COMPARATIVE EPIDEMIOLOGY OF THE PERSISTENTLY TRANSMITTED SCRLV AND THE NON-PERSISTENTLY TRANSMITTED BYMV, AND DEVELOPMENT OF MOLECULAR HYBRIDIZATION ANALYSIS AS A DIAGNOSTIC METHOD FOR SCRLV.

by

Kithsiri Wimal Jayasena B.Sc.(Hon.) Cey.

Department of Plant Pathology,

Waite Agricultural Research Institute,

The University of Adelaide

South Australia

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SUMMARY

Comparisons were made simultaneously of the spread of the non-persistently transmitted bean yellow mosaic virus (BYMV) and the persistently transmitted subterranean clover red leaf virus (SCRLV) in field plots of Vicia faba L. minor and Trifolium subterraneum L. The study was made in a Mediterranean environment.

Spread from a primary source was mapped following the artificial introduction of virus alone, or virus with vector at the centre of the plots. A gradient originated from the source of BYMV placed centrally in the plots and its shape was independent of whether or not the source was colonized with the vector aphid species Aphis craccivora Koch. and Aulacorthum solani (kltb.). By contrast, SCRLV spread from the source only when plants were also artificially infested with the vector A.solani (kltb.). This suggests that the spread of SCRLV is dependent on vector colonization. The gradient of infection observed for BYMV and SCRLV in the control plots where neither virus nor vector were provided centrally indicated that both viruses spread into the plots from an outside source, presumably from the nearby plots.

An attempt was made to evaluate the importance of secondary spread of both viruses by assessing the degree of clumping of infected plants that occurred outside the primary sites of virus introduction. With BYMV, clumping of infected plants occurred whether virus was introduced with its vector or not as well as in control plots. With SCRLV clumping occurred only when the vectors were introduced on virus source

plants at the beginning of the experiment.

The patterns of distribution of both viruses in the plots were examined using ordinary run analysis (Madden et al., 1982). With BYMV, when the virus source was provided either with or without vectors, the distribution of infected plants was nonrandom. This suggests that BYMV was spreading in the experimental plots from plant to plant. The distribution of SCRLV was nonrandom where the virus source was provided centrally with the vector but it was random when the SCRLV source was provided centrally without the vector. Such random distribution indicates that virus was not spreading from plant to plant and is consistent with the virus coming from an outside source.

In T.subterraneum L. plots similar gradients of infection were observed when a virus source was introduced with vectors at the centre. When virus and vector were not introduced at the centre (control plots) the observed gradient was from the outside and only on the sides proximal to the plots where both viruses were provided with vectors. Analysis of the development of clumps of adjacent infected plants according to their time of appearance showed that more clumps appeared in the plots where the virus and vector were provided artificially compared with the control plots, and that peak clumping occurred at the time of peak rate of spread of both viruses.

Time of spread of both viruses was determined by exposing trap plants at 4 weekly intervals throughout the 30 month trial period. Both viruses spread in the spring when vectors were flying, but negligible spread of the viruses was observed in the

autumn despite aphid flight activity.

Times of flight of the four main aphid vector species were continuously monitored with yellow water traps. A major spring and a minor autumn flight peak were observed for Aphis craccivora koch., Macrosiphum euphorbiae (Thomas), Aulacorthum solani (kltb.) and Myzus persicae (Sulz.). Analysis of weather data showed that the aphid flights occurred predominently in weeks when the mean weekly temperature was in the range 13 - 17°C. Weekly rainfall above 7 mm per week appeared to affect the flights only when mean weekly temperatures were outside the range 13 - 17°C.

A replicated trial was done in one crop growing season to determine whether treatment with the insecticides Disyston, Metasystox or Malathion, or a barley barrier row, influenced the spread of BYMV or SCRLV from infected to healthy V. faba L. plants. Their effect on the aphid population in the plots was also determined by sampling V. faba L. plants. The results indicate that although the insecticide treatments reduced the aphid population, they did not affect the pattern of spread of either BYMV or SCRLV. However the barley barrier influenced the pattern of movement of BYMV and not SCRLV while no effect was seen on the aphid population. The overall incidence of SCRLV, in the plots receiving Metasystox and Disyston was less than that obtained with the other treatments but no marked difference was observed between treatments for BYMV.

An electronmicroscopic study was made of T.subterraneum L.cv Mt.Barker infected with SCRLV. In thin sections obtained from

phloem of both leaves and stems, small isometric virus-like particles were detected in the transfer cells, but not in xylem or mesophyll cells. Healthy T.subterraneum L. contained no virus-like particles. Virus-like particles similar in size (30.4 nm diameter) and appearance but serologically distinct from those of potato leaf roll virus were also detected in purified preparations from SCRLV infected T.subterraneum L. plants.

Subterraneum clover red leaf virus (SCRLV) was purified from infected Trifolium subterraneum L. plants or Pisum sativum L. by either of two methods, both of which employed cellulase digestion of crude extracts. The PEG (polyethylene glycol) method using infected P.sativum L. plants gave a higher yield of virus (1.34 mg/kg) than the other method (24 µg/kg) where PEG was not used. Only traces of virus were recovered from tissue of infected T.subterraneum L. These virus particles had a bouyant density of 1.31 g/cm^3. in Cs_2SO_4 and A_{260}/A_{280} ratio of 1.5. The relationship of this isolate to the New Zealand isolate of SCRLV was studied with gel diffusion tests and showed a reaction with N.Z. antiserum. In immunosorbent electron microscopy tests, large numbers of particles of the SCRLV Tasmanian isolate became attached to grids coated with antisera prepared to the New Zealand isolate of SCRLV.

Polyacrylamide-urea gel electrophoresis of disrupted virus particles revealed two RNA species and one DNA species. The slow moving RNA and the fast moving RNA had estimated molecular weights of 2.08×10^6 and 1.08×10^6 respectively in these denaturing gels.

H^3 - labelled complementary DNA (cDNA) reverse transcribed from high molecular weight RNA of purified virus was specific for the detection of SCRLV, in that it showed no hybridization with nucleic acids from either healthy plants (T.subterraneum L., Pisum sativum L.) or plants (Physalis floridana Rydb.) infected with the serologically related potato leaf roll virus (PLRV) or nonviruliferous aphid vector Aulacorthum solani (kltb.) but hybridized with homologous RNA and nucleic acids from SCRLV infected plants of two species (T.subterraneum L., P.sativum L.) and viruliferous aphid vector A.solani (kltb.). The cDNA detected SCRLV in individuals and groups of the A.solani (kltb.) and the average virus content was greater than 157 pg per aphid. The enzyme linked immunosorbent assay (ELISA) confirmed the results of molecular hybridization analysis (MHA).

STATEMENT

This thesis contains no material which has been accepted for the award of any degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material published or written by any other person, except where due reference is made in the text.

I give my consents to the thesis being made available for photocopying and loan if accepted for the award of the degree.

Signed.

K.W.Jayasena.

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Abbreviations

BCMV	Bean common mosaic virus
BLRV	Bean leaf roll virus
BYDV	Barley yellow dwarf virus
BYMV	Bean yellow mosaic virus
CaMV	Cauliflower mosaic virus
CRLV	Carrot red leaf virus
LMV	Lettuce mosaic virus
LNYV	Lettuce necrotic yellows virus
PLRV	Potato leaf roll virus
PeLRV	Pea leaf roll virus
PMV	Pea mosaic virus
PWV	Passion fruit woodiness virus
PVY	Potato virus Y
RCNMV	Red clover necrotic mosaic virus
SCMV	Sugarcane mosaic virus
SCRLV	Subterranean clover red leaf virus
SCSV	Subterranean clover stunt virus
SDV	Soybean dwarf virus
SPMV	Sweat pea mosaic virus
VTMoV	Velvet tobacco mottle virus

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CHAPTER I

GENERAL INTRODUCTION

1.1 Properties of non-persistently and persistently borne aphid transmitted viruses

Watson and Roberts (1939), Day and Irzykiewicz (1954) and Sylvester (1958) have described the mode of virus acquisition, retention and inoculation by aphids and classified aphid-borne viruses into the three categories, non-persistent, semi-persistent and persistent. Black (1959) introduced the word circulative for the persistent viruses to emphasize the route of virus transport, and Kennedy et al. (1962) suggested the inclusion of all non-persistent and semi-persistent viruses into one group and used the term stylet-borne. These definitions are based either on the period of retention of the virus in the aphid, or the site of retention or route of transport of the virus inside the aphid (Pirone and Harris, 1977). Since there is evidence that non-persistently or semi-persistently transmitted viruses are non-circulative within the aphids (Harris, 1977) the term non-circulative has been suggested by Harris (1977) as an alternative to the term stylet-borne. Garrett (1971) concluded that non-persistent viruses are carried in the cibarium and transmission is effected by the injection of the viruses from the cibarium. Non-persistently transmitted viruses can be acquired and inoculated within a few minutes and survive in the vector for less than one hour (Watson and Plumb, 1972). Loebenstein and Raccah (1980) summarized the characteristics of non-persistent virus transmission as;

short acquisition and inoculation periods, no incubation period follows acquisition, virus is lost after short feeding periods, aphids lose infectivity after moulting, there is low virus - vector specificity, viruses are acquired from the epidermal cells of infected plants.

Some potyviruses and cauliflower mosaic virus require a helper component for transmission (Kassanis and Govier, 1971; Govier and Kassanis, 1974; Lung and Pirone, 1974; Paguio and Kuhn, 1976; Simons, 1976; Pirone, 1977; Pirone, 1981). This helper component might act by binding the virus to sites in the aphid (Govier et al., 1977; Lopez-Abella et al., 1981) thus rendering it transmissible.

Persistent viruses are ingested by aphids, circulated through the hemolymph to the salivary glands, and later injected into plant cells with salivary secretions during feeding (Black, 1959; Sylvester, 1980; Gildow and Rochow, 1980). Characteristics of persistently borne aphid-transmitted viruses were summarized by Sylvester (1969a; 1980); high level of virus vector specificity, a latent period between the acquisition and inoculation feeds, long retention of the virus in the vector and retention of infectivity through a moult. Some persistently transmitted viruses multiply in their vectors (propagative viruses) (Stegwee and Ponsen, 1958; Sylvester and Richardson, 1966; O'Loughlin and Chambers, 1967; Sylvester, 1969b; Peters and Black, 1970; Sylvester et al., 1974), and others do not (Mueller and Rochow, 1961; Nault et al., 1964; Sylvester 1969b; Paliwal and Sinha, 1970; Kellock, 1971; Clarke and Bath, 1973). Potato leafroll virus is persistently transmitted (Oortwijnbotjes

1920). The early reports that it multiplies in its vector (Stegwee and Ponsen, 1958) have recently been disproved by Eskandari et al. (1979) when they found that it does not multiply in the vector Myzus persicae.

Semi-persistent viruses are characterized by having no latent period in the vector, the virus is held in the aphid for a longer period than with non-persistent viruses (Sylvester, 1962).

Bean yellow mosaic virus (BYMV) and subterranean clover red leaf (SCRLV) which are the subject of this thesis are transmitted by aphids non-persistently (Pirone and Harris, 1977) and persistently (Kellock, 1971; Wilson and Close, 1973), respectively.

