would support the suggestion by Clark and Lister (58) that with TSV the small fourth RNA necessary for infectivity is encapsulated in the middle nucleoprotein component together with RNA species in common with the top nucleoprotein component. Evidence indicates that viruses in the ilarvirus group have closely similar infectivity requirements in relation to their RNA's (119).

Method

The following procedure was adopted to obtain estimates of molecular weights of RNA species.

Prunus necrotic ringspot virus isolate '154' was partially purified from heavily infected cucumber. Nucleic acid was obtained from the virus nucleoprotein using a sodium dodecyl sulphate/phenol extraction procedure (211).

Yields by this method were generally low and an alternative procedure using sodium perchlorate was adopted (285).

Polyacrylamide gel electrophoresis after the method of Peacock & Dingman (210) was carried out in quartz tubes, pre-electrophoresed for 2h at 6m A per tube.

Rat liver ribosomal RNA containing two RNA species (M.W. 1.80 and 0.63 x 10^6) was used as a standard.

For separation the gels were subjected to electrophoresis for 1.5h with a current of 4m A per tube.

Gels were removed and scanned on a Joyce Loebl Chromoscan using a 5V Rikadenki recorder. Gels were also stained overnight with a 0.01% solution of toluidine blue (58).

Molecular weights for the RNA species were estimated by comparing their mobility in gels with the two RNA species from rat ribosomes assuming a linear relationship between log molecular weight and distance moved (21, 179).

pera from p 54.

Results

Polyacrylamide-gel electórphoresis analysis of viral RNA was carried out using virus from purifications of isolate '154' which had showed a maximum absorption by the middle component in density gradient absorption profiles. Plots of the distance migrated in PAGE by the different RNA species gave closely similar values.

Two small peaks gave molecular weight estimates of approximately 1.4 and 1.0 x 10^6 daltons respectively and two large peaks gave molecular weights of approximately 0.7 and 0.3 x 10^6 daltons respectively (see Fig. 2).

Infectivity of the RNA preparations was not tested.

Discussion

By comparison TSV, the type member of the ilarvirus group, has three molecules of RNA with M.W's of about 1.1, 0.9 and 0.7 x 10^6 and a subgenomic RNA of M.W. 0.3 x 10^6 (58).

The three larger molecules are assumed to conform with the top, middle and bottom viral components which separate out in density gradient centrifugation (58).

With PNRSV from rose different isolates may vary markedly in the relative proportions of top, middle and bottom which separate in density gradient centrifugation. Isolate '154' which was used for this RNA analysis consists almost entirely of middle component.

The relatively large amount of the smaller RNA species would suggest that there had been considerable *in vitro* degradation of RNA during extraction.

Summing the molecular weights of the two smaller species of RNA gives a value similar to that of the 1.0×10^6 species suggesting that they may have been, at least in part, derived from this by breakage.



FIGURE 2. Optical density profile of nucleic acid from prunus necrotic ringspot virus isolate '154' after polyacrylamide gel electrophoresis for 1.5h at 4 mA per tube. Numbers are estimated molecular weights in millions of daltons. Migration from right to left. Relative absorbance at 260nm. Such an assumption would give a yield of nucleic acids - bottom (1.4×10^6) , middle (1.0×10^6) and top (0.7×10^6) - in similar proportion to the three nucleoprotein components which separate in this isolate when centrifuged in a density gradient, that is, predominantly middle component.

Clark & Lister (58) had found in their work with TSV nucleic acid analysis that similar degradation of RNA from the middle component nucleoprotein occurred.

Although identical molecular weight does not necessarily imply the same identity of two RNA species the evidence suggests that for the PNRSV isolate tested the middle viral component in density gradient centrifugation contains an RNA species in common with the top component together with a smaller species, unique to the middle component.

This would be similar to the evidence for RNA species in TSV nucleoprotein (58) which is in accord with Fulton's (104) biological evidence of common function for top and middle components of TSV.

Unfortunately no RNA analysis was made of other rose isolates with different proportions of top, middle and bottom components. This could have proved interesting in view of the wide variation between isolates.

-59-

Antiserum Production and Testing

Methods

Purified virus from isolate '154' was diluted to give approximately 1 mg/ml.

One millilitre of virus suspension was emulsified in the cold with 1 ml of Freund incomplete adjuvant and injected intramuscularly into the hind leg of a rabbit. Similarly prepared injections were given intramuscularly after 8 days and again after a further 8 days. These were followed 8 days later with an intravenous injection in the marginal vein of the ear. After the intravenous injection sample bleedings were taken at 4 days, 8 days, 11 days and 17 days. After 3h at room temperature to clot the bleedings were stored overnight under refrigeration, centrifuged at 3000 rpm for 20 min (Sorvall centrifuge 1000g), the serum decanted off and mixed with an equal volume of glycerol. Titres were determined by the microprecipitin test using drops in a petri dish and covering with parafin oil.

The same procedure was followed using purified virus from isolate '101'.

The identities of both antisera were checked by Ouchterlony gel double diffusion in petri dishes. The serology agar was 0.9% agar in phosphate buffered saline (0.01M K/K_2PO_4 pH 7.0 in 0.85% NaCl) with 0.02% sodium azide added after autoclaving.

Results

The homologous titres of the bleedings were 1/32 after 4 days, 1/64 after 8 days, 1/256 after 11 days, dropping off to 1/32 after 17 days.

An intravenous booster injection 30 days after the previous intravenous injection was similarly monitored and the titre again reached a maximum of 1/256 11 days after the booster.

The titre against clarified sap of healthy cucumber was never greater than half.

The antiserum to isolate '101' gave a maximum titre ll days after intravenous injection of 1/128.

The symptoms on the original rose sample '154', from which isolate '154' was obtained, were severe decline without any mosaic symptoms.

Rose sample '101', from which isolate '101' was obtained, was a symptomless 'Diamond Jubilee' which had been under observation and had been a source of propagation material for six years. Neither the parent plant or any of the progeny had shown any virus-like symptoms.

The antisera to isolates '101' and '154' were reacted in optimal concentrations by gel double diffusion against both homologous and heterologous, partially purified virus of isolates '101' and '154'. The resultant precipitation lines coalesced with no apparent spur formation indicating complete identity of the isolates with respect to their serological properties.

Other virus isolates from roses infected with PNRSV, but showing various mosaic and other symptoms distinct from the symptoms shown by rose samples '101' and '154', were tested by the same method using the antisera to isolates '101' and '154'. Despite the differences in symptom expression on rose no serological differences could be detected.

Both antisera were also checked against an isolate of PNRSV obtained from Plant Diseases Division (PDD), Dept. Scientific and Industrial Research, Auckland.

The identity of isolate '154' was further checked by reacting infected cucumber cotyledons with a range of antisera in gel diffusion tests. Positive reactions were obtained with the following PNRSV -type

-61-

antisera: PNRSV-RA (100) rose yellow net vein virus, RYNVV (108), rose 464A (100), all supplied by Fulton; PNRSV from PDD and the serologically closely related Danish plum line pattern virus DPLPV (100), also from Fulton.

Very faint reactions were also obtained with two ApMV-type antisera - ApMV-C (100) and RMV (99) - both from Fulton. This was not unexpected as PNRSV and ApMV both have a low proportion of common antigen (100).

There were no reactions with antisera to prune dwarf virus (PDV "B") (100), PDV (PDD) and TSV (PDD) except some non-specific reactions at antiserum concentrations of one quarter or higher.
-63-

CHAPTER 4

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Of the recently developed serological techniques ELISA has proved to be one of the most versatile. It is now widely used in many medical, veterinary, agricultural and horticultural situations. It is highly sensitive and can be used for both qualitative and quantitative determinations. There are several methods of applying the technique and the double antibody sandwich is the method generally used for detection of virus in plants. This method can be briefly described as follows:

1. Purified specific antibody (to the antigen to be measured) is attached to the solid phase which is then washed.

2. The test solution thought to contain antigen is then incubated with the sensitised solid phase which is then washed.

3. Enzyme labelled specific antibody is then incubated with the solid phase followed by washing.

4. Enzyme substrate is added. The colour change is proportional to the amount of antigen in the test solution in step 2.

The development of the test and its use in detecting viruses in plants is described in the following.

The use of glutaraldehyde for coupling and cross-linking of enzymes and proteins was pioneered by Avrameas and co-workers (9, 10, 11). The enzyme-protein conjugates were used for the microscopic detection of antigens or antibodies in cellular preparations after histochemical colouration of the enzymes (9). By using enzymes with reaction products of different colours two antigens could be revealed simultaneously in a single preparation (284).

The principle of using antigen absorbed to a solid phase to react with enzyme-conjugated antibody was outlined by Engvall & Perlmann (77, 78).

These methods were used to test for the presence of malarial antibodies in humans using the microplate method coated with malarial antigen (276). It was not until test plates were coated with antibody rather than antigen that the double antibody sandwich method was developed and applied to the detection of viruses in plants (274). The technique proved to be extremely sensitive, enabling assay of the viruses at concentrations as low as 10 to 100 ng/ml both in purified preparations and in crude plant extracts (274).

Extracts of some woody plants inhibited the virus-specific reaction while others caused non-specific reactions. It was found that the inhibition could be overcome by dilution of sap to 1:100 and the non-specific reactions could be reduced or eliminated by the addition of polyvinyl pyrrolidone at 1-2% (W/V) to the extraction buffer (55).

The technique was further used to assess differences in virus content within leaves and between different floral parts (56).

A detailed investigation of the characteristics of the microplate method of ELISA for detection and assay of plant viruses explored a range of variations of concentrations, buffer additives, sample sources, extract dilutions, incubation times and other effects (53) which led to a standardised procedure on the ELISA technique for plant viruses (54).

Various adaptations and variations of the technique were investigated to study particular aspects and applications, serotyping of PNRSV (12), homologous and heterologous reactions (169), short reaction times to speed the test (171), isolation of specific antibody for the test by acid sucrose density gradient centrifugation (186), the use of polystyrene sticks rather than plates (80, 81), the re-use of antibody coated microplates (14) and the re-use of non-sandwiched antibody-enzyme conjugates (15).

The use of the ELISA technique for testing for various viruses in a range of crops was rapidly exploited by many workers, prune dwarf virus in *Prunus avium* (46), potato leafroll virus in potato (47, 127, 183),

-64-

ArMV, PNRSV and hop mosaic virus in hop (259), plum pox virus in *Prunus* spp. (1, 56), two viruses in maize (222), strains of PNRSV in hop and plum (12), viruses in soybean seed and plants (175), tomato ringspot virus in red raspberry (65), seed-borne mosaic virus in pea (131A), citrus tristeza virus in citrus (13) and rugose mosaic in cherry (195). Concurrently with the investigation described in this report, but independent of it, ArMV, PNRSV and SLRSV were detected in roses by Thomas (252) using ELISA.

An investigation of the relative sensitivity of four different serological methods for the detection of ArMV, PNRSV and SLRV in rose showed that the latex flocculation assay was up to 250 times more sensitive than gel immunodiffusion but ELISA and serologically specific electron microscopy (SSEM) were respectively 200 and 1,000 times more sensitive than the latex test (252).

Thomas (252) also reported that several viruses could be detected simultaneously using SSEM, although the viruses could only be identified if they were morphologically distinct. He calculated that up to 100 prepared grids a day could be examined using SSEM.

On occasions Thomas (252) found that ELISA failed to detect viruses in rose sap due to non-specific reactions and possibly strain difference.

Discussion

The following discussion is appended here as it is pertinent to Thomas' paper just reviewed.

Although at the time the writer was unaware of Thomas' work, which was being carried out concurrently, the two investigations support each other in demonstrating the application of the ELISA technique for rapid, routine indexing of rose plants for known viruses.

Later in this report the writer describes how the occasional failure of ELISA to detect viruses in rose sap, due to non-specific reactions and strain differences, can be overcome with standardised selection of samples, adequate dilution and prolonged incubation.

-65-

A method using a modification of the ELISA technique to test simultaneously for several viruses in rose sap is also described later in this report.

With these modifications ELISA can be used for reliable rapid routine indexing of roses for known viruses. Although not as sensitive as SSEM it could be more convenient for general use. The ELISA method is capable of handling at least as many samples per week as SSEM but without the availability of an electron microscope.

Methods and Results

The general method of ELISA testing for the detection of viruses in this study closely follows that described by Clark, Adams & Barbara (55) and Clark & Adams (53, 54).

Each batch of coating and enzyme labelled gamma-globulin was tested for optimum concentrations after the method of Clark & Adams (54). In general a concentration of coating gamma-globulin of 1 ug/ml with enzyme labelled conjugate diluted 1:800 and plant test samples diluted 1:100 were found optimal.

A standardised method for collecting and preparing rose samples was used.

Young leaflets with the lamina just unfolded about the midrib were used. For each test approximately 0.1g of tissue was quickly macerated with a mortar and pestle in 1 ml of extraction buffer and a further 9 ml of extraction buffer added and stirred giving a sample concentration of approximately 1 in 100.

This method was used as it was found difficult to adequately macerate the small sample in 10 ml of buffer.

The use of a cork borer for taking leaf samples was found to be sufficiently accurate but this needed to be checked at different seasons and for different hosts as the same size cork borer was not always appropriate.

The purification of gamma-globulin, the conjugation of alkaline phosphatase enzyme (Sigma type VII) with purified gamma-globulin, and the buffers used in the test all followed the same method (54).

Incubation times were 2 h at 37° C for coating gamma-globulin, overnight at 4° C for test samples and 6 h at 37° C for enzyme-labelled gamma-globulin.

-67-

Enzyme substrate, p-nitrophenyl phosphate was used at the rate of one 5 mg tablet to each 8 ml of substrate buffer.

Initially the substrate was incubated at room temperature for lh and the reaction then stopped by adding 50 ul of 3 *M* NaOH to each well.

Initially assessment of results by visual observation proved unreliable as weak reactions were frequently undetected. Measurement of absorbance at 405 nm presented some difficulties as the volume available was insufficient for testing on the available spectrophotometers. Use of an ISCO UA5 flow through absorbance monitor to draw through successive wells proved convenient but insufficiently accurate. In later work where comparative quantitative results were required four wells were used for each test and the contents combined to give an average reading from a Pye Unicam SP 800 spectrophotometer.

Where only a qualitative test for the presence or absence of virus was required, as when surveying a large number of plants to determine their health status, a visual test was devised.

It was found that by incubating at 37°C rather than room temperature, and extending the time of incubation of substrate weak reactions not reliably visible after 1 h, developed sufficiently to be reliably assessed by visual observation. This method could not be used for quantitative results as the more rapidly reacting wells would go to completion using up the available substrate during the extended incubation period.

For this type of rapid survey plates were incubated at 37°C for 1 h and then visually assessed before storage overnight at 4°C, again assessed, then held at room temperature for a further 2 h or more before finally recording.

During and after assessment a careful watch was kept on healthy control wells but these did not show any colour change for at least 5-6 h after final assessment.

-68-

In order to adapt the method for rapid screening of field crops an equal weight of young leaves from each of ten rose bushes were macerated together to make one test sample. One infected bush combined with nine healthy still gave a reliably detectable reaction.

Where a crop may be infected by several different viruses it is desirable to be able to test for the occurrence of any of the viruses.

Purified gamma-globulins against PNRSV, ApMV and ArMV were each diluted in coating buffer to three times their optimum concentrations and then mixed to give the optimum concentration of each gamma-globulin in the coating buffer. Similarly the enzyme-labelled gamma-globulins to the three viruses were diluted to optimum concentrations of each in PBS-Tween containing 2% PVP and 0.2% ovalbumin (see Fig. 5).

Using these mixtures a reliable positive reaction was obtained not only in the presence of any one of these viruses in a young leaf of infected rose but also when the infected sample was combined with equal samples by weight of nine other healthy roses.

Bulking up of rose samples was not attempted beyond this point and three viruses were the most tested simultaneously but from the strength of the positive reactions obtained the use of larger numbers could well be successful.

For routine screening in this study four rose plants were tested simultaneously for three viruses. Where a positive reaction was obtained from a batch test the individual plants were tested for specific viruses.

The antisera used for preparation of purified gamma-globulin for ELISA testing were as follows: PNRSV - '154', prepared as described under "Serology", from a PNRSV isolated from rose sample '154' of the sample collection.

Antisera obtained from R.W. Fulton, University of Wisconsin, U.S.A., were PNRSV-RA titre 1:1280 from sour cherry necrosis (100),

-69-

Danish plum line pattern titre 1:320 from Danish plum (100), PDV-B titre 1:640 from plum (100), ApMV-c titre 1:1280 Cropley isolate (100) and TSV titre unknown.

Antisera obtained from B.D. Harrison, Scottish Horticultural Research Institute, U.K., were ArMV titre 1:2048 (source unknown) and SLRSV titre 1:2048 (source unknown) and TSV antisera from the antisera collection held at Massey University.

After purification of the gamma-globulin from each antiserum the optical density was measured at 280nm and diluted to give approximately lmg/ml of gamma-globulin (OD 1.4) except SLRSV which gave a yield after purification of less than 0.4mg/ml of gamma-globulin (OD 0.6).

No difficulty was experienced, nor was any apparent variation in the reliability of the testing methods observed, irrespective of the time of the year, providing young unfolding or just unfolded leaflets were used as test material.

When young leaves were not available in mid-summer and late autumn suitable growth could be induced by pruning and forcing the plants to be tested.

Some equally conclusive tests were also carried out in mid-winter by cutting dormant canes from the plants to be tested, wrapping the canes in damp newspaper, sealing in polyethylene film and storing at room temperature. When the buds on the canes had produced 10 to 20mm etiolated shoots these could be used as suitable test material.

Similarly in early spring when the first shoot movement from a dormant bud had reached lOmm or more in length the young shoot provided suitable test material. Although the use of such young shoots is practical it is not as convenient as unfolding leaves as it is necessary to weigh each sample rather than use a cork borer to obtain the required amount for testing.

Plants grown in greenhouses provided suitable young material at any time of the year.

1. ELISA indexing of "disease collection"

In order to carry out indexing by ELISA, purified gamma-globulin was prepared from '154' antiserum with a titre of 1/256 and diluted to 1 mg/ml.

One ml was diluted in coating buffer to give 100 ml of 10 ug/ml gamma-globulin.

One ml was conjugated with alkaline phosphatase (Sigma type VII) enzyme and diluted to 200 ml with PBS-Tween containing 2% PVP and 0.2% ovalbumin.

The remainder of the purified gamma-globulin was stored in siliconetreated glass tubes, 1 ml/tube, in a deep freeze.

Determination of optimum concentrations of samples, coating gammaglobulin and enzyme-labelled gamma-globulin was carried out after the method of Clark & Adams (54) using cucumber infected with the homologous virus and healthy cucumber as test samples.

With this preparation samples diluted 1:100 and enzyme-labelled conjugate diluted 1:200 were optimum but the optimum concentration of coating gamma-globulin was between 10 and 1 ug/ml. After further testing 5 ug/ml was used.

A series of tests were then carried out using infected and healthy rose to determine the best type and age of rose material to use for testing.

The greatest absorbance difference (at 405 nm) between healthy and infected rose, occurred with young rose leaflets at the stage they were just unfolding about the midrib.

A spectrophotometer set to measure absorbance of the hydrolysed substrate at 405nm was zeroed on a buffer only blank. Absorbance of healthy controls varied between 0.05 and 0.15 whereas absorbance of infected samples fell within the range 0.24 to 0.3. No samples registered an absorbance in the range 0.15 to 0.24. This indicated that the test gave a clear distinction between infected and healthy rose material.

The effect of increasing incubation times and temperatures was investigated and it was found that incubation at 37^oC for 1h, rather than room temperature, greatly increased the visually observable colour of the wells containing infected material without making any observable difference to the wells containing healthy material.

Spectrophotometer readings of absorbance at 405nm showed that although absorbance of the healthy controls had increased to range between 0.2 and 0.25 the absorbance of the infected samples had increased to range between 0.43 and 0.52.

The range within which no absorbance values were registered was therefore 0.25 to 0.43, range of 0.18 absorbance rather than the range of 0.09 absorbance, which did not register with incubation at room temperature.

In order to assess the possibility of using visual observation, rather than measurement of absorbance to determine the presence or absence of virus, plates were initially incubated for 1 h at 37° C then stored overnight at 4° C and incubation under regular observation continued the next day. As soon as any sign of discernable colour appeared in the healthy control wells the reaction was stopped by adding 50 ul of 3 *M* NaOH to each well.

By this time all wells containing infected material were clearly bright yellow giving a distinctive test for the presence of virus. As the reaction in many of the wells appeared to have gone to completion using up the available substrate this method would only be suitable for qualitative rather than quantitative assessment.

-72-

In order to test the reliability of the method all the samples in the disease collection were tested for the presence of PNRSV using gamma-globulin purifed from the '154' antiserum.

All plates were given extended incubation either until no further colour developed in the test wells or colour started to develop in the control wells.

Each plate was visually assessed as positive or negative for the presence of PNRSV and then the absorbance of each test measured at 405 nm.

The positive and negative results obtained by measurement of absorbance agreed with the results obtained by visual observation.

The use of extended incubation and visual observation alone, where large numbers of plants have to be screened for presence or absence of a virus, greatly speeds the operation as further test plates can be loaded and processed while earlier tests are developing.

This series of tests using gamma-globulin from '154' antiserum was carried out during February-March 1980, a time of the year when it is considered difficult to index for PNRSV. Successful testing was dependent on obtaining young leaves at the optimum stage of development for testing. It was necessary to prune some samples in order to force suitable young growth.

Additional tests using older fully mature leaves were done with some 'optimum' growth samples for comparison. Although these indexed positive or negative, in agreement with the results using young leaves from the same sample, the tests were less decisive in that absorbance readings for infected old leaves were at the bottom of the range recorded for infected plants and absorbance readings for old leaves from healthy plants were at the top of the range recorded for healthy plants. The virus concentration appeared to decrease with increasing maturity of the tissue and non-specific reactions tended to increase, thus reducing but not eliminating the differential between a positive and a negative test. By the following spring (1980) a range of antisera to various types and strains of ilar and nepo-viruses had been obtained as previously described.

Initially gamma-globulin was purified and conjugated from PNRSV-RA and ApMV-C both obtained from and prepared by Fulton (100).