1.2 The relationship of the Tasmanian isolate of subterranean clover red leaf virus (SCRLV-T) to the luteovirus group

The luteovirus group comprises viruses which cause yellowing symptoms in infected plants (Fenner, 1976). The first well characterized member of the group was barley yellow dwarf virus (BYDV) and other members of this virus group have properties similar to the type member. The properties characteristic of this group, have been summarised by Matthews (1979) as; virus particles are isometric and about 25 nm in diameter, the nucleic acid is a positive sense single-stranded (ss) RNA, molecular weight (MW) = 2.0×10^6, coat protein is a single polypeptide with MW= 24×10^3, virus particle sedimentation coefficient ($s_{20}W$) is 115-118S, virus particles are strongly immunogenic, and most members are

serologically related, virus particles appear to be confined to the phloem tissue of infected plants, the viruses are not transmitted by mechanical inoculation, they are circulative and transmitted persistently by specific vectors.

Subterranean clover red leaf virus (SCRLV) has been tentatively assigned to the luteovirus group (Matthews, 1979; Rochow and Duffus, 1981), on the basis of its symptomatology and vector relationships. Ashby et al. (1979) suggested that SCRLV may be related to soybean dwarf virus (SDV) and filaree red leaf virus which have been classified respectively as a member and a possible member of the luteovirus group (Matthews, 1979; Rochow and Duffus, 1981). Jayasena et al. (1981) suggested that on the evidence of particle morphology, cytopathology, and biological properties, SCRLV is a member of the luteovirus group (see Chapter 6).

1.3 The relationship between BYMV-S and other members of the potyvirus group

The term potyvirus has been derived as an abbreviation of the name of its type member, potato virus Y. This group comprises 34 characterized members and some 38 possible members (Matthews, 1979). The main characteristic properties of this group, as summarized by Matthews (1979) are; particles are flexuous and rod-shaped, 680-900 nm in length by 11 nm in diameter, with helical symmetry and pitch c. 3.4 nm, coat protein is a single polypeptide of molecular weight (MW) = 32-34 x 10^3, genomic RNA is single stranded with a MW of 3.0-3.5 x 10^6 and is

5% by weight of the particle, particles have a sedimentation coefficient ($s_{20}W$) 150-160S and a buoyant density of 1.31 g/cm^3 in CsCl, serological relationships exist between some members with different biological properties, individual viruses have a narrow host range, transmission is by mechanical inoculation, by aphids in a non-persistent manner, and some are transmitted through seed. Cylindrical inclusions which appear as pinwheels in cross section are induced in the cytoplasm. They consist of a protein, coded for by viral RNA which is unrelated to either host protein or viral coat protein (Dougherty and Hiebert, 1980).

Bean yellow mosaic virus (BYMV) particles are c. 750 nm long, c. 15 nm in diameter (Brandes, 1964) with a pitch of 3.4 nm. Inclusions are induced in infected cells which resemble those produced by other viruses of the potyvirus group (Bos, 1970). BYMV was assigned to the potato virus Y group by Brandes (1964), Harrison et al. (1971) and Matthews (1979).

Edwardson (1974) and Edwardson and Christie (1978) attempted to divide the potyviruses into 3 sub-groups, since cytoplasmic inclusions appeared to be characteristic of specific viruses. Moghal and Francki (1981) proposed that inclusion bodies could be used to divide potyviruses into only two sub-groups. In one sub-group curved pinwheels with scrolls were induced in plant cells infected with BCMV, PWV, PVY and SCMV and in the other sub-group pinwheels with laminated aggregates were induced by BYMV, PMV, SPMV and LMV.

The S isolate of BYMV used here is a distinct member of the BYMV group (Randles et al., 1980) on the basis of host range, serology, mean particle length (Edwardson, 1974), the type of inclusion bodies induced in both cytoplasm and nuclei (Christie and Edwardson, 1977) and the amino acid composition of its coat proteins. On the basis of molecular hybridization assay (MHA) S was again shown to be a strain of BYMV (Abu-Samah and Randles, 1981).

1.4 Economic importance of bean yellow mosaic virus (BYMV)

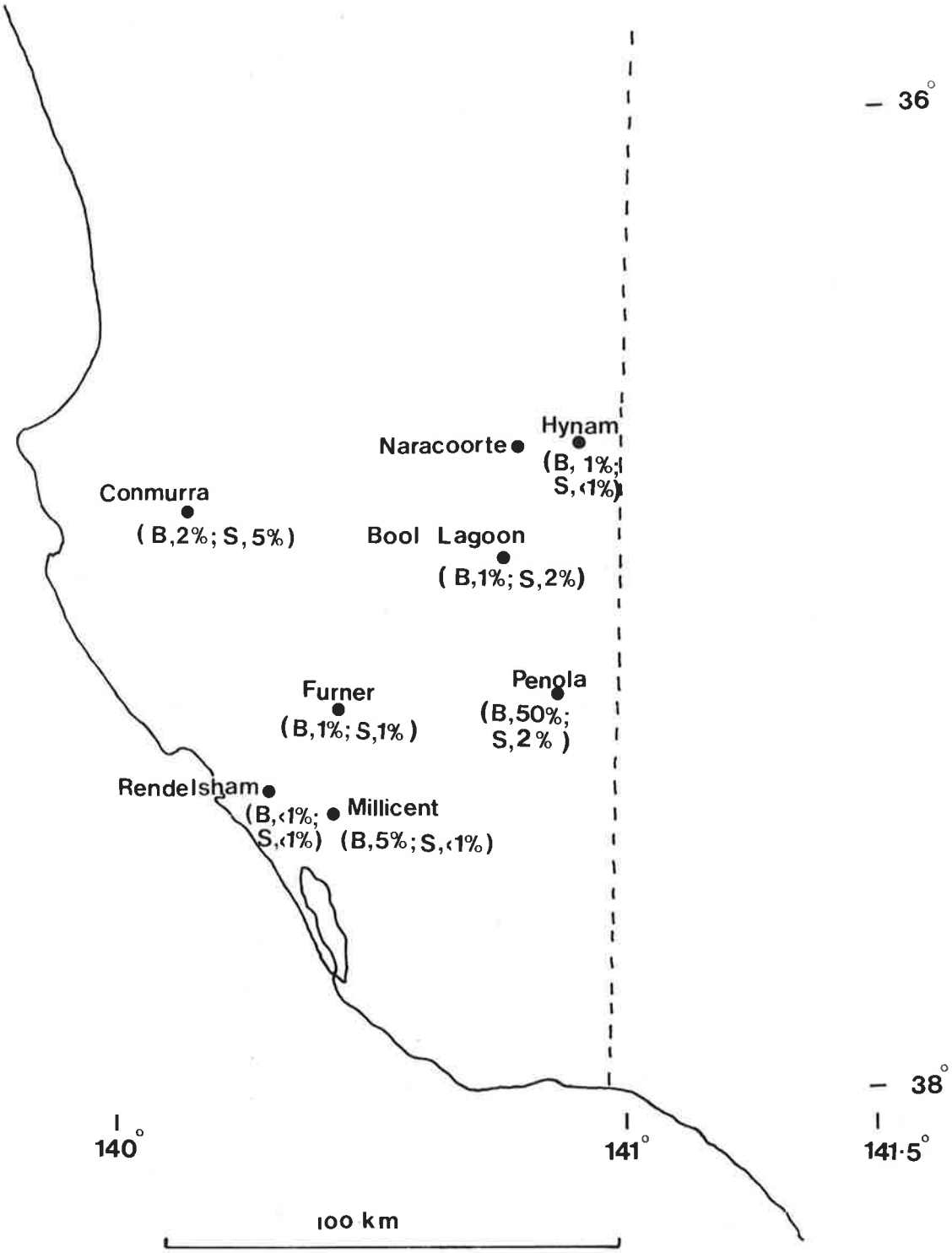
BYMV was first described by Pierce (1934). Bos (1970) and Hollings and Brunt (1981) have summarised the physical, chemical and biological properties of BYMV. The disease is widespread throughout the world where legumes are grown and cause losses of seed in V.faba L.

BYMV is reported from most parts of Australia but was not recognized as an important disease in South Australia until after 1975 when broad bean and tick bean (V.faba L. major and V.faba L. minor) were grown more extensively for stockfeed and commercial seed. In 1978 the incidence of BYMV in V.faba L. crops in the south-east of South Australia, was estimated to be between 10% and 85% (Randles, pers. com.) and between 1% and 50% in 1979 (Fig. 1), based on symptoms.

1.5 Economic importance of subterranean clover red leaf virus (SCRLV)

SCRLV was first reported in Australia (Anon, 1968) and appears to be confined to Australia (Kellock, 1971; Johnstone, 1978) and New Zealand (Wilson and Close, 1973). Work in New

Fig. 1 The areas where V.faba L. crops were surveyed and sampled in the south-east of South Australia in late October 1979. Data in parenthesis shows percent plants infected with BYMV (B) and SCRLV(S) at the survey time.


– 36°
Hynam
(B, 1%;
S,<1%)
Naracoorte
Conmurra
(B,2%; S,5%)
Bool Lagoon
(B,1%; S,2%)
Penola
(B,50%;
S,2%)
Furner
(B,1%; S,1%)
Rendelsham
(B,<1%;
S,<1%)
Millicent
(B,5%; S,<1%)
– 38°
140°
141°
141·5°
100 km

Zealand (Toh, 1973; Wilson and Close, 1973; Ashby *et al.*, 1976; Teh, 1978), Victoria (Kellock, 1971) and Tasmania (Johnstone and Rapley, 1979; 1981) has demonstrated its vector specificity, transmission, host range, effect on yield and the effect of aphicides on disease control. Irrigated ungrazed pastures which had been kept aside for hay and seed production (Anon, 1968) became unproductive as a result of SCRLV infection. SCRLV infection is also thought to be associated with infertility in sheep due to high levels of oestrogenic compounds present in SCRLV infected *T.subterraneum* L. plants (Johnstone, pers. com).

Infected *V.faba* L. shows yellowing and leaf rolling symptoms (Wilson and Close, 1973; Teh, 1978; Johnston, 1978), and yield losses in *V.faba* L. can reach 90% (Johnston and Rapley, 1979). Johnstone (1978) reported that in Tasmania, the seed yield of *V.faba* L. in commercial crops was reduced by SCRLV infection to less than an average of 2000 kg/ha over eleven years. This has led to the reduction of the area planted.

SCRLV was first recognized in South Australia in 1975 in *Trifolium subterraneum* L. (Randles, pers. com.) and now appears to be widely distributed. During the field survey in the south east of South Australia in 1978 the incidence of symptoms in *V.faba* L. was up to 20% (Randles, pers. com.). In 1979 the incidence was between 1% and 5% (Fig. 1).

1.6 Pattern of virus spread and distribution

The survival of plant viruses depends upon an effective means of transmission (Andrewes, 1965). To have an effective means of spread it is important to ensure that the number of infective plants does not fall so low that transmission to other susceptible plants becomes unlikely. The critical level for virus survival depends on the effectiveness of the tranmission process, number and distribution of host plants, their susceptibility, size, longevity, and potency as sources of inoculum (Thresh, 1978). Plant viruses are transmitted by a range of agents (Matthews, 1979). For example, biology, feeding behaviour and worldwide distribution of aphids make them ideally suited for transmitting plant viruses (Harris, 1980).