Optimum concentrations were determined for both preparations at 1 ug/ml of coating gamma-globulin, 1:800 dilution of enzyme-labelled conjugate and 1:100 dilution of test samples.

In order to check the previous indexing using '154' antiserum to PNRSV, and to test for the presence of ApMV, the disease collection was again tested.

For each test two plates were used, one coated with ApMV-C gammaglobulin and tested with ApMV-C conjugate, the other coated with PNRSV-RA gamma-globulin and tested with PNRSV-RA conjugate. The diluted test samples were loaded in an identical pattern on both plates. Controls were healthy rose, PNRSV- infected rose and ApMV infected *C. quinoa* and buffer blanks.

Visual assessment only of plates after prolonged incubation was used to determine presence or absence of virus.

The same 120 samples from the disease collection which had indexed positive with gamma-globulin from '154' antiserum also indexed positive with gamma-globulin from PNRSV-RA antiserum. The other 101 samples were negative with respect to PNRSV.

All plates tested with ApMV-C were negative except for controls and one rose sample, '72' (see Fig. 4).

This was the only occurrence of ApMV in rose detected in this study and this sample was subsequently used as a control in further tests for ApMV.

-74-

The source of the sample was a plant of 'Masquerade' growing in a public rose garden on the southern approaches to Taihape. The symptoms were stunting, leaf distortion and large gold leaf blotches.

Although these symptoms were similar to chlorotic blotching and chlorotic mottle symptoms expressed by roses infected with PNRSV they differed in degree, tending to be larger and more golden than symptoms associated with PNRSV.

The growth of roses, infected with PNRSV and showing mosaic type symptoms other than chlorotic stipple, is generally normal in size or only slightly reduced. The growth of 'Masquerade' infected with ApMV was grossly reduced and stunted with severe leaf distortion.

These severe symptoms may in part account for the limited occurrence of ApMV in roses as infected plants showing these symptoms are not likely to be used for propagative material.

Although the symptoms on 'Masquerade' were typical of those described and illustrated for ApMV on rose (238, 253) transmission of typical symptoms to *R. multiflora* did not occur until almost three years after budding. This is contrary to the experience of Thomas (253) who reported that transmission of ApMV to *R. multiflora* in the U.K. produced very severe chlorosis.

Despite several attempts ApMV was not successfully transmitted to herbaceous hosts from rose sample '72'.

In order to check that the transmissions from roses in the disease collection to herbaceous hosts were in fact PNRSV a selection of 40 different isolates in herbaceous hosts was made. These isolates were selected from as wide a range of symptom expression as possible, both in the rose source plants and on herbaceous hosts. These isolates in herbaceous hosts were tested by ELISA for the presence of PNRSV and ApMV. All tests were positive for PNRSV and negative for ApMV.

After five days incubation of substrate a very slight colour change could be observed when cucumber infected with Fulton's ApMV serotype from rose was tested with PNRSV gamma-globulin (see Fig. 3).

-75-

This was surprising in view of the reports of serotypes intermediate between PNRSV and ApMV in rose (45) and in hops (12). However, Koenig (169) found that the specificity of ELISA was so great that with some viruses conjugates prepared to one strain failed to detect other serologically closely related strains. With other viruses cross-reactivity was broader, but distant and even intermediate serological relationships between viruses were usually not detected.

In the present study, although there were obvious strain differences in the reactivity of different isolates of PNRSV, positive and negative tests were quite distinctive using young tissue and extended incubation times.

Presumed differences between strains of PNRSV were apparent in the relative rate of colour development. Some strains produced an obvious reaction after 1 h incubation at 37°C. Other strains did not produce an apparent reaction until after further storage overnight at 4°C. Further strains would show a reaction after continued incubation for several hours at room temperature.

Storage thereafter for several days at room temperature would produce no further colour reactions of greater intensity than the healthy controls but those wells which had shown a colour change would proceed to completion giving a strong contrast between wells containing infected material and those containing healthy material. At this stage differences between strains were no longer apparent.

The different rates of reactivity may have been due to varying concentrations of virus in the tissue samples tested rather than serotype differences. Even if the effect was due to differences in viral titre within the tissue it did appear to be strain related. Relative rates of reaction between different isolates were similar in several tests using young tissue. Variation in tissue age and tissue source within the one isolate also gave a similar variation in rate of reaction. This would be due to variation in viral concentration within the plant as described later.

Some evidence of cross reactivity between PNRSV infected material and ApMV gamma-globulin could just be discerned but only after six days incubation at room temperature. This slight reaction was unlikely to cause confusion as positive tests for ApMV in young rose leaves on the same plates showed a strong reaction after only 1 h incubation at $37^{\circ}C$.



FIGURE 3. ELISA plate, five days after adding substrate, using PNRSV-RA (Fulton) gamma-globulin fraction. Samples are from cucumber leaves of isolates from the "disease collection" and tests are repeated in four adjacent wells - horizontal rows. Controls are in columns 9, 10, 11, 12. Row C, buffer blank; row D, uninoculated cucumber; row E, cucumber infected with PNRSV-G (Fulton); row F, cucumber infected with Fulton's ApMV serotype from rose. Other positive and negative tests are clearly distinguishable.



FIGURE 4. ELISA plate, one day after adding substrate, using ApMV-C (Fulton) gamma-globulin fraction. Forty six samples are from young leaves of roses '45' to '90' in the "disease collection" and each test is duplicated in two adjacent horizontal wells. Row F, wells 7 and 8 contain rose '72' ApMV; row D, well 2, contains ApMV (Fulton's serotype from rose) in *C. quinoa*; and row E, well 2 contains PNRSV-G (Fulton) in cucumber. Other controls are row D, well 1, healthy rose and row E, well 1, buffer bank.



FIGURE 5. ELISA plate, six days after adding substrate, using the following gamma-globulin diluted to their respective optimum concentrations: PNRSV-RA (Fulton) columns 1 to 4; ApMV-C (Fulton) columns 5 to 8; a combination of these two antisera with ArMV (Harrison) antiserum in columns 9 to 12. Each row of 12 wells was loaded with the same sample, as follows: row A, rose '21' infected with PNRSV; row B, *R. multiflora* grafted with rose '72' infected with ApMV; row C, buffer control; row D, as in row B but diluted fourfold with healthy rose; row E, old leaves of rose '72' infected with ApMV; row F, healthy rose; row G, PNRSV (isolate '101') in cucumber; row H, ApMV (Fulton's serotype from rose) in *C. guinoa*.

ELISA indexing of "High health mother plants"

This collection consisted of groups of four plants of each cultivar. Each group either originated from two to four budsticks imported from the breeder of the cultivar or from one or two budsticks obtained from amateur and commercial sources known to be endeavouring to maintain plants free of known viruses. It seemed reasonable to assume that if a cultivar was infected, the virus would occur in all four plants. However, in case this were not so, equal size samples were taken from each of the four plants of a cultivar and combined for a single test.

Initially, to check whether one infected plant would still give a positive reaction with ELISA, plates were tested using equal samples per plant of one infected rose combined with either three, six or nine healthy roses.

With extended incubation time even one infected plant in 10 was easily detected by visual observation with the colour developing almost to completion before there was any sign of colour change in healthy controls.

Using separate plates for PNRSV and ApMV testing and bulking equal samples from each of the four bushes of a cultivar together, 274 cultivars in the high health mother plant blocks were indexed.

All cultivars were negative with respect to ApMV but 14 cultivar beds, or just over 5 percent indexed positive for PNRSV.

None of the mother plants of these 14 cultivars had, in the seven to eight years they had been under observation, shown any symptoms normally associated with PNRSV. During this period they had been used as a source of propagative material for ordinary nursery production and no symptoms normally associated with PNRSV had been observed in their progeny.

-78-

However six of the larger flowered cultivars, now found to contain PNRSV, were suspected to be not quite normal on account of a very slight lack of petal texture. As no material of these cultivars, which was better in this respect, had been located these mother beds were retained. This observation suggested that a slight loss of petal texture may be associated with some strains of PNRSV.

The individual plants in each of the beds which had indexed positive were then tested separately for PNRSV.

In 11 cultivars all four of the plants in a bed were found to be infected with PNRSV, indicating that this material had been infected at source. Of these 11 cultivars only one had been imported direct from the raiser. The other ten had been obtained from enthusiastic amateurs who endeavoured to maintain elite stock for competitive showing. The other three cultivars were not one hundred percent infected. The variety 'Lulu' was infected with PNRSV in only one plant and the variety 'Fresco' was infected in two of the three plants, one plant having died. It was suspected that these two varieties must have been propagated on understock which was not of high health status, as two of the four plants of 'Lulu' and one of the three plants of 'Fresco' were also found to be infected with rose petal fleck. The bed of 'Baby Masquerade' was the only one in this collection known to have come from two sources, namely direct from the raiser budded on suspect understock, and cuttings from a private importation. Half the plants in this bed were infected with PNRSV.

It is of interest that all the mother plants found to be infected with PNRSV appear to have either been infected at source or infected by budding on understock not of high health status.

-79

This provides some evidence that PNRSV is not transmitted in roses from one plant to another by either pollen or vectors. This high health mother plant collection, comprising over 1000 plants, was growing for the first four years of its existence in close proximity to large numbers of roses known to have a high incidence of symptoms associated with PNRSV and would have been subjected to a heavy loading of pollen from infected bushes and possibly infected vectors. If a significant percentage transmission had occurred, random infection, otherwise inexplicable, would be expected.

At the conclusion of this investigation all cultivars in the mother plant collection found to be infected, were destroyed.

ELISA indexing simultaneously for a range of viruses

Using the methods already described for testing simultaneously for several viruses, both the disease collection and the "High Health mother blocks" were again indexed for six different viruses. Prune dwarf virus, DPLPV and TSV gamma-globulins were combined in one plate and ArMV, SLRSV and TobRSV were combined in a second plate. Each rose sample was loaded on to both plates.

Controls for this multiple testing consisted of the usual buffer blank, a seedling rose, which was apparently healthy as it consistently tested negative. The following virus sources were used as positive controls:

PDV	-	infected plum ex PDD.
DPLPV	-	cherry aucuba mosaic ex PDD.
TSV	_	Massey freeze dried collection no 1013.
ArMV	-	cucumber infected from Massey freeze dried collection
		no 1037, and also infected cucumber ex PDD.
SLRSV	-	Massey freeze dried collection.
TobRSV	-	cucumber infected from Massey freeze dried collection,
		and also infected cucumber ex PDD.

All the above virus controls gave positive reactions with the appropriate antisera. Danish plum line pattern virus reacted positively with PNRSV antisera.

Those samples, which had already indexed positive for PNRSV, also reacted to varying degrees with the combined PDV, DPLPV, TSV plates. Testing of these samples for the individual viruses showed in all cases a reaction for DPLPV but no reaction for either PDV or TSV. This was not unexpected as DPLPV is a strain of PNRSV.

All tests with the combined ArMV, SLRSV, TobRSV plates were negative except the cultivar 'Molly McGredy' in the "High Health mother blocks". Testing of this variety for the individual viruses showed a positive reaction for ArMV, but not for either SLRSV or TobRSV. The four plants in the 'Molly McGredy' mother block were individually indexed for ArMV, three were positive and one was negative.