Sometimes information on the vector involved in field transmission and the source of inoculum can be obtained by studying the distribution of diseased plants. As Thresh (1974) stated, the rate of virus spread between plants varies according to the type of virus, crop, environment and mode of transmission. Spread can be observed within crops and between crops and it is possible to distinguish between both modes of spread (Thresh, 1976). For example, virus spread within crops typically produces very steep gradients of infection, from primary sources within the crop, which arises from the use of infected plants (Doncaster and Gregory, 1948), infected seed (Broadbent et al., 1951); Paguio and Kuhn, 1974); Demski, 1975), weed hosts (Duffus, 1971) or crop residues from previous plantings (Duffus, 1963). Localized distribution has been observed with

viruses transmitted non-persistently by aphids (Doncaster and Gregory, 1948; Broadbent et al., 1951; Broadbent, 1957) and slow moving nematodes (Taylor and Thomas, 1968).

Where annual crops are growing in a restricted growing season whether virus diseases spread into or within crops is a crucial feature of virus epidemiology (Thresh, 1976). Few virus diseases spread exclusively into a crop from outside sources but the best known examples are tomato spotted wilt (Bald, 1937) and lettuce necrotic yellows (Stubbs et al., 1963; Randles and Crowley, 1970; Thresh, 1978; Martin, 1979). Most viruses first spread into crops, then within crops. For example, beet curly top virus which persists in its vector can move long distances to initiate new outbreaks (Thresh, 1978) and later spread within the crops (Clark, 1968). Incoming vectors tend to alight and accumulate on the perimeter of the crop (Doncaster and Gregory, 1948; Orlob and Medler, 1961) commonly on the windward edge (Taylor and Johnson, 1954). Such an "edge" effect has been reported for viruses which are persistently transmitted by aphids (Stubbs et al., 1963; Rochow et al., 1965); by beetles (Croxall et al., 1959); by leaf hoppers (Rose, 1974); by thrips (Bald, 1937; Carter, 1939); and by nematodes (Legg, 1964; Taylor and Thomas, 1968).

Various patterns of distribution of diseased plants occur (Kerr, 1980) such as; random distribution - often caused by seed borne infection and very common for virus diseases of legumes; aggregation - this indicates initial random distribution followed by spread within crop; regular distribution - fits

regular pattern; patch distribution - characteristic for soil borne diseases caused by viruses transmitted by nematodes and fungi; flat gradient - mainly depend on the behaviour of vectors and the gradient due to winged form vectors which transmit viruses persistently; steep gradient - very common on non-persistently transmitted viruses and viruses transmitted by slow moving vectors where the spread is only for a short distance.

The possibility of plants becoming infected from a point source decreases with the increasing distance (percent infection is inversely proportional to the power of distance) and transforming the values for infection and/or distance to produce linear regressions facilitates the comparison between gradients and aids statistical analysis (Thresh, 1976). To obtain a mathematical relationship between the distance and infection, as well as to approach the analysis of dispersal gradients, various equations have been used by Wolfenbarger (1946); Gregory and Read (1949); Gregory (1968); Nelder and Wedderburn (1972); Lambert et al. (1980); Taylor (1980).

From an epidemiological point of view it is critical to identify the type of disease pattern and its spread in the field (Campbell and Pennypacker, 1980). Random patterns of spread suggest that the pathogen is not spreading from plant to plant, conversely aggregation of diseased plants suggests that pathogens spread from plant to plant (Madden et al., 1982). Different techniques for studying the pattern of spread of the pathogen have been considered by

Cochran (1936); Todd (1940); Swed and Eisenhart (1943); van der Plank (1947); Iyer (1948); Freeman (1953); Pielou (1969); Kranz (1974); Gibbons (1976); Converse et al. (1979); Madden et al. (1982).

1.7 Scope of this thesis

An objective of epidemiology is to understand the contribution of host, vector, virus and environment to the distribution and incidence of a virus disease. Because of the complexity of interactions between these factors most work has been limited to studying aspects which directly affect control. Less attention has been directed towards the ecology of the vector. While times of flight of vectors have been obtained by trapping, vector population dynamics as they affect virus spread have been little studied. Such studies have been commenced with LNYV (Martin, 1979; Martin and Randles, 1981), a virus with a relatively simple epidemiology. In contrast, Randles and Crowley (1967) describe a virus-vector-host system for cauliflower mosaic virus in South Australia which is more complicated because of the interaction of several vector species and several plant host species.

Factors influencing the successful spread of aphid-transmitted viruses are the proximity of virus sources and the timing of the peaks of migratory flights of the alate aphids of vector species. The importance of virus sources near or within crops has been demonstrated by Broadbent and Gregory (1948); Duffus (1963); Heathcote and Cockbain (1966); Wallis (1967); Hamptom (1967); Nelson and Tuttle (1969);

Adlerz (1974) and Demski (1975). Correlations have been established between number of winged aphids trapped and spread of persistently transmitted viruses in crops such as potato (Broadbent, 1950; Hille Ris Lambers, 1972; Bacon et al., 1976); sugar beet (Watson and Healy, 1953; Hollings, 1955; Heathcote, 1974), and lettuce (Gonzalez and Rawline, 1969). Other workers have shown that the aphid species most frequently trapped in the field are not necessarily the most important in virus spread (Broadbent et al., 1951; Dickson et al., 1956; Zettler et al., 1967; Gonzalez and Rawlins, 1969). The early migration of aphid vectors (Gill, 1970; Gutierrez et al., 1971) and the ability of viruliferous aphids to undertake long distance flights (Wallin and Loonan, 1971) are also considered as important factors.

In Australia, the epidemiology of non-persistently and persistently transmitted aphid borne viruses has been studied mainly in the cool temperate regions but very little is known of virus epidemiology in the Mediterranean climatic region which has a winter-spring growing season, and a hot dry summer during which annual plants mature and die.

This thesis consists of two main parts. The objective of the work described in the first part of this thesis was to investigate the comparative epidemiology of the non-persistently transmitted BYMV and the persistently transmitted SCRLV in a Mediterranean environment at the same time so as to overcome difficulties in the analysis of data from different sites and times of cropping. The investigations concentrate on: aphid population changes and flight activity

in the area of the trial with special reference to vectors of BYMV and SCRLV; the time of spread of BYMV and SCRLV; the patterns of spread of BYMV and SCRLV in experimental plots in which the sources of virus and vector were manipulated; the effects of controlling the vector on virus spread.

The second part of the thesis describes studies of SCRLV in infected tissue by electronmicroscopy; the purification of SCRLV and its nucleic acid; and synthesis of complementary DNA (cDNA) to SCRLV-RNA.

The results obtained in the first part have been used to describe the time of aphid migration, the time of spread of both BYMV and SCRLV in the experimental area, and the relationship between both viruses and their vectors.

The results obtained in the second part have been used to confirm the view previously based on biological properties (Rochow and Duffus, 1981) that SCRLV is a luteovirus. MHA is shown to have potential in identification of SCRLV in plants and vectors, and thus may have an application to epidemiology.

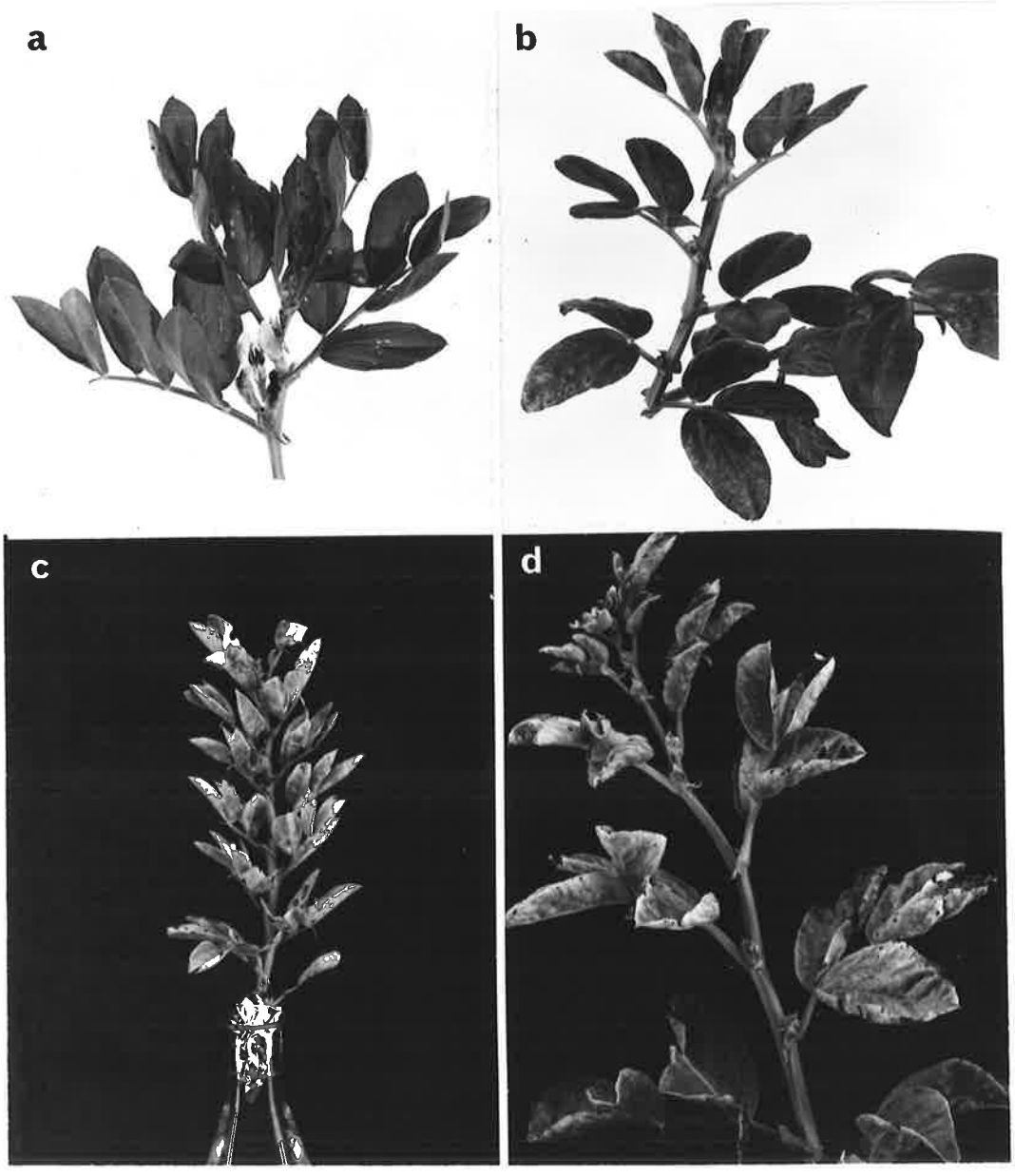
Part of the work described in this thesis has already been published.

(i) Jayasena, K.W., Hatta, T., Francki, R.I.B., and Randles, J.W. (1981). Luteovirus-like particles associated with subterranean clover red leaf virus infection. J. gen. Virol. 57: 205-209.

(ii) Jayasena, K.W. and Randles, J.W. (1984). Patterns of spread of the non-persistently transmitted bean yellow mosaic virus and the persistently transmitted subterranean clover red leaf virus in Vicia faba. Ann. appl. Biol. (In press).

(iii) Jayasena, K.W., Randles, J.W. and Barnett, O.W. (1984). Synthesis of a complementary DNA probe specific for detecting subterranean clover red leaf virus in plants and aphids. J. gen. Virol. 65:00 (In Press).

Fig. 2 Field symptoms of BYMV-S(b), SCRLV-T(c) and double infection with BYMV-S and SCRLV-T (d) on V.faba L. minor line 383A, (a) healthy. Systemic vein banding resulting from BYMV-S infection was detectable in double infections (d).


a
b
c
d

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Maintenance of virus isolates

2.1.1 Bean yellow mosaic virus

A South Australian isolate of bean yellow mosaic virus (BYMV-S) (Randles et al., 1980) was used in all the field experiments. It was maintained in Vicia faba L. cv. Aquadulce, and transferred by mechanical inoculation. Prior to mechanical inoculation, two week old V.faba L. cv. Aquadulce seedlings were kept in darkness for 24 hours, leaves were dusted lightly with 500 mesh carborundum powder and inoculated with infective sap extracted in water. Excess inoculum was washed off with tap water. Plants were maintained in an insect free glasshouse provided with supplementary cooling and heating.

BYMV-S produces distinctive dark green vein banding symptoms on V.faba L. (Randles et al., 1980) (Fig. 2b). Since this isolate is unable to systemically infect Phaseolus vulgaris L. (Randles et al., 1980; Abu-Samah, 1982) its identity was checked regularly by mechanical inoculation to P.vulgaris L.cv. Hawkesbury Wonder; Pisum sativum L.cv. Green feast; V.faba L.cv. Aquadulce; Chenopodium amaranticolor Coste and Reyne and Chenopodium quinoa Willd.

2.1.2 Subterranean clover red leaf virus - Tasmanian isolate

The isolate of subterranean clover red leaf virus came from Tasmania and was provided by Dr.G.R.Johnstone. This isolate was maintained on Trifolium subterraneum L.cv. Mt.Barker or Bacchus Marsh by aphid transfer (see Section 2.3) in which

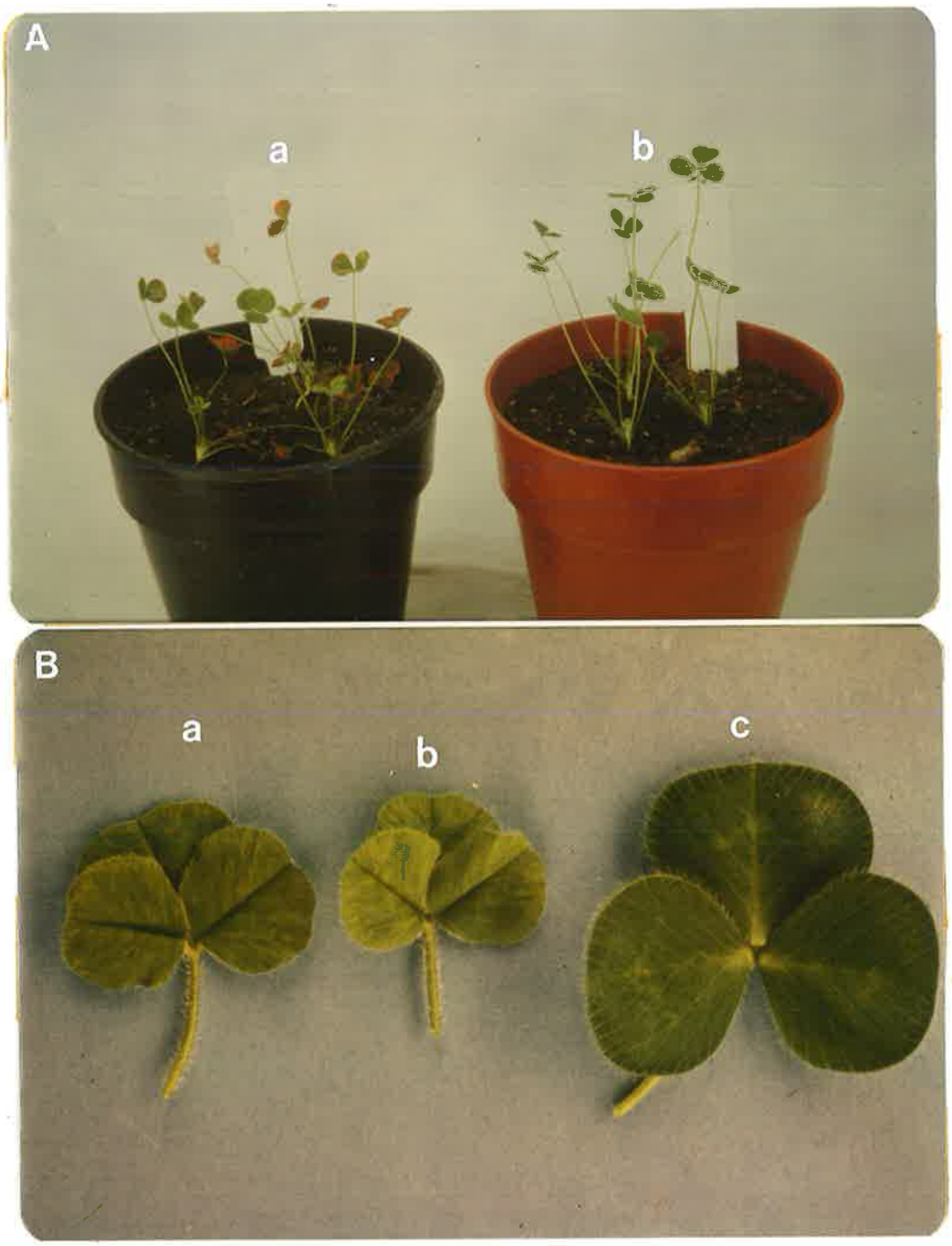
Fig. 3 A - Symptoms of SCRLV-T on T.subterraneum L. cv. Mt.Barker (a), compared with healthy plants (b), 30 days after the time of inoculation

B - Symptoms of BYMV-S infection on T.subterraneum L. cv. Mt.Barker leaves.

a - Mosaic - early stage (3 weeks after inoculation).

b - Yellow and mosaic - later stage (6 weeks after inoculation).

c - healthy.


A
a
b
B
a
b
c

A.solani (kltb.) were fed on diseased plants for 3 days and transferred to virus-free T.subterraneum L. seedlings for an inoculation feed of 3 days. The symptoms produced were similar to those described by Kellock (1971) in that red leaves were induced in T.subterraneum L. cv. Mt.Barker (Fig.3Aa).

2.2 Establishment and maintenance of aphid colonies

2.2.1 Aphis craccivora Koch.

A.craccivora Koch, apterae were collected from V.faba L. plants in the field at the Waite Institute. Two aphids were selected from the group and placed separately on seedlings. These two pots were kept separately in a double mesh-lined cage (18" x 18" x 36" height) in an insect-proof glasshouse compartment. The cultures were maintained on healthy V.faba L. plants.

2.2.2 Aulacorthum solani (Kltb.)

A virus-free colony was established from viruliferous A.sonali (Kltb.) provided by Dr.G.R.Johnstone, Department of Agriculture, Tasmania. SCRLV is transmitted by A.solani (kltb.) and it resembles other members of the leaf roll group of persistently transmitted viruses (Kellock, 1971; Rowhani and Stace Smith, 1979). Because Miyamoto and Miyamoto (1966) reported transovarial transmission of potato leaf roll virus (PLRV) in Myzus persicae (Sulz.) it was considered necessary to avoid this possibility when attempting to establish a virus-free colony of A.solani (kltb.). The procedure adopted for raising this colony was as follows.

Ten apterous aphids were collected from the viruliferous aphid colony received. They were allowed to larviposit separately for 24 hrs. on 10 detached Datura stramonium L. leaves in petri dishes containing moist filter paper. Three first instar nymphs were taken from the progeny of each of the 10 apterous aphids and transferred individually onto healthy T.subterraneum L. cv. Mt.Barker seedlings to allow detection of SCRLV. The 10 apterous maternal aphids were checked by placing them singly onto T.subterraneum L. seedlings. It was observed that none of the nymphs from the progenies transmitted the virus whereas all ten maternal aphids transmitted SCRLV.

A virus-free colony was established from these progenies. Since it is difficult to rear this species in large numbers (G.R.Johnstone, pers. comm.) cultures were maintained on a mixture of Datura stramonium L., Nicotiana clevelandii Gray. Vicia faba L. major cv.Aquadulce and Trifolium subterraneum L. cv. Mt.Barker in double mesh-lined cages as previously described.

2.3 Virus transfer

Apterous A. solani (kltb.) were collected from the virus-free colony with a moistened hair brush. To avoid breakage of stylets, aphids were induced to withdraw their stylets by a gentle touch before transfer to diseased subterranean clover plants which were then covered with a tapered perspex cylinder. Since the acquisition, transmission, availability thresholds, and the latent period after acquisition feeds were 6 hrs., 20 mins., 4 days, and 12 hrs. respectively

(Kellock, 1971) aphids were allowed to feed on diseased plants for 3 to 4 days at room temperature. Groups of 5 were then placed on one trifoliate leaf of healthy 2 weeks old T.subterraneum L. plants. The aphids were then killed by spraying with the insecticide "Metasystox" (DEMETON-S-METHYL) and the plants were kept in the glasshouse.

Leaf cages were used to confine apterae on host plants. Leaf cages were made with 2 mm. thick transparent perspex tubes of 1.5 cm diameter. One cm. wide rings were cut from the tubes and one end of the ring was glued with terrylene net. Balsa-wood discs of 2 cm. diameter were used to support the leaf cages on the other side of the leaf. Cages and the supporting discs were held together by light-weight aluminium hair clips. These cages were used to confine up to 8 apterae.

2.4 Preparation of partially purified virus for electron microscopy

Partially purified virus preparations were negatively stained with either 2% uranyl acetate (UAc) in water, or 2% Na-phosphotungstate (Na-PTA) pH 7.0. Drops of virus suspension were placed on 400 mesh copper grids which had been coated with a carbon stabilized Formvar membranes, and ionized by glow discharge, for a few seconds before application of sample. The grids were drained then, stained for 30 seconds with a drop of UAc or PTA and the excess drained off with filter paper. The air-dried specimens were then examined with the JEM 100 CX electron microscope.

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CHAPTER 3

FIELD STUDIES OF DISEASE GRADIENTS

3.1 INTRODUCTION

Localized spread is mainly characteristic of aphid-borne virus diseases transmitted in a non-persistent manner (Broadbent, 1957; van Hoof, 1979) whereas distant or long-range spread is frequently observed with persistently transmitted viruses (Adams, 1967). It is sometimes possible to distinguish between infection resulting from localized spread, and that resulting from spread over longer distances from outside sources (Thresh, 1976). These two types of spread have a different role in epidemiology because localized spread within crops entails spread within the same crop plant environment, whereas the spread between and into plantings, from outside, involves the invasion of new and sometimes distant places where conditions may be quite different (Thresh, 1976). The amount and extent of spread are further influenced by the size, density and susceptibility of the crop, by temperature and other conditions influencing the abundance and activity of the aphid vectors (Thresh, 1976).