The 'Molly McGredy' mother plants had originated as four plants selected from fifteen plants which had been propagated from several budsticks imported in November 1971 from the raiser of the variety. The raiser had advised at the time that they were of doubtful virus status as the only source from which budwood could be collected was from standard plants budded on *R. rugosa* understock which he had imported from Holland.

Some of this understock was suspected to be infected with either ArMV or SLRSV. This was subsequently shown to be the case (157, 267, 268).

These mother plants had consistently remained symptomless, although a few leaves showing the vein banding symptom were subsequently observed on some of the progeny from them.

This agrees with observations in the U.K. that although SLRSV shows severe symptoms, ArMV infected plants were frequently symptomless or showed symptoms of 'rose mosaic' (157).

It would appear that ArMV was carried to the U.K. from Holland on *R. rugosa* standard understocks and thence to New Zealand on this variety. There did not however appear to be any further spread, other than to the progeny from these plants, as ArMV was not detected on any other roses tested.

ELISA Indexing of "old rose collection"

One hundred and eighty eight old and species type roses in this collection were initially indexed in groups of 4 cultivars, for the presence of PNRSV, ApMV, TSV, PDV, DPLPV, ArMV, SLRSV and TobRSV. The tests were all negative for these viruses except PNRSV and DPLPV.

Fifteen groups of four roses showed a positive reaction for these strains of PNRSV. The individual plants in each of these groups were then tested for the presence of PNRSV. Two of the groups had two infected plants in each and the other thirteen groups each contained one infected plant giving a total of seventeen plants (approximately 11%) infected with PNRSV.

Considering the time that most of these varieties have been in cultivation the incidence of PNRSV was surprisingly low. This could be partly accounted for by the fact that a number of varieties of this type of rose are frequently propagated by cuttings rather than budding and partly due to the propagation and build up of these varieties being less intensive during the period when PNRSV was becoming widespread in more modern cultivars.

Non rose hosts and rose rootstocks

In order to detect graft transmission of PNRSV from rose to peach 'Golden Queen' seedlings young leaves of the peaches were tested by ELISA for the presence of PNRSV. The results were decisively either positive or negative with no doubtful intermediates.

Those peaches which tested positive had all been budded with PNRSV infected rose. Those peaches which tested negative had either been budded with rose free of PNRSV or the rose bud had died shortly after grafting to peach.

Although no further indexing of the peaches, other than visual observation, was carried out it would appear that the ELISA technique is a useful indexing method for the detection of PNRSV in peach.

Young leaves of the apple understocks which had been budded with roses from the disease collection were also indexed by ELISA for the presence of ApMV. All tests were negative except ApMV controls (rose sample '72' and Fulton's ApMV isolate from rose (99) in *C. quinoa*).

This result was not surprising as ApMV had not been detected in any of the rose samples budded to apple.

In order to check that ELISA would detect ApMV in apple, samples were included of apples from the Massey University orchards showing typical ApMV symptoms. These samples were strongly positive.

In association with work being carried out by Dr J.B. Sweet, some cherry samples were also tested by ELISA. Young leaves of seedlings from PNRSV infected trees tested either positive or negative for PNRSV indicating that some seed transmission was occurring.

Most of the old trees of cultivar clones gave a positive test for the presence of PNRSV when young leaves were used as a sample. It was noted however, that older leaves gave a weaker test.

Subject to the choice of suitable sample material the ELISA method appeared suitable for indexing for PNRSV in cherry.

Testing by ELISA was also carried out on a number of commercial rose understock clones in current use by nurseries. Most of these clones were found to be infected, in at least some of the samples taken, with PNRSV.

Those clones which appeared free of virus were new clones recently derived from seedling *R. multiflora*, except one clone which had been in commercial use for many years appeared free of virus. This clone was subsequently found to be infected with the virus-like flower disease RPF.

The fifteen cultivar clones and the six rootstock clones imported from the University of California, USA, and originating from their foundation stock of heat treated and indexed roses were also tested by ELISA.

Tests were made for the presence of PNRSV, ApMV, TSV, PDV, DPLPV, ArMV, SLRSV and TobRSV. Tests for all the above viruses were negative for all clones.

Distribution of virus within the rose plant

Although decrease in ELISA absorbance values is not strictly linear in relation to dilution, the method is sufficiently quantitative to give a fair measure of relative virus concentration in plant tissues having been used to determine the relative distribution of plum pox virus in the various parts of plum flowers (53), and the presence of virus in seed of soybean (175) and pea (131A). Detection of PNRSV in pollen of rose has also been reported (242).

Method

As young unfolding leaves had been found to be more reliable for indexing than older leaves, it appeared that the concentration of virus within the plant varied with age of tissue.

In order to estimate how this concentration varied, not only with age but also in various plant organs, two tests were made with a range of plant parts of various ages using the ELISA technique. Both tests were made with tissue from sample '101', a symptomless 'Diamond Jubilee' rose.

Test 1 The enzyme-substrate reaction was allowed to continue for 24h (lh at 37° C, overnight at 4° C followed by 6h at room temperature) before measurement of absorbance.

<u>Test 2</u> The reaction time was shortened to lh at 37° C and overnight at 4° C before measurement of absorbance.

Although the vegetative material used in the two tests was similar in age and source the flower bud used in the first test was less advanced than that used in the second test. In the first test the bud was still almost completely enclosed in the sepals with petal colour just showing under one reflexing sepal. The partially developed innermost petals and the developing carpels recorded a lower absorbance than the other flower parts.

However, in the second test the flower bud had opened to the stage where the outer whorl of petals had unfurled and reflexed. At this stage there was little difference in absorbance between the petals, stamens and carpels.

Results

Results are presented in Table 1.

The vegetative tissue showed a trend to increase in absorbance with increase in metabolic activity. The old mature tissue of the previous season's cane showed little difference to the healthy controls excepting in the metabolically active cambium area.

The recently mature cane (Test 1, 1/11/80, Test 2, 4/11/80) showed a similar trend but was less pronounced.

It was apparent in the young material that either an unfolding or just unfolded leaf blade was the most suitable tissue to use when indexing for the presence of virus by ELISA.

Although the single test of a mature root was negative it cannot be inferred that the virus was not present in the root system as young metabolically active roots were not tested.

Similar tests were carried out on the distribution of ArMV in an infected plant of 'Molly McGredy' but these were only visually assessed.

A similar pattern of distribution was observed with no apparent reaction from old mature tissue, a weak reaction with cambium, a strong reaction with younger tissues and the strongest reactions with just unfolded leaves.

-87-

In order to test for the possibility of seed transmission of PNRSV in rose, seed was gathered from a range of rose bushes known to be infected with PNRSV and expressing either rose mosaic or rose wilt type symptoms in varying degrees of severity. Seed was also collected from infected symptomless bushes.

All the seed collected resulted from open pollination therefore it was not known whether they had been fertilised with either healthy or infected pollen.

The embryos were carefully excised from ten seeds from each plant and all parent plant tissue was removed. Indexing of extracts of each group of ten embryos for PNRSV by ELISA was negative in all tests.

There was no evidence in this series of tests that a PNRSV infected seed-bearing parent transmitted the virus to it's seeds.

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<u>TABLE 1</u> Results of two tests using ELISA to determine the incidence and relative concentration of prunus necrotic ringspot virus in various rose tissues.

VIRUS-LIKE FLOWER DISORDERS

Rose flowers may show either one or both of two similar, but distinguishable, graft transmissible groups of symptoms. These appear to be two separate diseases of different etiological cause. One of these was described by Hunter (153, 154) as rose colour break (RCB) in 'Queen Elizabeth'. In his papers he makes mention of a "somewhat similar" condition in the rose cultivar 'Super Star' but does not distinguish this from RCB. In New Zealand the variety 'Super Star' very commonly shows symptoms of the disease which has come to be known in New Zealand as rose petal fleck (RPF) and there is no known record of the occurrence of RCB as described in 'Queen Elizabeth' occurring in 'Super Star'.

It would seem probable that the symptoms observed by Hunter in 'Super Star' were in fact RPF rather than RCB. This would be supported by an article in the "N.Z. Gardener" the following year by Fry (88) a co-worker of Hunter's.

Fry (88) states "Each of the above symptom types ('Queen Elizabeth' and 'Super Star') is perpetuated by buds taken from infected plants and each remains true to type when transmitted to previously healthy plants. This suggests that each is caused by a distinct virus entity..."

A 'virus-like' condition in rose flowers in the U.K. was mentioned in an illustrated talk given by Dr R.R. Frost and reported in "The Gardeners Chronicle & HTJ" (87). A subsequent note in "Plant Pathology" by Farrar & Frost (79) makes it quite clear both from the description and from the illustrations that this disorder is of the rose petal fleck type and not the flower colour-break originally described by Hunter (153) from 'Queen Elizabeth'. Unfortunately this same outbreak was briefly mentioned in a paper on virus diseases of roses in the U.K. (157) under the heading "Rose flower-break".

It has been confirmed by Sweet (pers.comm) from his experience of the disorders in both the U.K. and New Zealand that what he has seen in the U.K. was of the petal fleck type rather than the colour break described by Hunter.

The use of the term "colour break" by Hunter is perhaps unfortunate as the symptoms are not of the type classically associated with flower colour breaking in other flowering plants. The symptoms are more characteristic of the greening or virescence symptoms typical of diseases which are increasingly becoming associated with mycoplasma-like organisms (25).

On the other hand the symptoms of the disease known as RPF are closer to those classically termed "colour break" being a typical "dark breaking" of colour giving small dark streaks or elongated flecks (25). Similarly this type of symptom in other unrelated plant species is generally not apparent in yellow or white flowered varieties.

Despite these apparent anomolies in the use of the term "rose colour break" for the disease described by Hunter, his description of the disease has priority of publication and should be retained meantime, at least until more is known of the causal agent.

Rosa chinensis Jacq. var viridiflora Dipp., the so-called green rose, has been associated with mycoplasma-like bodies and a virescence induced in petunia, *Petunia hybrida* Vilm. with the aid of dodder, *Cuscuta campestris* Yunck (217, 218).

Although this conditions is quite distinct from RCB, the association of mycoplasma with greening in rose is of interest.

Method

Double budding a test bud and an indicator bud onto a healthy understock is to date the only satisfactory method of testing for the presence of RPF and RCB.

Rosa multiflora 'ISU 60' cuttings were prepared and grown as previously described (Indexing on *R. multiflora* 'ISU 60'). A bud of the plant to be tested and a bud of a healthy indicator cultivar were T-bud grafted onto the clean stem below the stock top with the indicator bud above the test bud.

When the grafts had taken the stock tops were excised immediately above the test bud. Where necessary growth from the test bud was reduced to force growth from the indicator bud. Flowers of the indicator were examined for transmission of symptoms.

During this current investigation the cultivar 'Anabell' was used for most of the indexing for RPF as this variety also reliably indicated the presence of RCB and when both diseases were transmitted to one plant both symptom types could be reliably distinguished superimposed in the one flower.

The cultivars 'Super Star' and 'Fragrant Cloud' were also used on occasions when testing for RPF only. Because no attempt was made to transmit RCB to these varieties it is not known whether they would also serve as indicators for both diseases.

The cultivar 'Bridal Pink' was used occasionally when indexing for RCB only as RPF is barely distinguishable in this variety.