Many procedures adopted to control plant diseases depend mainly on the separation of diseased and healthy plants (Gregory and Read, 1949). To effectively separate diseased and healthy plants, a knowledge of the characteristics of disease spread is essential (Gregory, 1968; Thresh, 1976; Adams, 1978). To reduce the effects of viruses by these and other means it is important to understand characteristics of

the field spread of these viruses and particularly their patterns and rates of spread (Zimmerman and Nitzany, 1964).

This chapter describes a study of the rate and patterns of spread of BYMV, which is transmitted non-persistently by several aphid spp. (Hollings and Brunt, 1981) and SCRLV, which is transmitted persistently (Johnstone, 1978) by a single sp. of aphid.

This study was undertaken for the following reasons. These two viruses were first recognized as important pathogens in South Australia during 1975 when V.faba (L.) was first grown extensively for stockfeed and commercial seed. In 1978, the incidence of BYMV in different crops was between 10 and 85% and of SCRLV was up to 20% (Randles, 1978 ; unpublished data). In 1979, the incidence of BYMV was between 1 and 50% and for SCRLV 1 and 5% (Fig.1). Although the importance of SCRLV was not known in South Australia it has been shown in Tasmania, SCRLV can cause yield losses of 90% in V.faba (L.)(Johnstone and Rapley, 1979). No systematic studies of the epidemiology of either virus have been done in a mediterranean environment such as that found in South Australia. So far only the host range and strain relationships of BYMV have been studied in South Australia and other states of Australia (Randles et al., 1980; Abu Samah and Randles, 1981). For SCRLV data has been obtained for vector specificity (Kellock, 1971; Johnstone,1978), host range (Teh, 1978), yield loss in V.faba (L.) in relation to time of aphid infestation (Johnstone and Rapley, 1979) and effects of chemical control of the aphid vectors (Johnstone and

Rapley, 1981) in a cool temperate climate. There is no specific information on virus spread, to use as a model to study the patterns and rates of spread of non-persistently (BYMV) and persistently (SCRLV) transmitted aphid borne viruses under mediterranean conditions.

An attempt has been made in this chapter to study the epidemiology of these viruses in experimental plots in which both viruses were provided artificially either with or without their vectors. These studies were done in plots of the pasture species T.subterraneum (L.) and the field crop species V.faba (L.)

The selection of winter planting for V.faba (L.) in this experiment was in accordance with the time of cropping for Australia and the climate in the Strathalbyn district resembles that of a typical mediterranean environment (winter-spring growing season followed by a hot dry summer during which annual plants mature and die).

Selection of an area to conduct these field experiments took special account of the isolation of the area from other legume crops, and access to irrigation.

The V.faba L. line 383A and T.subterraneum L.cv. Mt.Barker were selected for these experiments because they were both physiologically suitable to the area, and because each of the viruses could be easily recognized in single (Fig. 2b, 2c, 3a, 3b) or mixed infections (Fig. 2d).

3.2 MATERIALS AND METHODS

3.2.1 Establishment of V.faba L.in the field.

Seeds of V.faba L. line 383A had 100 percent

Fig. 4 a - 10 x 10m plots of V.faba L. separated by bare ground (11.5m) used for the field experiment; ▼ shows the water traps, and white arrow shows the trap plants adjacent to the experimental plots.

b - A single plot showing the central position of the plants infected with BYMV and SCRLV.

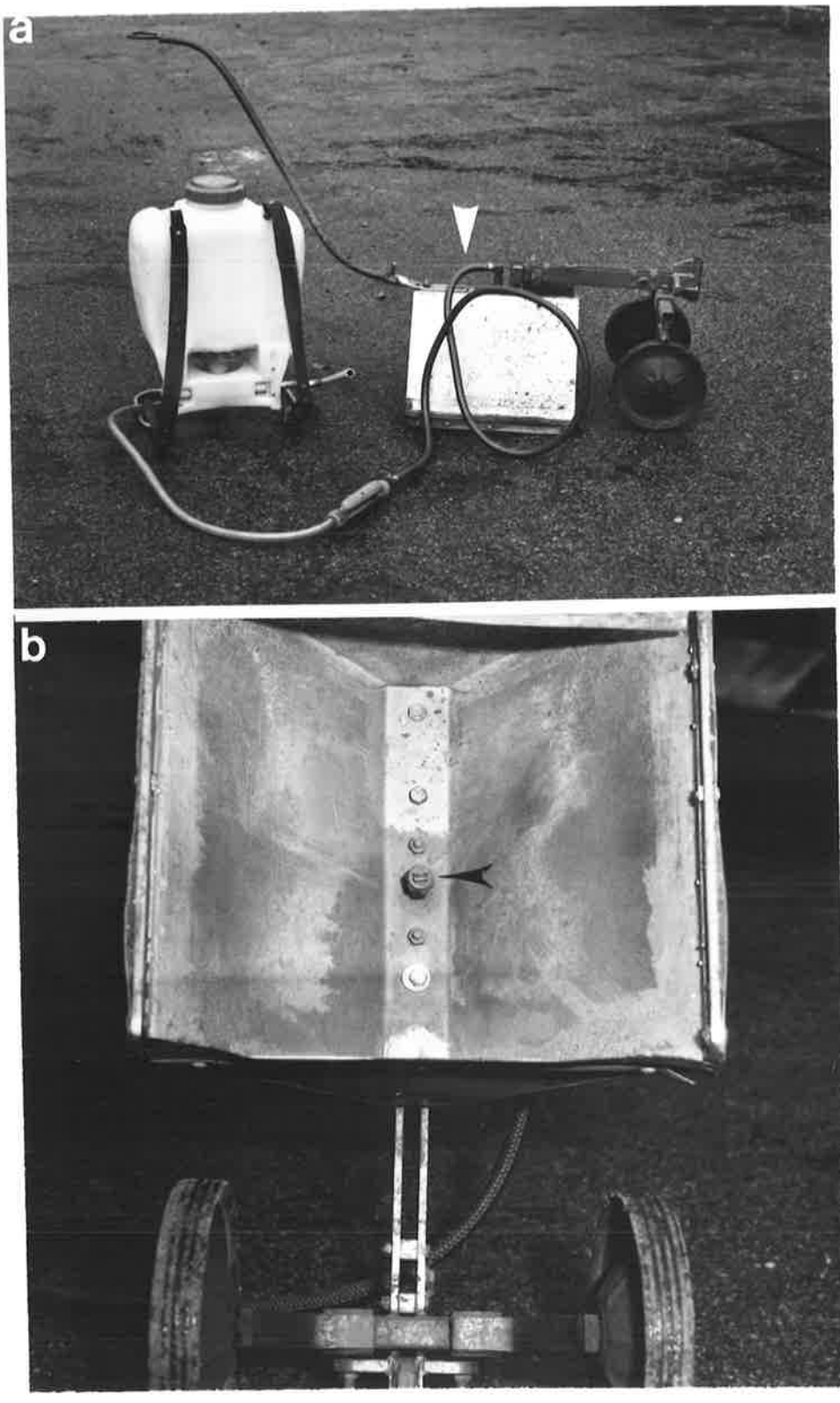

a
b

germination. Prior to planting seed was mixed with Nodulaid Group E (@ 40 g/20kg of seed - Agricultural Laboratories, N.S.W.) and Benlate (@ 24 g/20 kg of seed - methyl 1 - (butylcarbamoyl) benzimidazol-2-carbamate). For the preliminary trial (winter-spring, 1979) seeds were sown on 20th July with a tractor mounted seeder (Connor-Shea Series 2 linkage type drill) at 60 seeds per m^2. The space between plants and rows was 10 and 18 cm. respectively. The plots were 10 x 10 m. spaced 11.5 m. apart. The 3 treatments (BYMV and SCRLV provided with their vectors A.craccivora Koch., A.solani (kltb) respectively (BYMV-Vv, SCRLV-Vv); BYMV and SCRLV provided without vectors (BYMV-V, SCRLV-V); no virus or vector provided (BYMV-C, SCRLV-C)) and 3 replicates were arranged in a latin square design.

Because the trial in 1979 had problems with weed control and irregular spacing, the 1980 trial was modified. Seed was hand-sown @ 3 seeds per hill in rows 50 cm. apart, and within row spacing was 50 cm. (Fig. 4b). Sowing date was 28 May 1980. The lay-out of the experimental plots is shown in Fig. 4a. At the centre of the plot 16 seeds were planted in a square 30 x 30 cm. to be inoculated with both BYMV and SCRLV (Fig. 4b). Thinning or filling of empty spaces was not done in either trial. No fertilizer was applied to the plants. In the 1979 trial weeds were removed manually, but in the 1980 trial they were controlled by manual cultivation and by spraying with "Round up" (a.i. Glyphosate 360 g/L-Monsanto) @ 540 a.i. g/ha. Spray was applied using a plant guard (Fig. 5)

Fig. 5 a - The spray-guard used to protect V.faba L. during herbicide application shown attached to the knapsack spray.

b - Inside view of plant spray guard; arrow shows position of spray nozzle.


a
b

which had been fixed to the nozzle of the sprayer. During the latter part of the growing season (October - November) plants were watered by furrow irrigation. Sprinkler irrigation was not used so as to avoid the possibility of washing aphids from plants.

3.2.2 Establishment of T.subterraneum L. in the field

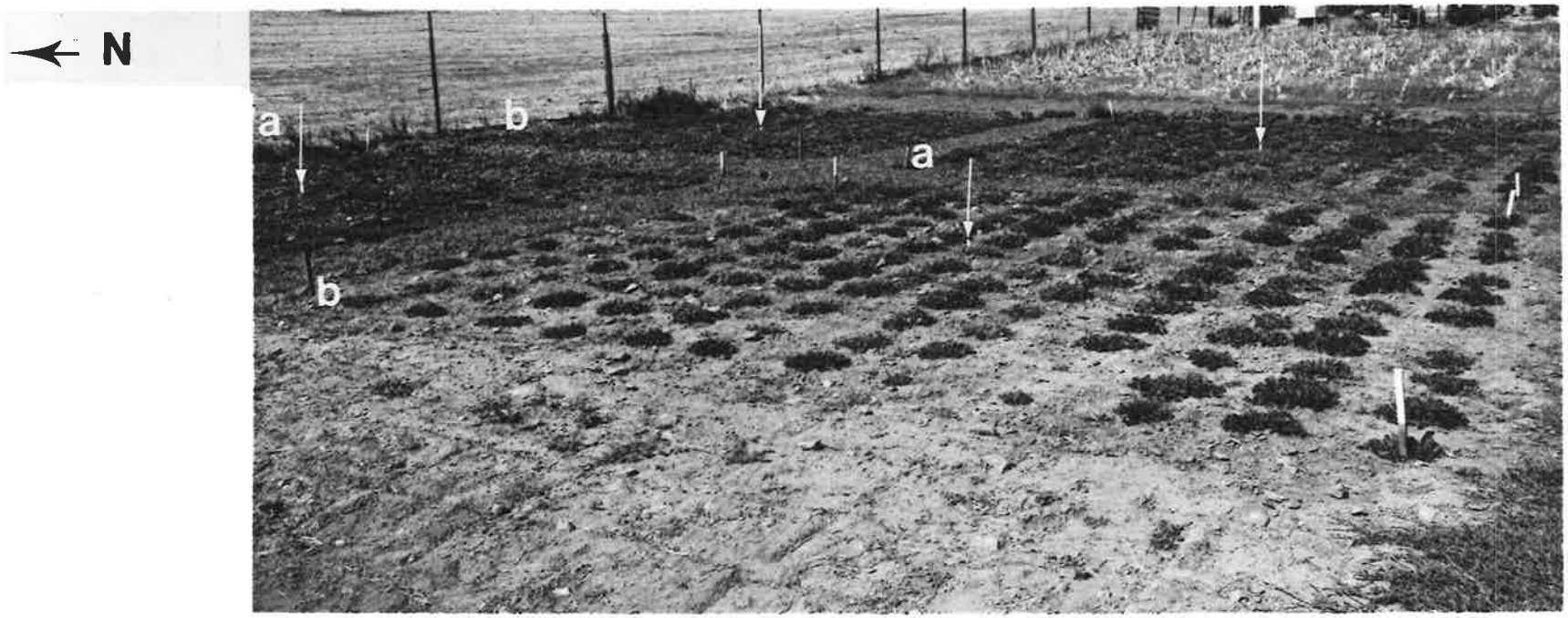
T.subterraneum L. cv. Mt.Barker seeds were planted on 21 January 1980 by hand @ 2 seeds per hill at Alverstoke Orchard, Waite Institute. Four plots each 6 x 6m square were sown with a space of 1 m between plots. The space between plants and rows was 50 cm. Each plot consisted of 13 rows. No fertilizer was added to the plants during the experiment and weeds were removed manually. The plants were watered by furrow irrigation when necessary. The layout of the trial is shown in Fig. 6.