The expression of symptoms in all RCB indicators used was variable. In some indexings the whole flower of the indicator was severely affected whereas in others the symptoms were confined to the veins of the outer petals.

Transmission of RCB to *P. hybrida* with the aid of dodder was attempted using *Cuscuta epithymum* Murray. Five plants of the rose 'Anabell' were grown in pots placed adjacent to pots containing plants of petunia 'Rosy Morn' grown from seed. Dodder was grown so as to parasitize both the petunia and the rose. Seedling petunias from the same batch were grown as controls.

Because of apparent variation in the flower colour of the seedling petunia controls, cuttings were rooted from a single self coloured pink petunia. The experiment was repeated using petunia plants from this clone.

Results

Frequent indexing by double budding with a wide range of infected cultivars and healthy susceptible varieties has shown that there are two distinct diseases involved as each disease remains true to type when transmitted.

It appears that there is no close relationship between the two causal agents as there is no evidence of cross protection, and when plants are infected by budding with both diseases the two symptom types can be discerned, superimposed in the same flowers.

Rose colour break

Flowers affected by RCB may be grossly malformed. The flowers fail to open normally and the outer petals become more or less green, very distorted with the appearance of a crisp lettuce-like leaf, with more or less crimped outer margins, and may appear either fleshy or leathery depending on the variety affected. Clear pink varieties such as 'Queen Elizabeth' and 'Bridal Pink' may develop paler areas on the petals and the outer green tinged petals may develop deep rose-coloured blotches on the reverse. Deep salmon varieties such as 'Anabell' develop a darkening and greening of the outer petals. The main veins of the petals appear prominent and darker in colour when viewed from the outer surface of the petal. (See Fig. 6).

The variety 'Whisky' generally becomes much deeper in colour with redorange blotching associated with distortion of the outer petals resulting in a crinkled or crumpled appearance with fleshy or leathery texture.

In all varieties on which symptoms have been observed, the severity of symptoms tend to vary from plant to plant rather than from flower to flower on the same plant.

The outer petals, of an affected flower, show more severe symptoms than the inner petals. In a severely affected flower the outer petals are reduced to half their normal size and may be completely green with darker green veining. The inner petals are generally more or less normal in



FIGURE 6. Two flowers of the rose cultivar 'Bridal Pink' both showing severe symptoms of rose colour break. Normal flowers of 'Bridal Pink' are smooth-petalled and colour a clear rose-pink. colour, but the main veins appear prominent on the reverse of the petals and darker in colour than normal.

On mildly affected plants the flowers only show the prominent main veins of darker colour on the outer surface of the outer petals and the inner petals appear normal.

In the field, affected plants in a crop may be recognized at a distance by a tendency of the petals of spent flowers to become red and papery and to remain on the plant for a much longer period than is normal for healthy flowers of that variety.

This symptom alone is not necessarily diagnostic of the disease as some cultivars normally retain old petals on spent flowers.

A greening or virescence of the outer petals on healthy plants of some cultivars may also occur late in the season and appears to be a normal reaction to the onset of winter and colder conditions.

The symptoms of RCB are suggestive of a disease caused by mycoplasmatype organisms.

Virescence was not induced in petunia by attempted transmission of RCB from rose with the aid of dodder, *C. epithymum*.

Rose Petal Fleck

The symptoms of rose petal fleck (RPF) vary in colour intensity depending upon the variety affected. Symptoms are generally most obvious in varieties in the orange-red to scarlet colour range, less obvious in pink, salmon, crimson red and dark red varieties and almost indiscernable in those varieties coloured yellow, apricot or white. (See Fig. 7).

In orange-red or scarlet varieties rose petal fleck shows scattered groups of flecks of darker colour particularly towards the margins and on the inner surface of the petals. Although the flecks are elongated in a direction parallel to the veining pattern they are not confined to the veins nor do they follow or affect the larger veins of the petal.


FIGURE 7. Two fully open flowers of the rose cultivar 'Fragrant Cloud'. The flower on the left is from a healthy indicator bud of 'Fragrant Cloud' double budded with a healthy test bud. The flower on the right shows symptoms of rose petal fleck induced in a healthy indicator bud by double budding with an infected test bud. Adjacent flecks are usually linked laterally giving a complex "chinese character" effect to the flecked areas. Expansion growth of the developing petal appears to be retarded in the flecked areas causing some deformation of the petal with resultant loss of smooth surface texture. Affected petals tend to become slightly rugose or crinkled with a "crepe paper" type texture.

Yellow, apricot and white varieties tend to only show this change in texture without any obvious colour change. Infection of varieties in this colour range may be indiscernable except when healthy blooms of the same variety are available for comparison. Varieties in this colour range may act as virtually symptomless carriers. Indexing for the presence of this disease can be done by budding the plant to be tested with a healthy bud of a variety which shows symptoms when infected e.g. 'Super Star', 'Fragrant Cloud', 'Orangeade', 'Hartina', 'Anabell' and others.

RCB	RPF
Virescence of outer petals	No virescence
Increased pigmentation and prominence of main veins	Flecks of darker pigmentation towards edges of petals. Not associated with main veins
Vein symptoms more prominent on reverse of petals	Characteristic flecking symptoms more apparent on inner surface of petals
Darker veins appear swollen and fleshy	Flecked areas appear shrunken
Generally observed occurring on pink varieties	Most obvious on orange- red and scarlet varieties

Table 2: Analysis of difference between rose colour break (RCB) and rose petal fleck (RPF)

Because no clear distinction between the two diseases has been made in the literature there has tended to be confusion both overseas and in New Zealand as to which of the two disorders is present.

In New Zealand the occurrence of RCB is relatively rare. The symptoms have only been observed occurring 'naturally' on a few plants in nurseries, cut flower crops, public and private gardens in a few varieties such as 'Queen Elizabeth', 'Bridal Pink', 'Vol de Nuit', 'Whisky' and 'Anabell'. This would infer that the causal agent has not become widespread in nurserymen's understocks. Also, because the symptoms on the flowers are relatively obvious, affected plants are unlikely to be used as a source of budwood for grafting.

On the other hand, the occurrence of rose petal fleck in New Zealand is widespread. Some ten to fifteen years ago many, if not most, rose nurseries were using understock with a high percentage infection of RPF. The disease was so widespread that the presence of flecking was considered normal for many of the varieties in commerce at that time. It was not until clean budwood of such varieties was worked on healthy understocks and the normal appearance of the varieties became available for comparison that the extent of the disfigurement became appreciated.

Despite the widespread occurrence of RPF there does not seem to be any evidence of vector involvement in its spread. One infected plant of the variety 'Rosenelf' was observed in a large bed of that variety in a public garden. Its position was noted and the bed was observed from time to time over a period of eight years. At the end of that period it was still the only plant showing symptoms. Similar observations over a four year period on a bed of 'Anabell' did not show any spread of symptoms from the originally infected plants to adjacent apparently healthy plants. In this instance the bed contained some plants showing symptoms of RCB as well as other plants showing symptoms of RPF. There was no apparent spread of either disease.

Because RPF had become so widespread and accepted as normal by the rosegrowing public affected plants in gardens have tended to be retained as long as they continue to produce a reasonable crop of flowers.

Although plants free of RPF have been widely available for a number of years these have mostly gone for new plantings rather than for replacement of older infected bushes.

That the incidence of RPF was until recently very high in private gardens was indicated by inspection of the blooms entered competitively by home gardeners at local rose shows. Of 14 shows inspected during November 1979 all showed at least some symptoms of RPF on a third to a half of the total number of blooms on display. No doubt a number of those which appeared symptomless would in fact have also been infected with RPF.

In November 1977, twelve of the blooms receiving the highest awards at the N.Z. National Rose Show were taken with the permission of the owners and indexed by double budding using a healthy indicator. Although no RPF symptoms could be discerned on any of the original blooms five of

-97-

the twelve produced RPF symptoms in the indicator flowers.

By November 1982, probably due to increased awareness of the problem by rose nurserymen and rosarians, the observable incidence of RPF at the N.Z. National Rose Show was less than 1% of the blooms on display. No doubt again there would have been a number of infected blooms on which symptoms were not apparent.

Origin of rose petal fleck

The first observation of the disease now known as RPF was the appearance of abnormal flecking on some plants of the variety 'Rosenelf' about 1960 (Salinger, pers. comm). Shortly afterwards a similar condition was noted in a range of varieties but particularly those with orange or scarlet flowers such as 'Super Star', 'Fragrant Cloud', and 'Orangeade'. The symptom appeared to be more pronounced in spring and autumn and initially it was thought to be a seasonal climatic effect.

In 1970 a commercial importation of 45 new varieties of rose was made by the author from rose breeders in the U.K., Germany and Denmark. The majority of the buds of each variety were budded onto a commercial nursery understock and a few of each were budded onto cuttings taken from *R. multiflora* seedlings.

When the resultant plants flowered, it became immediately apparent that some of the varieties were almost 100% infected with RPF on the commercial understock but showed no sign of the disease on the understock from seedlings.

It was apparent that the disease was being transmitted from infected commercial understock but the majority of the varieties were not showing any distinct symptoms other than a slight loss of petal texture in some cultivars.

In order to test this hypothesis the apparently symptomless varieties budded on commercial understock were indexed by double budding using cuttings from seedlings as understock and healthy buds of the cultivar 'Hartina' which had expressed symptoms of RPF when budded on the commercial understock, as an indicator.

The symptomless varieties were indexed both from plants on commercial understock and from plants on cuttings from seedlings.

The 'Hartina' indicator showed no symptoms from any of the plants on cuttings from seedlings but 95% of those indexed from symptomless plants

on commercial understock produced characteristic RPF symptoms on the 'Hartina' indicator (Gardner unpubl. results).

Budding of healthy indicator varieties direct onto the commercial understock resulted in almost 100% transmission of RPF.

This high level of infection in the commercial understock was probably due to the standard practice in rose nurseries at that time of taking understock cuttings from the tops of budded plants and collecting budwood from plants of the previous season's production.

In subsequent years it was observed that RPF was widespread through New Zealand rose nurseries and that the main foci of distribution appeared to have been from nurseries that either grew or had grown "old and species" type roses as part of their production. This observation was of particular interest as many of the so-called "old" roses have either flecked petals and/or relatively thin petal texture. This is considered normal for those varieties by those who are knowledgeable about this type of rose.

As far as could be ascertained, all plants of an "old" variety which showed this flecking were similar in this respect.

It was postulated that rather than being normal for these "old" varieties to be flecked, it could be that all the plants were infected with RPF and that these "old" varieties could have been the source from which RPF was disseminated through understock cuttings to infect modern varieties.

In order to test this hypothesis a range of "old" varieties showing this flecking characteristic and a number with what seemed abnormally thin petal texture were indexed by double budding with healthy 'Super Star' on healthy rootstocks.

All indexings proved positive for RPF.

Although it is impossible to prove in retrospect what may have occurred in the 1950's, or before, it would seem likely that these "old" roses were the source of RPF in New Zealand.

-100-

This would not necessarily have occurred in other countries where it is common practice to bud roses onto seedling understocks rather than cuttings.

CHAPTER 6 CONCLUSIONS

-102 -

It is apparent that the old nursery practice of making rose understock cuttings from the tops of budded plants has been the major factor in disseminating virus and virus-like diseases of roses in New Zealand.

Initially, when rose gardens and nurseries were being surveyed for material for the 'disease collection', the problem was not one of finding apparently infected material but rather one of deciding what material to use.

In the five years since this investigation was initiated there has been an obvious reduction in the percentage of apparently infected plants in nurseries and gardens.

This has no doubt occurred as a result of an increased awareness of the problem, by rose nurserymen and rose growers.

The occurrence of symptomless carriers of at least some of the virus and virus-like diseases is likely to continue to be a problem in the future. Such symptomless carriers may either develop symptoms themselves or infect, by undesirable propagation practices, cultivars which express symptoms.

The use of ELISA to directly index for the presence of viruses in roses has been a very useful tool not only for rapid surveying of rose populations to detect infected plants but also to determine the relative reliability of other means of indexing.

The techniques of extended incubation time, bulking up of test samples and simultaneous testing for several viruses, developed during the course of this investigation, have enabled a large number of plants to be rapidly and reliably indexed.

In all cases where infection of PNRSV was detected by ELISA, it could be confirmed by other indexing methods. However, none of the other indexing methods used would have unequivocally detected PNRSV in all cases without either repetitive testing or lengthy observation. Although this investigation did not make any progress beyond previous speculation (31, 111) as to the etiology of rose proliferation it has, by associating viral rose wilt type symptoms in mature plants with a strain of PNRSV, separated this complex (89) into two distinct disorders of different etiology.

This was largely made possible by the use of ELISA as a number of isolates of PNRSV from plants with rose wilt symptoms were difficult to detect by bud grafting and mechanical transmission.

The extensive occurrence of PNRSV in all areas where roses are grown would indicate that the build-up of this disease in roses in New Zealand has occurred over a lengthy period.

The isolated occurrence of ApMV in one plant of 'Masquerade' and ArMV in the variety' Molly McGredy' would indicate that these two diseases have not become established in commercial understock clones.

The small section of this investigation on the properties of PNRSV particles indicates that this virus appears to conform with other members of the ilarvirus group. The MW of the largest RNA species obtained by the methods used is higher than that obtained by other workers for other members of the group (58, 120). This may be due to the method used for preparing the RNA's or that the strain of PNRSV associated with rose wilt symptoms differs in this respect.

The differentiation of RPF from RCB has not been adequately described in the literature. The causal agents of these two virus-like diseases of roseswere not detected and the only means of indexing for these diseases is by double budding with cultivars producing obvious flower symptoms.

The fact that RPF is frequently symptomless in many cultivars poses a potential threat to its continued spread if undesirable propagation methods are used. Similarly the propagation of these "old" rose varieties which are totally infected with RPF may serve as a fresh source of inoculum to infect modern roses.

-103-

Inoculum from such "old" roses is probably the original source of the current high level of infection in New Zealand roses.

Infection with RPF has apparently been building up in roses and rose understock in New Zealand for at least 20 to 30 years.

The disease RCB is of much less common occurrence and appears unlikely at this stage to become epidemic.

Increased awareness of virus and virus-like diseases of roses in New Zealand, the availability of high health propagative material of both understocks and cultivars and the adoption of the mother block system by the nursery industry for production of propagative material, will in the future, reduce the incidence of these diseases.

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1	II	111	IV	v	VI	VII	VIII	IX	х
Collection	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	Ind 'Shirofugen'	dexing on cucumber	C. quinoa	ELISA test for PNRSV
1	Fragrant Cloud	N. Levin	C8	-	-	G ¹ /5 N ⁰ /5 S.A.	043	¥2	-
2	Dimples	N. Levin	C8	-	-	G ⁰ /5 N ⁹ /5 S.A.	1.00	3	
3	Molly McGredy	N. Levin	A1	Al	A5 B1	G ⁵ ∕5 N ⁵ ∕5 S.D.	+	+	+
4	Molly McGredy	N. Levin	Al	A1	A5 B1	G ⁴ ∕5 N ⁴ ∕5 S.D.	+	+	+
5	Western Sun	N. Levin	A1	A5	A4 B1	G [™] /5 N [™] 5 S.D.	+	+	+
6	Western Sun	N. Levin	A1	-	A3 B1	G ¹ ∕5 N ³ ∕5 S.D.	+	+	+
7	Hartina	N. Levin	Bl	Bl	B 1	G ⁰ /5 N ⁹ /5 S.A.	-	-	-
8	Hartina	N. Levin	Bl	B 1	B1	G1/5 N1/5 S.A.	-	-	-
9	News	N. Levin	B1 C6 C9	B1 C6 C9	B1	G% N% S.A.	-	-	-
10	News	N. Levin	B1 C6 C9	B1 C6 C9	Bl	G% №% S.A.	-	-	-
11	Kronenbourg	N. Levin	-	-	Bl	G ³ ∕ ₅ N ⁹ ∕ ₅ S.A.	-	-	-
12	Western Sun	N. Levin	Al	A3 A5	A3 A5 B1	G ² /5 N ³ /5 S.A.	+	+	+
13	National Trust	N. Levin	C8	-	Bl	G ¹ /5 N ¹ /5 S.A.	-	-	-
14	Show Time	A. Palm North	A3	A3	A3	G ¹ ∕s № ⁵ ⁄s S.D.	+	+	+
15	Fragrant Hour	N. Levin	A7	A7-10	A7 A10	G ⁵ ∕ ₅ № ⁵ ⁄ ₅ S.D.	+	+	+
16	Whisky	N. Levin	в2	B2	B1 B2	G [™] /5 N [™] /5 S.A.	-	-	-
17	Whisky	N. Levin	B2	B2	B1 B2	G ⅓ N% S.A.	-	-	-
18	Whisky	N. Levin	C18	C18	B1	G ³ ∕5 N ⁰ ∕5 S.A.	-		(-)
19	Whisky	N. Levin	B2	В2	B1 B2	G% N% S.A.	-	-	-
20	Honey Favourite	P. Palm North	A5	A5	А5	G ² ∕s N ⁵ ∕s S.D.	+	+	+
21	Honey Favourite	P. Palm North	A3	A3 A5	A3 A5	G∜5 N∛5 S.D.	+	+	+
22	Honey Favourite	P. Palm North	A3	A3 A5	А5	G∛5 N∛5 S.D.	+	+	+
23	Honey Favourite	P. Palm North	A3	A3 A5	A3 A5	G∜5 N⁵∕5 S.D.	+	+	+
24	Kim	P. Palm North	C6	-	-	G ⁵ ∕5 N%5 S.A.	-	5	

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APPENDIX I - SUMMARY OF SAMPLE SOURCE, SYMPTOMS AND INDEXING OF ROSE DISEASE COLLECTION

I	11	111	IV	v	VI	VII	VIII	IX	x
Collectio number	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	Ind 'Shirofugen'	exing on cucumber	C. guinoa	ELISA test for PNRSV
25	Tanestry	P Palm North	47 - 10	47 10	17.10	o ⁴ / ¹⁵ / ²			
26	Vi-	P. Pale North	A7 - 10	A7 - 10	A/ A8	G75 N75 S.D.	+	+	+
27		P. Palm North	C6	-	-	G% N% S.A.	-	-	-
27	Ole	P. Palm North	Bl	B1	B1	G ¹ /s N ⁹ /s S.A.	-	-	-
28	Seven Seas	P. Palm North	C6 C8	-	-	G ² /s N ⁰ /s S.A.	-	-	-
29	Ginger Rogers	P. Palm North	A6	A3 A6 A7 - 10	A2 A6	G% N% S.A.	+	+	+
30	Apricot Nectar	P. Palm North	A3	A3	A3	G ² / ₅ № ⁰ / ₅ S.A.	+	+	+
31	Silver Lining	P. Palm North	A1	A2 A3	A2 B1	G ⁰ / ₅ N ⁰ / ₅ S.D.	+	+	+
32	Tony Jacklin	P. Palm North	Spray Burn	-	-	G ⁰ / ₅ N ⁰ / ₅ S.A.	-	-	-
33	Unnamed budded	P. Palm North	A7 - 10	A7 A9	A6 A7	G ² /5 № ⁰ /5 S.A.	+	+	+
34	Orange Sensation	P. Palm North	C8	-	-	G% №% S.A.	-	-	-
35	Trio	P. Palm North	C11	C11	-	G ⁴ /5 N ⁵ /5 S.A.	-	-	-
36	Silver Lining	P. Palm North	A4 A5	A4 A5	A4 A5 B1	G∛5 N∜5 S.D.	+	+	+
37	Unnamed budded	P. Palm North	A3	A3	A3	G [™] /5 N [™] /5 S.D.	+	+	+
38	Tony Jacklin	P. Palm North	Spray burn	-	-	G ³ ∕5 N ⁹ ∕5 S.A.	<u>82</u>	-	-
39	Fragrant Cloud	N. Levin	-	-	-	G% N% S.A.	-		-
40	Fragrant Cloud	N. Levin	C18	-	84.0	G ³ /5 N ⁰ /5 S.A.	-	-	_
41	Hartina	N. Levin	-	L.)	-	G% N1/5 S.A.	+	+	+
42	Hartina	N. Levin	-	-	A7 A8	G % N % S.D.	+	+	+ *
43	Anabell	N. Levin	-	A7 - 10	A7 - 10	G% N% S.D.	+	+	+
44	News	N. Levin	-	-	-	G ² /s N ⁰ /s S.A.	-	_	12
45	Peter Frankenfeldt	N. Levin	C16	-	-	G ³ /s N ⁰ /s S.A.	-		_
46	Matangi	N. Levin	-	-	_	$G^2 \neq N^1 \neq S \Rightarrow$		_	-
47	Old Master	N. Levin	-	_	_	C ⁰ 4 N ⁰ /- C A		_	
48	Old Haster	N. Levin	-	-	-	G% N% S.A.	-	Ē	-