3.2.3 Inoculation of V.faba L. with BYMV and SCRLV and establishment of vectors

In the treatments where the virus and vector were provided (Vv) as well as those where only the virus was provided (V) 8 of the 16 infector plants at the centre of the plot were inoculated mechanically with BYMV-S (see section 2.1.1). Inoculations were done on 11 August 1979 and 26 June 1980, when plants were at the two-leaf stage.

V.faba L. cv. Aquadulce and T.subterraneum L. cv. Mt.Barker plants showing BYMV-S and SCRLV symptoms respectively in the glasshouse were used to raise viruliferous A.craccivora Koch. and A.solani (kltb.) colonies respectively. Apterae of each of A.craccivora Koch. and A.solani (kltb.)

Fig. 6 Plot arrangement of 1980 trial showing 6 x 6m T.subterraneum L. planting with inter-plot spacing of 1 m. Plot a - SCRLV infected A.solani (Kltb.) infested T.subterraneum L. plants placed in a pot at the centre (white arrow). Plot b - BYMV infected, A.craccivora Koch. infested T.subterraneum L. plants placed in a pot at the centre (white arrow).


N
a
b
a
b

were collected from these colonies and placed in vials containing infected stems just before they were taken to the field. In treatment V, 8/16 at the central plants which were not inoculated with BYMV-S were inoculated with SCRLV by caging 50 viruliferous A.solani (kltb.) per plant with leaf cages. In treatment Vv, 100 viruliferous apterae of A.craccivora Koch and A.solani (kltb.) were placed on all 16 central source plants and covered with mesh cages to protect them from natural enemies as well as to allow aphids to acclimatize to the field conditions. The cages measured 45 x 45 x 65 cm. high. The sides and the roof were of fine terylene net and the bottom was open. The cages were held down to the ground by elastic straps between two pegs in the ground. Cages were removed one week after the introduction of aphids to the central infector plants.

In treatment V, leaf cages were removed after one week and sprayed with Pyrethrum (Active constituent 4 g/l Pyrethrins and 16 g/l Piperonyl Butoxide) in 1979 winter-spring trial or with Metasystox (Demeton-S-Methyl) in the 1980 trial. Observations were made subsequently to ensure that the aphids failed to colonize these plants.

On the day the viruliferous aphids were introduced on the central infector plants, some of the infector plants were mechanically inoculated with BYMV-S.

3.2.4 Establishment of viruliferous BYMV and SCRLV aphids in the T.subterraneum L. plots

A number of mature potted T.subterraneum L. cv.

Mt.Barker plants which were showing symptoms of BYMV and SCRLV were used to raise viruliferous A.craccivora Koch. and A.solani (kltb.) respectively. Two days before the infested plants were put in the field (3 February 1980) aphids on the plants were thinned by removing alates and alatiform nymphs of A.craccivora Koch. so that the infested diseased plants (BYMV) had mostly apterous aphids. No alate forms were observed on A.solani (kltb.) infested SCRLV infected plants.

Of the four plots, 2 diagonally opposite plots, at the centre received a pot each containing BYMV infected plants infested with A.craccivora Koch. Similarly the other two plots received SCRLV-infected plants infested with A.solani (kltb.). The pots were sunk in the soil so that the rim of the pots were at soil level (Fig. 6,13). All the plots received either a BYMV infected, A.craccivora Koch. infested pot or a SCRLV infected, A.solani (kltb.) infested pot. The plots containing BYMV infected plants (Fig.6 b) were the control plots for spread of SCRLV without a central virus source. Conversely the SCRLV infected plots (Fig. 6a) were the control plots for BYMV spread without a source.

3.2.5 Survey procedure

In the 1979 trial, plants were tagged at radial intervals of 1 m up to 5 m from the centre of the plot. Forty plants were tagged at random (10 plants per 90^{o} sector) by choosing the numbers from a table of random numbers. These tagged plants were inspected one month after the inoculation and then at fortnightly intervals for symptoms of SCRLV and BYMV.

In the summer-autumn of 1980 trial with T.subterraneum L. and the winter-spring 1980 trial with V.faba L. the survey procedures were modified by inspecting every plant in the plots so that the position of plants showing symptoms could be mapped. Even though there were two to three plants per hill, it was assumed that these together acted as one test site for infection. To minimize the probability of overlooking plants with mild symptoms, and to avoid viewing the highly reflective surface of the plant leaves during the surveying period as Hampton (1967) suggested, the observations were done with the observer standing above the shaded plant.

The percentage of infection at different distances from the inoculated point source were determined from the map (Fig. 7 and 8) by counting the total number of plants and diseased plants at specific distances.

Percentage of infection =

$$100 \times \frac{\text{Number of diseased plants at a given radius or a given row}}{\text{Total number of plants available at that radius or that row}}$$

Results are expressed by radii and rows for 1979, 1980 trials respectively.

3.2.6 Indexing of infected plants from the field

To check the field identification of virus on the basis of symptoms at each survey time 2-3 cuttings from infected plants (V.faba L.) in each of the 9 plots were collected, placed in polythene bags, chilled on ice and brought back to the glasshouse for indexing.

Since SCRLV symptoms produced in V.faba L. resemble

those induced by bean leaf roll virus (BLRV) and subterranean clover stunt virus (SCSV) (Johnstone, 1978), it was necessary to confirm the presence of SCRLV in diseased plants in the experimental plots. M.persicae (Sulz.) (Wilson, 1968) and A.craccivora Koch. (Grylls and Butler, 1956) are reported to be the efficient vectors of BLRV and SCSV respectively. Therefore, to index the diseased plants these two aphid species were also used. M.persicae (Sulz.) and A.craccivora Koch. do not transmit SCRLV in Australia (Kellock, 1971; Johnstone, 1978).

Cuttings which showed yellow cupping were indexed by allowing groups of 40 individuals from virus-free colonies of each species (A.solani (kltb.), M.persicae (Sulz.) and A.craccivora Koch.) an acquisition feed of 3 days then a 3-day inoculation feed on the indicator plant T.subterraneum L. cv. Mt. Barker. Cuttings which showed mosaic symptoms in the field were indexed by mechanical inoculation on V.faba L. cv. Aquadulce C.amaranticolor Coste and Reyne and P.vulgaris L. cv. Hawkesbury Wonder (see sections 2.1.1, 2.1.2).

T.subterraneum L. cv. Mt.Barker plants showing red leaf and mosaic symptoms were indexed as described for V.faba L.

3.3 RESULTS AND DISCUSSION

3.3.1 Confirmation of BYMV and SCRLV in diseased V.faba L. and T.subterraneum L. plants in the experimental plots

SCRLV produces bright to dull yellow interveinal chlorosis in V.faba L. and leaves upward rolled have a harsh feel (Johnstone, 1978) (Fig. 2c). BYMV-S produces distinctive dark-green vein banding symptoms on V.faba L (Fig. 2b)

(Randles et al. 1980; Abu Samah, 1982). Both these viruses can be recognised in single and dual infections (Fig. 2d) respectively.

In T.subterraneum L. SCRLV induces red leaf symptoms (Fig. 3a) (Kellock, 1971; Ashby, 1976), BYMV infected T.subterraneum L. plants produce mosaic and mottling on sytemically infected leaves (Randles et al., 1980; Abu Samah, 1982) (Fig. 3b).

V. faba L. with yellowing and cupping symptoms which were indexed on T.subterraneum L. cv. Mt.Barker were all positive for SCRLV and not for BLRV or SCSV. Aphids which fed upon T.subterraneum L. plants showing red leaf symptoms were able to induce red leaf symptoms on transfer to test seedlings of T.subterraneum L. cv. Mt.Barker. Neither M.persicae (Sulz.) nor A.craccivora Koch transmitted red leaf symptoms to T.subterraneum L. cv. Mt.Barker from the field isolates. Therefore the symptoms observed in V.faba L. and T.subterraneum L. in experimental plots were due only to SCRLV.

The cuttings from the plants V.faba L. and T.subterraneum L. showing mosaic symptoms when indexed to test plants, showed symptoms similar to the BYMV-S isolate described by Randles et al.,(1980);Abu Samah (1982). These results together with the absence of plants showing other symptoms, indicate that SCRLV-T and BYMV-S were probably the only viruses causing disease in the plot.

3.3.2 Confirmation of the presence of BYMV, SCRLV in inoculated source plants

The centre plants which were inoculated artificially (treatments Vv and V) with both BYMV and SCRLV at the beginning of the experiment were indexed at the end of the experiment as described in sections 2.1.1, 2.1.2. These plants were all infected with BYMV and SCRLV.

3.3.3.1 Pattern of spread of BYMV and SCRLV in experimental plots

The results of a preliminary trial to observe the pattern of spread of BYMV and SCRLV at Strathalbyn in winter-spring 1979 in *V.faba* L. are shown in Table 1. In treatment SCRLV-C, only SCRLV was found to spread from the outside towards the inside of the plot. At a radius of 5 m (measured from the centre of the plot) more plants were infected, at the 3 m radius fewer plants were infected with SCRLV and at the 1 m radius no infection was observed. It was concluded that SCRLV was spreading from a neighbouring reservoir. No BYMV spread was observed in treatment BYMV-C (Table 1).

In treatment SCRLV-V, SCRLV had apparently spread outwards from the central point source as well as into the crop from outside (Table 1). At a 1 m radius from the centre more of the plants were infected, then a reduction to the 4 m radius and then an increase at the 5 m radius. A possible explanation for this, some of the apterous *A.solani* (kltb.) introduced to infect the plants at the centre, may have survived the contact insecticide spray (Pyrethrum) and these may have colonized the

Table 1: Mean percentage of plants infected with BYMV and SCRLV at known radii from the centre of the plot at the last 4 survey times, 1979.

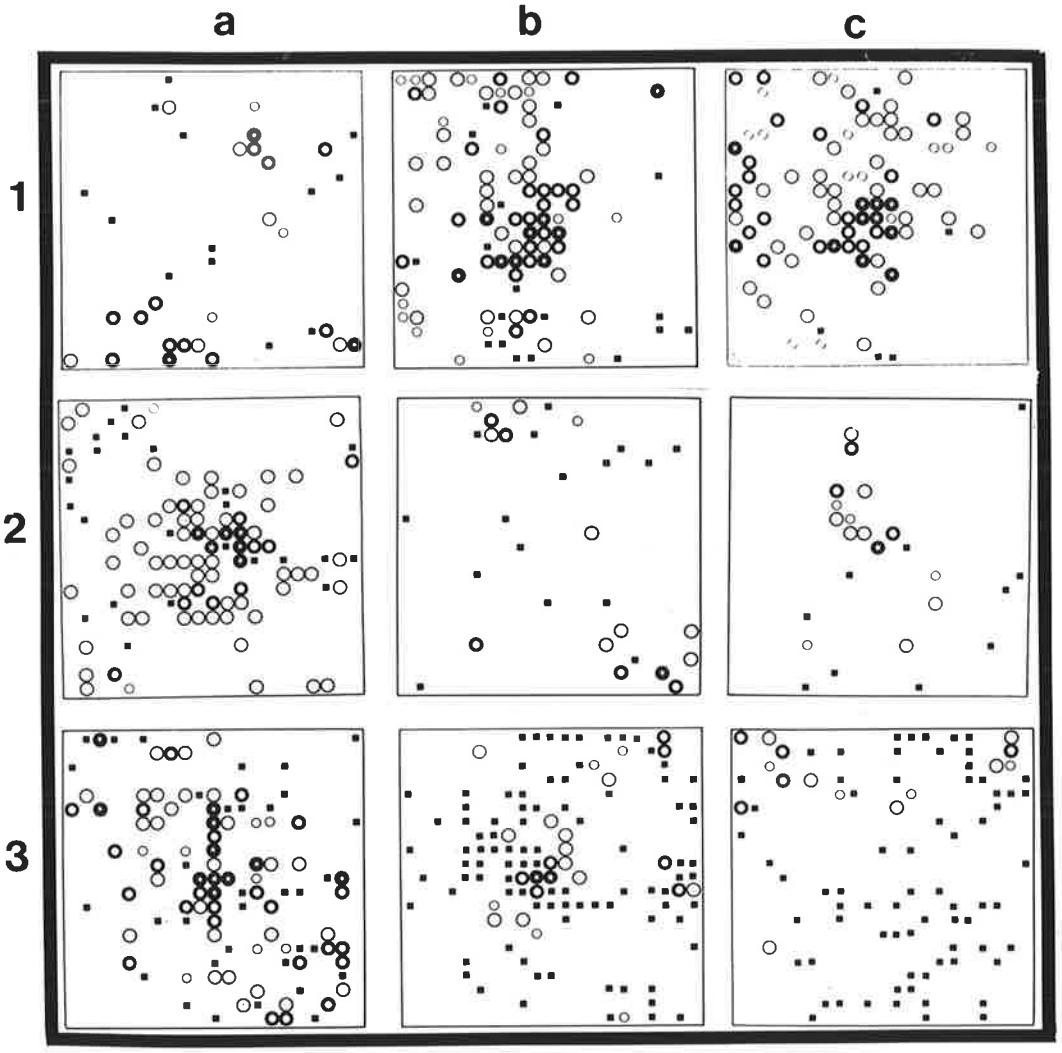
Treatment	Distance (m)	Percent infection BYMV 5/10	BYMV 18/10	BYMV 2/11	BYMV 17/11	SCRLV 5/10	SCRLV 18/10	SCRLV 2/11	SCRLV 17/11
Vv*	1	4.1	6.66	6.66	6.66	0	8.33	8.33	8.33
	2	0.83	1.66	1.66	1.66	0.83	4.16	5.83	5.83
	3	0	0.83	0.83	0.83	0	2.5	2.5	4.16
	4	0	0	0	0	0	1.66	1.66	2.5
	5	0	0	0	0	0	0.83	0.83	0.83
V	1	0.83	1.66	1.66	1.66	0	4.16	5.0	5.0
	2	0	0	0	0	0	0.83	2.5	2.5
	3	0	0	0	0	0	0	2.5	2.5
	4	0	0	0	0	0	0.83	1.66	1.66
	5	0	0	0	0	0	0.83	2.5	2.5
C	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0.83	0.83
	4	0	0	0	0	0	0	1.66	2.5
	5	0	0	0	0	0	0.83	5.0	5.0

Note:- In first two surveys (8/9 and 22/9), I observed no plants infected either with BYMV or SCRLV.

Vv* = Virus + vector; V = Virus only ; C = No virus or vector.

Fig. 7 The distribution of BYMV infected V.faba L. plants at five survey times around a point source (◘) at the centre where BYMV and A.craccivora Koch. were artificially provided (BYMV-Vv) and in the control plots (BYMV-C). First data is shown at the 9 week survey (●) and then at 2 weekly intervals in the series ◘ , ○ , ○ .
Row spacing was 0.55m. Layout shown as in the field, except that between plot spacing was 11m. Position of the missing or dead plants are shown by ■ .

BYMV



Vv–1b, 2c, 3a
V –1c, 2a, 3b
C –1a, 2b, 3c

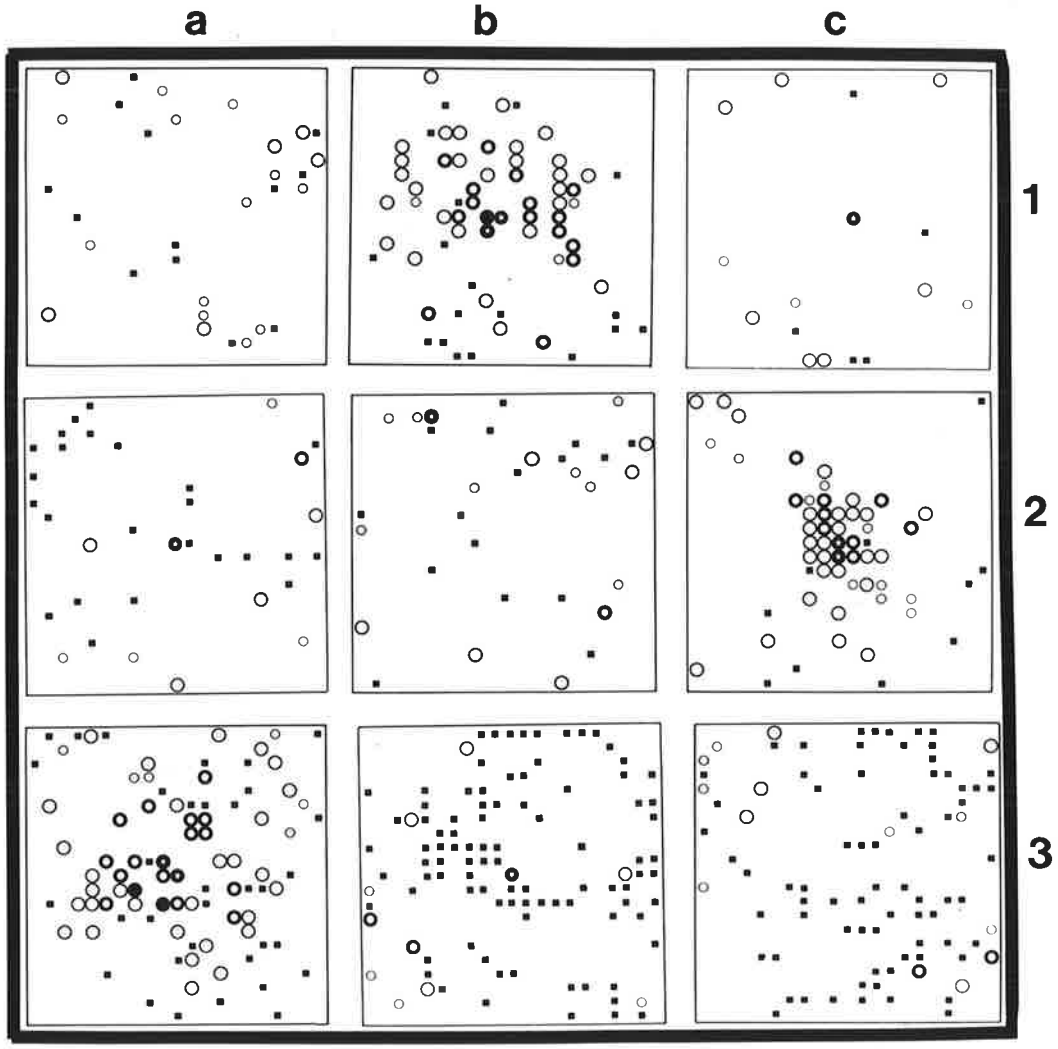
virus source plants and caused virus spread from inside towards the outside of the plot. In treatment BYMV-V, BYMV spread was only observed up to the 1 m radius from the central point source. Presumably BYMV spread would have arisen from nonviruliferous migrating alate aphids acquiring the virus from the central point source in the treatment BYMV-V plots and contributing to the spread of virus.

In treatments BYMV-Vv and SCRLV-Vv a high percentages infection of both BYMV and SCRLV were observed close to the central source with a gradual reduction towards the outside of the plots (Table 1). For both viruses the percentage infection at each 1 m radius was higher than treatments BYMV-V, SCRLV-V, BYMV-C and SCRLV-C, suggesting that viruliferous vectors already present at the centre of the plots were responsible for the spread of both viruses from inside towards the outside of the plots.

From the above experiment it was concluded that both viruses spread, when vectors were not artificially introduced. However, in treatment SCRLV-V, SCRLV spread had occurred even though contact insecticide had been sprayed to kill the aphids at the point source. It seems likely that the insecticide did not kill all the aphids artificially introduced at the centre to infect the plants. Therefore, results in treatment SCRLV-V may be in doubt.

Fig. 8 The distribution of SCRLV infected V.faba L. plants at five surveys around a point source (⊛) at the centre where SCRLV and A.solani (Kltb.) were artificially provided (SCRLV-Vv), where SCRLV only was provided (SCRLV-V) and in the control plots (SCRLV-C). First data is shown at the 7 week survey (●) and then at 2 weekly intervals in the series ●, ◉, ○, ○. Row spacing was 0.55m. Layout shown as in the field, except that between plot spacing was 11m. Position of the missing or dead plants are shown by ■ .

SCRLV



Vv–1b, 2c, 3a
V –1c, 2a, 3b
C –1a, 2b, 3c

3.3.3.2 Pattern of spread of BYMV and SCRLV in V.faba L. in winter-spring 1980

The effects of introducing BYMV and SCRLV with (Vv) or without (V) aphid vectors, into the experimental plots are shown in Fig. 9.

The incidence of BYMV in BYMV-C treatment and SCRLV in SCRLV-C treatment showed a gradient extending inwards from the outside rows (Fig.9). This indicated that an outside source, possibly the adjacent plots, were contributing to a low incidence of both viruses in the outer rows of each plot. In treatments BYMV-C, SCRLV-C the final overall mean incidence for BYMV and SCRLV were 4.3% and 3.9% respectively (Table 2).