1	11	111	IV	v	VI	VII	VIII	IX	х
ct lo er				Symptoms on		Inc	lexing on		ELISA
Colle	Cultivar	Source	Original plant	Progeny	R. multiflora	'Shirofugen'	cucumber	C. guinoa	test for PNRSV
49	Old Master	N. Levin	-	-	-	G ³ ∕5 N ⁹ ∕5 S.A.	-	-	-
50	First Love	A. Hawera	-	A3	A3	G% N⅔ S.A.	+	+	+
51	Dulce Vita	A. Palm North	-	A3	A3	G ^{\$} ∕5 N ^{\$} ∕5 S.D.	+	+	+
52	Clg. Bettina	A. Blenheim	A6	(A3) A7	A7	G ³ ∕ ₅ N ² ∕ ₅ S.D.	+	+	+
53	Pharon	A. Blenheim	A6	A6 A7 - 10	A7 B1	G ³ ∕5 N ² ∕5 S.A.	+	+	+
54	Royal Highness	A. Blenheim	A5	A3 A5	٨٥	G ³ / ₅ N ⁵ / ₅ S.A.	+	+	+
55	Zambra	A. Blenheim	-	A7 - 10	A6	G ³ / ₅ N ⁴ / ₅ S.A.	+	+	+
56	Apricot Silk	A. Blenheim	-	-	-	G ¹ / ₅ N ⁹ / ₅ S.A.	-	<u> 25</u>	-
57	Sancho	N. New Plymouth	Spider mite	-	-	G% N% S.A.	-	-	-
58	Westerland	N. Hastings	A5	A5	A5	G ³ / ₅ N ⁴ / ₅ S.D.	+	+	+
59	Golden Fleece	N. Hastings	C14	-	-	G ¹ / ₅ N ⁹ / ₅ S.A.	-	-	-
60	Peep-o-day	N. Hastings	Spray spotting	-	-	G% N% S.A.	- 6	-	_
61	Double Delight	N. Hastings	Spray spotting) _ ;	-1	G ¹ / ₅ N ⁰ / ₅ S.A.	<u>(</u> -	-	-
62	Tahiti	P. Napier	C9 (varietal)	<u>-</u>)	-	G% N% S.A.	-	-	-
63	Blue Moon	P. Napier	Spray spotting	-	-	G∜5 N%5 S.A.	-	- '	-
64	Louksor	P. Napier	A3	A5	A3	G ² / ₅ N ³ / ₅ S.A.	+	+	+
65	Louksor	P. Napier	A7 A10	A6 A7	A 6	G∛5 N∜5 S.A.	+	-	+
66	Ama	P. Napier	C7	C7	-	G% N% S.A.	-	-	-
67	Mischief	P. Napier	A7 - 10	A7 - 10	A7 A10	G∜s N∜s S.D.	+	-	+
68	Scarlet Queen Elizabeth	P. Napier	C18	C18	-	G∜5 № ² /5 S.A.	-	-	-
69	Queen Elizabeth	P. Napier	-	A6 - 10	A6 - 8 A10	G ³ / ₅ N ² / ₅ S.D.	+	+	+
70	Couvoisier	P. Napier	A6	A6 A7	A7	G ¹ / ₅ N ¹ / ₅ S.D.	+	+	+
71	Rondelay	P. Napier	A6	A7 - 10	A6 A7	G ³ / ₅ N ⁹ / ₅ S.D.	+	+	+
72	Masquerade (ApMV)	P. Taihape	A4	A4	A4	G ¹ /5 N ¹ /5 S.A.	-	-	-

(+ ApMV)

1	II	111	IV	ν	VI	VII	VIII	IX	x
Collection	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	In 'Shirofugen'	dexing on cucumber	C. guinca	ELISA test for PNRSV
73	Not known	P. Taihape	A10	A6 A7 A10	A7	G ⁵ ∕s N ⁵ ∕s S.D.	+	-	+
74	Not known	P. Taihape	C14	C14	-	G⁵⁄s N%s S.A.	-	-	-
75	Not known	P. Taihape	C6	C6	-	G3/5 N% S.A.	-	-	-
76	Not known	P. Taihape	C7	C7	-	G ¹ /5 N ⁹ /5 S.A.	-	- 0	-
77	Freude	A. Hastings	C6 C17	C6 C17	-	G ² /5 N ⁹ /5 S.A.	-	-	-
78	Anabell	N. Levin	B 2	В2	B2	G ¹ /5 N% S.A.	-	-9	-
79	Silver Lining	P. Palm North	A 3 A5	A3 A5	A3 A5	G% N% S.A.	+	+	+
80	Silver Lining	P. Palm North	A5 C6	A5 C6	A5	G% N% S.A.	+	+	+
81	R. multiflora	N. Levin	simazine chlorosis	7	-	G ² /5 N ⁹ /5 S.A.	-	-	-
82	Vol de Nuit	A. Lower Hutt	B2	B2	В2	G% N% S.A.	-	-	-
83	Not known	A. Lower Hutt	A7 - 10	A7 - 10	A7	G ⁴ /s N ⁵ /s S.D.	+	+	+
84	Josephine Bruce	P. Hamilton	A6	A6 A7	A7	NT	+	-	+
85	Command Performance	P. Hamilton	A9 A10	A7 - 10	A7	NT	+	-	+
86	Elizabeth of Glamis	P. Hamilton	C11	C11	-	NT	-	1-1-1-	14
87	Christian Dior	P. Hamilton	C9 C14	-	-	NT	-	-	-
88	Not known	P. Hamilton	С7	C7	-	NT	-	-	-
89	Oueen Elizabeth	P. Auckland	B1 C13	B 1	B1	NT	+	+	+
90	Command Performance	P. Hamilton	B2	B2	B2	NT	-	-	-
91	Grandpa Dickson	P. Auckland	A5	A5	A5	NT	+	-	+
92	Jiminy Cricket	P. Auckland	C6	C6	-	NT	-	-	-
93	Bewitched	P. Auckland	B2 (Hormone damage)	B2	B2	NT	-	-	-
94	Apricot Nectar	P. Auckland	A3	A3	A3	G ² / ₅ N ² / ₅ S.D.	+	-	+
95	Orangeade	P. Auckland	B1 C11	B1	B1	G ³ /s N ⁹ /s S.A.	-	-	-
96	Queen Elizabeth	P. Auckland	-	A7 - 10	А7	G% N ² /s S.D.	+	+	· +

I II	III	IV	v	VI	VII	VIII	IX	x
Cultivar Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	Ind 'Shirofugen'	dexing on cucumber	C. guinoa	ELISA test for PNRSV
97 Josephine Bruce	PDD Auckland	A8	A7 - 9	-	G ⁵ /s N ¹ /s S.D.	+	-	+
98 Frensham	PDD Auckland	A8 B1	A7 - 9 Bl	A6 A7 B1	G ² /5 N ³ /5 S.A.	+	+	+
99 Iceberg	PDD Auckland	A8	A6 - 9	A6	G ² /s N ¹ /s S.A.	+	+	+
00 Super Star	PDD Auckland	A8 B1	A7 - 9 Bl	B1	G ¹ ∕5 N ⁵ ∕5 S.A.	+	+	+
01 Diamond Jubilee	A. Palm North	-	-	-	G ³ /5 N ² /5 S.A.	+	+	+
02 Wee Man	N. Levin	C19	-	(G% N% S.A.	-	-	-
03 Melina	N. Levin	C7	C7 C2	-	G% N% S.A.	-	-	-
04 Not known	Not recorded	A7 - 10	A7 - 10	A7 - 9	G% N 3/5 S.A.	+	+	+
05 Josephine Bruce	Not recorded	C11	C11	B 1	G ¹ /s N ³ /s S.A.	-	-	-
06 Camelot	P. Auckland	-	A6 A7	A6 A7	NT	+	+	+
07 Queen Elizabeth	P. Auckland	C13	A5	A5	NT	+	-	+
08 R gallic a	P. Auckland	C6 C9 C15	C6 C9 C15	B1	NT	<u> </u>	-	-
09 Scarlet Queen Elizabeth	P. Auckland	B1 B2	A7 - 9 B1 B2	A7 B1 B2	NT	+	+	+
10 Bridal Pink	GH Auckland	B2	A7 - 9 B2	A7 B2	NT	+	-	+
ll Bridal Pink	GH Auckland	B 2	A7 B2	A7 B2	NT	+	•	+
2 News	N. Auckland	B1 C11	B1	B1	NT	_	-	-
13 Unnamed seedling	N. Auckland	C11	C11 ·	-	NT	-	<u> </u>	_
14 Unnamed seedling	N. Auckland	C3 C12	C12	-	NT	_	_	-
15 Queen Elizabeth	N. Auckland	B2 C9 C11	B2 C11	B2	NT	+	_	+
16 Not known	N. Auckland	Cl symptomless shoot	-	-	NT	-	-	-
7 Scarlet Queen Elizabeth	N. Hamilton	Bl	A7 - 9 B1	A7 B1	NT	+	+	+
18 Tiki	P. Te Awamutu	A3	-	A3 A5	NT	+	-	+
9 Chivalry	A. Te Awamutu	C11	C11	-	NT	-	-	-
20 Garden Party	P. Te Awamutu	C11	A7 - 10	A7 A8	NT	+	-	+
l Not known	N. Hamilton	A5	A5	A5	NT			

APPENDIX I - SUMMARY OF SAMPLE SOURCE, SYMPTOMS AND INDEXING OF ROSE DISEASE COLLECTION

I	II	111	IV	v	VI	VII	VIII	IX	х
Collectio number	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	In 'Shirofugen'	dexing on cucumber	C. guinoa	ELISA test for PNRSV
122	Not known	P. Te Kuiti	C13 C15	A7 - 10	A6 A7	NT	+	_	+
123	Rosenthal	P. Te Awamutu	С9	-	-	NT	-	-	-
124	Queen Elizabeth	N. Hamilton	B2	A6 B1 B2	A6 A7 B1 B2	NT	+	-	+
125	Duet	P. Te Awamutu	C15	А7	A3 A5 A7	NT	-	+	+
126	Not known	N. Hamilton	Cl symptomless shoot	-	-	NT	+	+	+
127	Fragrant Cloud	N. Hamilton	C17	A7 C17	A7 A10	NT	43	+	+
128	Royal Dane	P. Te Awamutu	C9 C12 C18	-	-	NT	-	-	-
129	Handel	A. Te Awamutu	C15	-	<u></u>	NT	+	-	+
1 30	Unnamed seedling	A. Te Awamutu	C11	-	-	NT	-	-	-
131	Unnamed seedling	N. Auckland	C3 C12	C12	-	NT	-	-	_
132	Command Performance	A. Palm North	C13	-	-	NT	-	_ X	-
133	Deep Secret	N. New Plymouth	C11	-	-	NT	-	-3	-
134	Carla	P. Hawera	B2	B2	В2	NT	-	-	-
135	Not known	P. Hawera	B1	B1	B1	NT	-	-	-
136	Fragrant Cloud	P. Hawera	C9 C12 C18	C9 C12 C18	-	NT	-	_·	-
137	Not known	P. Hawera	B2?	B 2	B2	NT	-	-	-
138	Unnamed budded	P. Palm North	В2	B2	B2	NT	-	-	-
139	Virgo	A. Palm North	-	-	B1	NT	-	-	-
140	Tiki	A. Palm North	B2	A5 B2	B 2	G% N% S.A.	+	+	+
141	Super Star	A. Palm North	B1 B2?	A7 - 10 B1	A7 B1	G ² /5 N ⁹ /5 S.A.	+	+	+
142	Scarlet Queen Elizabeth	A. Palm North	B1 C18	B1 C18	B1 C8	G ² / ₅ N ⁴ / ₅ S.A.	-	-	-
143	Carina	GH Auckland	A5	A3 A5	A5 C8	G ² /s N ³ /s S.A.	+	-	+
144	Bridal Pink	GH Auckland	В2	A3 A5 B2	A3 B2	G ¹ /5 N ⁹ /5 S.A.	+	+	+
145	Bridal Pink	GH Auckland	В2	A3	A3 A5 A8 B2	G ¹ / ₅ N ³ / ₅ S.A.	+	+	+
146	June Bride	P. Auckland	A7 A9	A3 A5	A5 A7 - 9	G75 N75 S.A.	+	-	+

I	11	III	IV	v	VI	VII	VIII	IX	х
Collection	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	Ind 'Shirofugen'	lexing on cucumber	C. guinoa	ELISA test for PNRSV
147	Diamond Jubilee	P. Auckland	A3	A3 A5	A3 A5	G ³ / ₅ N ¹ / ₅ S.A.	+	+	+
148	Diamond Jubilee	P. Auckland	C4 C5	A5	A5	G ¹ /5 N ¹ /5 S.A.	+	+	+
149	Duet	P. Auckland	A5 C11	A5 C11	A3 A5	G ² /5 N ⁹ /5 S.A.	+	+	+
1 50	Tiffany	P. Auckland	C11	C11	-	G 75 N 95 S.A.	-	-	-
151	First Love	P. Auckland	A7 - 10	A3 C2	A3 A7 - 9	G 75 N 75 S.D.	+	-	+
152	First Love	P. Auckland	A3	A3 A5	A3 A5	G ¹ ∕s N ³ ∕s S.D.	+	-	+
153	Bel Ange	P. Auckland	C8	C8	C8	G ² / ₅ N ⁹ / ₅ S.A.	-	-	-
154	Chrysler Imperial	P. Auckland	A7 - 10	A7 - 10	A7 - 10	G% N ⁷ 5 S.A.	+	+	+
155	Grus am Berlin	P. Auckland	A3	A3 A5	A5	G ³ ∕s N ⁷ ∕s S.D.	+	+	+
56	Astree	P. Auckland	A6	A6 - 10	A6 - 9 B1	G 1/5 N 1/5 S.D.	+	+	+
57	Molly McGredy	P. Auckland	A6	A6 - 10	A6 - 9	G ⁵ ∕s N ⁷ ∕s S.D.	+	+	+
58	Grande Amore	A. Cambridge	A5	A5	A5	G ² / ₅ N ² / ₅ S.A.	+	-	+
159	Invitation	A. Cambridge	A3 A5	A5	A3 A5	G ² /5 N ⁵ /5 S.A.	+	+	+
160	Heatwave	A. Cambridge	A3	A3	A2 A3 A5	G ² /5 N ³ /5 S.A.	+	+	+
161	Margaret	A. Cambridge	-	A2 A5 A7-9 B1	A3 A5 B1	G ¹ /5 N ³ /5 S.A.	+	+ .	+
162	Flamenco	A. Cambridge	B1	A2 A3 A5 B1	A 3 A5 B1 C8	G ² /5 N ⁵ /5 S.D.	+	+	+
163	Diamond Jubilee	A. Cambridge	C4 C5 C11	C4 C5 C11	-	G% N ^{2/5} S.A.	-	-	-
164	Red Planet	A. Cambridge	-	A3 A5 A7-10	AS	G ² /s N ⁵ /s S.A.	+	+	+
165	Jan Spek	P. Hamilton	C4 C5 C11	C4 C5 C11	-	G1/5 N1/5 S.A.	-	-	-
166	Josephine Bruce	P. Hamilton	A 5	A5	AS	G ² ∕5 N ³ ∕5 S.A.	+	+	+
167	Gabriel Cutler	P. Te Awamutu	-	A3 A7	AS	G ¹ / ₅ N ⁹ / ₅ S.A.	+	+	+
168	Elizabeth of Glamis	P. Te Awamutu	-	A3 A5 A7	A3 A5 A7	G ³ / ₅ N ⁴ / ₅ S.D.	+	+	+
169	Not known	A. Palm North	Little leaf	-	-	NT	-	-	-
170	Prominent	GH Cambridge	C5 C11	C5 C11	-	G ² /s N ⁵ /s S.A.	-	-	-
171	Sonia	GH Cambridge	C5 C11	C5 C11	-	G ¹ /5 N ¹ /5 S.A.	-)	-	-
172	73/86	N. Auckland	C5 C11	C5 C11	-	G ² /5 N ² /5 S.A.	-	-	-

1	II	111	IV	v	VI	VII	VI11	IX	х
Collection number	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflors	Ind 'Shirofugen'	dexing on cucumber	C. guinca	ELISA test for PNRSV
173	73/86	N. Auckland	C5 C11	C5 C11	-	G ¹ /5 N ² /5 S.A.	-	-	5
174	Harkness AP	N. Feilding	C8	-	-	G3/5 N95 S.A.	-	-	-
175	Diamond Jubilee	P. Palm North	A3	A3 A5	A3 A5	G ⁴ / ₅ N∛5 S.D.	+	+	+
176	Kings Ransom	P. Palm North	A3	A3 A5	A3 A5 B1	G ³ / ₅ N ³ ∕ ₅ S.A.	+	-	+
177	Ideal Home	P. Palm North	C7 C8	C8	C8	G ³ / ₅ N ⁹ / ₅ S.A.	-	-	-
178	Trio	P. Palm North	C5 C11	C5 C11	-	G2/5 N %5 S.A.	-	-	-
179	Ole	P. Palm North	B1	A5 A7 B1	A5 A7 B1 C8	G ³ / ₅ N ³ ∕ ₅ S.D.	+	+	+
180	Mirato	P. Napier	C7	C7	-	G1/5 N %5 S. A.	-	-	-
181	Petula Clark	P. Napier	C8	C8	C8	G1/5 N%5. S.A.	-	-	-
182	Vogue	P. Napier	A7 - 10	A3 A5 A7 - 9	A3 A5 B1	G% N∛s S.D.	-	+	+
183	Rotorua	P. Napier	B1	A3 A5 A7 A10 B1	A3 A5 A7 B1	G ⁵ / ₅ N ³ / ₅ S.A.	+	-	+
184	Scherzo	P. Napier	Spider mite	-	-	G ¹ / ₅ №%5 S.A.	-	-	-
185	Allotria	P. Napier	A6	A6 - 10	A6 - 10	G ³ / ₅ N ³ / ₅ S.A.	+	-	+
186	Fred Howard	P. Napier	A5	A2 A3 A5	A5	G ¹ / ₅ N ⁹ / ₅ S.A.	+	+	+
187	Chicago Peace	P. Napier	-	A3 A5 A8	A7 C8	G4/5 N4/5 S.D.	+	+.	+
88	Diamond Jubilee	P. Napier	C14	C14	-	G ⁰ / ₅ N ⁰ / ₅ S.A.	-	-	-
l 89	Vienna Charm	N. Hastings	C8	C2 C8	- 1	G % N% S.A.	-	-	-
90	Libery Bell	N. Hastings	C11	A6 - 9 C11	A6 A7	G ⁹ / ₅ N ¹ / ₅ S.D.	+	-	+
91	Grande Amore	N. Hastings	C5 C11	C5 C11	-	G ¹ / ₅ N ⁹ / ₅ S.A.	-	-	-
192	Sweet Afton	N. Hastings	A 3 A5	A 3 A 5	-	G∛5 N∜5 S.D.	+	+	+
193	Mde H. Guillot	N. Hastings	C9 C11	C9 C11	_	G∜5 N%5 S.A.	-	-	-
94	Whisky	N. Hastings	B2	A6 - 10 B2	A6 - 10 B2	G ¹ / ₅ N ¹ / ₅ S.A.	+	+	+
195	Natalie Wood	N. Hastings	C11	A7 - 10	A7 - 10	G% N% S.A.	+	+	+
196	Natalie Wood	N. Hastings	C11 C18	A7 - 10	A7 - 10	G ¹ / ₅ N ⁹ / ₅ S.A.	+	+	+
197	Kines Ransom	N. Hastings	-	-	A 5	62/2 N5/2 SD			

1	11	111	IV	v	V 1	VII	VIII	IX	х
Collection number	Cultivar	Source	Original plant	Symptoms on Progeny	R. multiflora	Ind 'Shirofugen'	exing on cucumber	C. guinca	ELISA test for PNRSV
198	Pascali	N. Hastings	-	A7	A5 A7 - 10	G ¹ ∕5 N ⁴ ∕5 S.D.	+	+	+
199	Peter Frankenfeldt	N. Hastings	C9 C11	C9 C11	-	G ¹ / ₅ N ⁹ / ₅ S.A.	-	-	-
200	Dortmund	P. Napier	C12	A6 C12	A7 A8 C14 B1	G% N ⅓ S.A.	+	+	+
201	Sarabande	P. Napier	C11	C11	-	G ¹ / ₅ N ⁹ / ₅ S.A.	-	-	-
202	Curiosity	P. Napier	variegated	variegated	-	G% N% S.A.	-	-	-
203	lceberg	P. Napier	A6	A6 A7	-	G ² /5 N ⁵ /5 S.D.	+	+	+
204	Merry Widow	P. Napier	A7 A10	A6 - 10	A6 A7	G ¹ / ₅ N [*] / ₅ S.D.	+	+	+
205	Interflora	P. Napier	C11	A7 - 10	A7 A8 B1	G% N ⅓ S.A.	+	+	+
206	Buccaneer	P. Napier	C11	A6 - 10	A3 A6 A7	G% N% S.A.	+	+	+
207	Circus	P. Napier	C7 C8	C7	C8	G ¹ / ₅ N ⁹ / ₅ S.A.	-	-	-
208	Virgo	P. Napier	-	A3 A6 A7	A6 A7 B1	G ¹ / ₅ N ³ / ₅ S.A.	+	+	+
209	Garden Party	P. Napier	A6	A6 A7	A6	G ² / ₅ N ⁵ / ₅ S.D.	+	+	+
210	Lady X	P. Napier	A6	A6 - 10	Α7	G ⁴ / ₅ N ⁴ / ₅ S.D	-	+	+
211	Masquerade	P. Napier	A3 A5	A3 A5	A5	G ⁵ ∕5 N ⅔5 S.A.	+	+	+
212	Ilona	GH Hastings	C11	C11 B1	B1	NT	-	-	-
213	Clg Mde Butterfly	N. Hamilton	A3	A3	A3 A5	NT	+	+ .	+
214	Virgo	N. Hamilton	-	A5 C11	A5 A7 - 9 Bl	NT	+	-	+
215	Western Sun	N. Hamilton	-	-	A 3 A5 B1	NT	+	+	+
216	Queen Elizabeth	N. Hamilton	-	-	A5 A7 - 9	NT	+	-	+
217	Sutters Gold	N. Hamilton	-	A2 A7 - 9	A5 A7	NT	+	+	+
218	Scherzo	N. Levin	Spring dwarf?	-	-	NT	-	-	-
2 19	<i>R. chinensis</i> 'Viridiflora'	N. Feilding	Green fl owers	Green flowers	-	NT	-	-	-
220	Command Performance	A. Palm North	C13	-	-	NT	-	-	-
221	Super Star	PDD Auckland	A8 B1	A7 - 9 B1	B1	NT	+	+	+

- III Source: N nursery; P public rose garden; A amateur home garden; GH - greenhouse cut flower grower; PDD - Plant Diseases Division, DSIR.
- IV Symptoms: According to the text, pp5-8, and as recorded from the original source plant at time of sample collection.
- V Symptoms: Observed at some stage on progeny, resulting from budding sample to Rosa multiflora.
- VI Symptoms: Transmitted by grafting and observed at some stage on *R*. multiflora stock tops and/or healthy cultivars used as indicators for rose petal fleck or rose colour break.
- VII Indexing on 'Shirofugen' cherry: G gummosis; N necrosis; results expressed as number of buds showing symptom/number of buds tested. S.A. - shoot alive following spring; S.D. - shoot dead following spring; NT - not tested.
- VIII Indexing on cucumber 'Crystal Apple' positive (+) or negative (-)
 transmission.
- XI Positive (+) or (-) transmission to Chenopodium quinoa
- X Positive (+) or negative (-) ELISA tests for prunus necrotic ringspot virus.

KEY