In the treatments where BYMV and SCRLV were provided artificially with vectors (BYMV-Vv, SCRLV-Vv) or without the vectors (BYMV-V, SCRLV-V) 100 percent infection was observed at the centre (e.g. all 16 plants at the centre of the plots were infected with BYMV and SCRLV). At the early stages, percent infection of BYMV and SCRLV in treatments BYMV-Vv, BYMV-V and SCRLV-Vv were low close to the infector source, but with time the number of plants infected close to the infector source increased (Fig. 9). This indicates that virus was spreading from the central source plants.

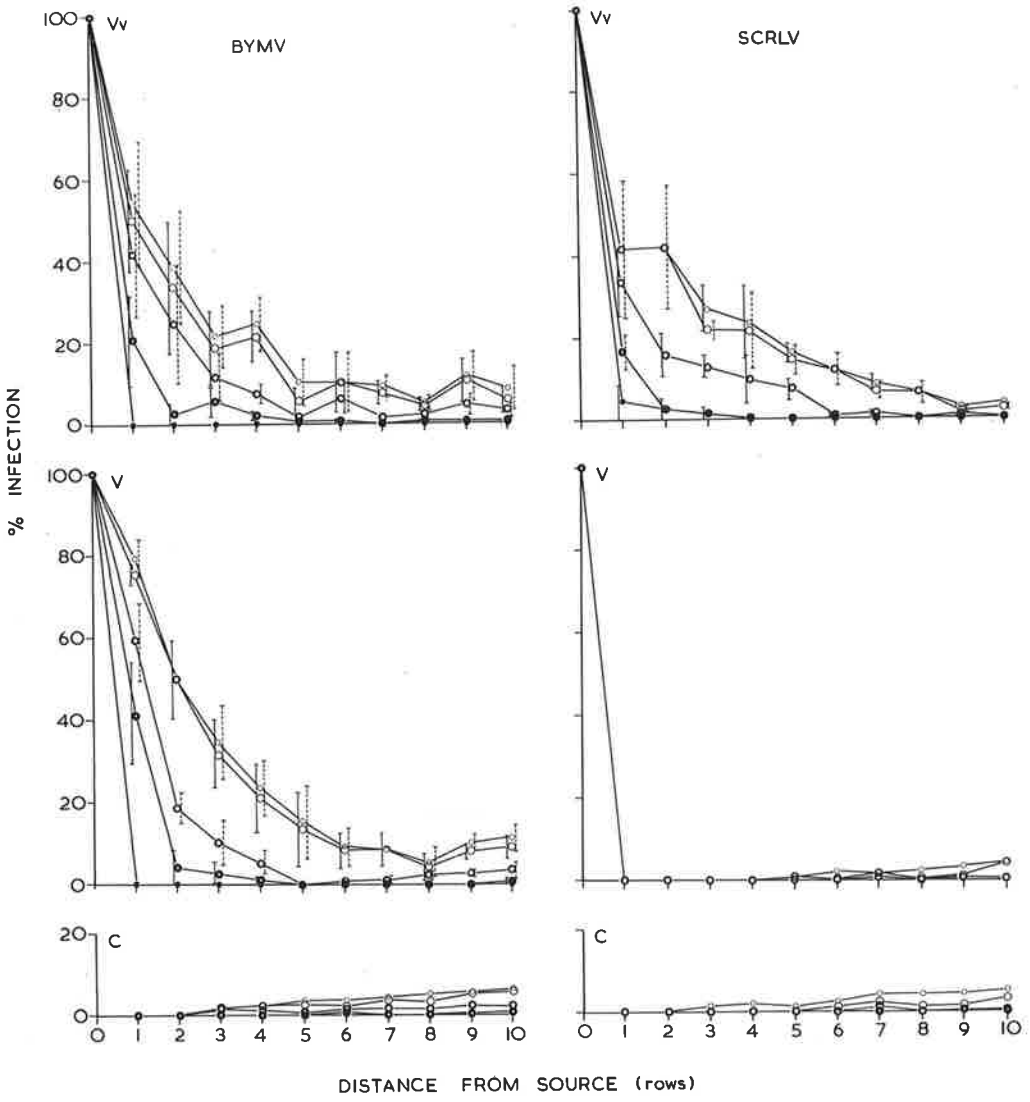
In treatment SCRLV-V, no plants were infected close to the infector source, however there was a gradient from the outside. This suggests that introduction of a virus source alone had no effect on the spread of SCRLV and the spread observed from outside was coming from outside sources, possibly

Table 2. Final incidence of BYMV and SCRLV in V.Faba L. in the 1980 trial

Treatment	Number of plants infected to number of healthy plants in 3 replicates (R_1)						Percent infection (mean)		S.D.		S.E. (±)	
	BYMV			SCRLV			BYMV	SCRLV	BYMV	SCRLV	BYMV	SCRLV
	R_1	R_2	R_3	R_1	R_2	R_3						
Vv*	68/406	77/422	14/430	53/406	47/422	47/430	12.74	11.70	8.25	1.17	4.77	0.67
V	77/416	24/349	80/436	9/416	10/349	10/436	14.57	2.43	6.66	0.37	3.85	0.21
C	15/428	23/425	15/370	16/428	18/425	14/370	4.32	3.91	0.98	0.27	0.56	0.15

*Vv = Virus + vector
V = Virus only
C = Control

Fig. 9 Spread of BYMV and SCRLV in treatments Vv (virus + vector); V (virus alone); and C (no virus, no vector). Results are expressed as the mean incidence (S.E ±) of disease (for 3 replicates) in rows from the central source plants and plotted with increasing time after inoculation of source plants at the centre of the experimental plots. First data is shown at the 7 week survey (●) and then at 2 weekly intervals in the series ●, ○, ○, ○.

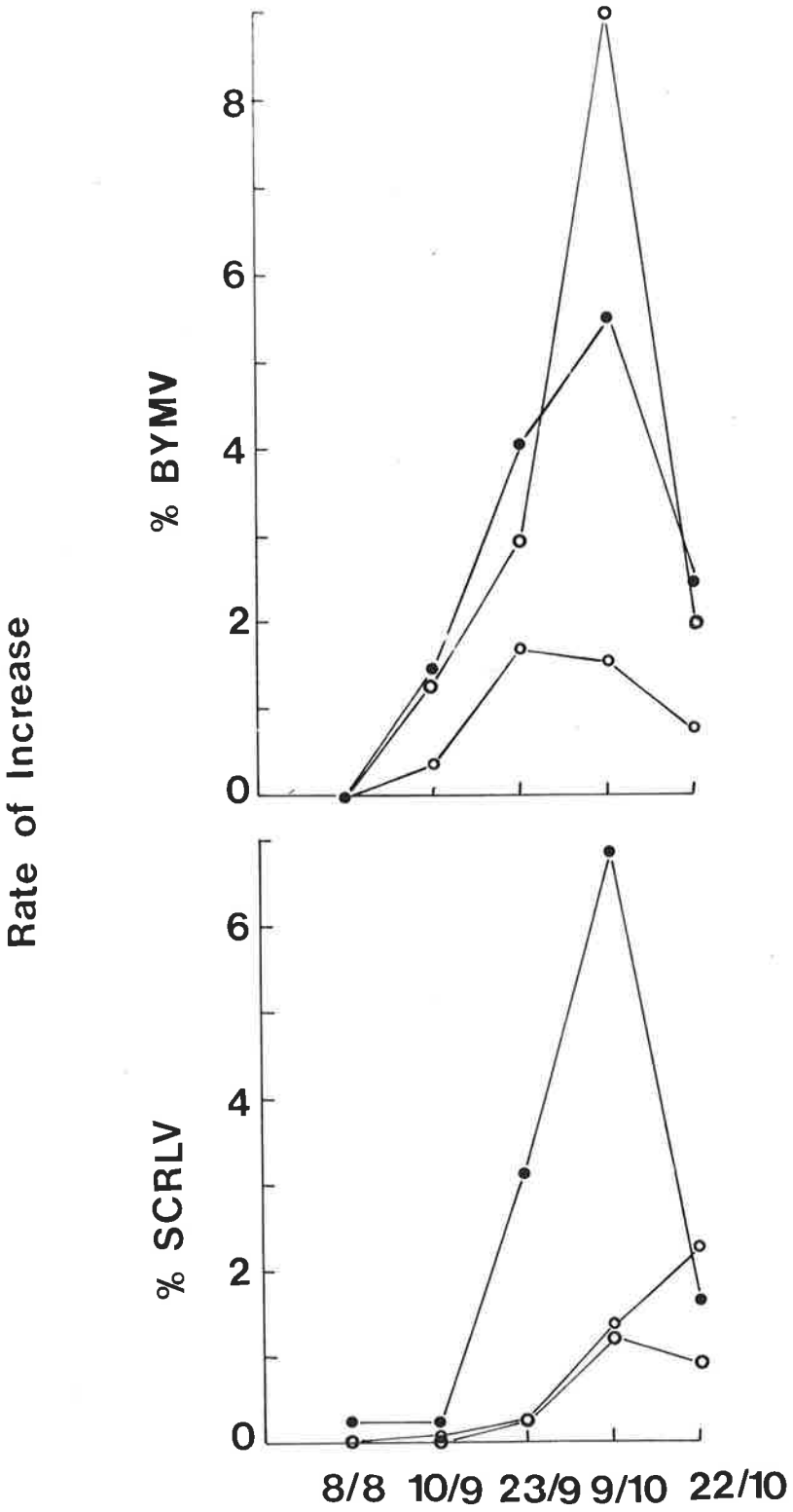

Vv
BYMV
Vv
SCRLV
V
V
C
C
% INFECTION
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
DISTANCE FROM SOURCE (rows)

from the adjacent plots (Fig. 9). Further, there was a close similarity between treatments SCRLV-V and SCRLV-C because overall mean incidence at crop maturity was 2.5% and 3.9% respectively (Table 2).

In treatment BYMV-V, the introduction of a virus source alone led to rapid spread of BYMV which was not seen in treatment SCRLV-V. This gradient was extending outwards from the central infector source plants. Analysis of the aphid trapping records during winter-spring 1980 trial period (Fig.16) revealed that there was alate aphid activity especially of M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. (Kennedy et al., 1962) and A.solani (kltb.) (Johnstone, 1980) the vectors of BYMV, in the experimental plot area. A possible explanation of BYMV spread in treatment BYMV-V may be that some of the nonviruliferous aphids could have acquired the virus from the central infected plants and contributed to the further spread from the centre towards the outside plot. At the time of crop maturity the overall mean incidence of BYMV in treatment BYMV-V was 14.6 (Table 2) and it may be estimated that 10.3% (BYMV incidence at crop maturity in treatment BYMV-V 14.6% - BYMV incidence at crop maturity in treatment BYMV-C 4.3% = 10.3%) was due to the BYMV source provided at the centre.

In treatments BYMV-Vv and SCRLV-Vv, a gradient extended outwards from the central infector plants (Fig.9). Further the gradient extending inwards from the outside rows probably also existed in treatments BYMV-Vv and SCRLV-Vv but it was partly overshadowed by the gradient from the central

Fig. 10 Mean fortnightly percentage rate of increase in incidence of BYMV and SCRLV in treatments Vv (●); V (◎); and C (○) for the trial shown in Fig. 9.


Rate of Increase
% BYMV
0
2
4
6
8
% SCRLV
0
2
4
6
8/8
10/9
23/9
9/10
22/10

infector plants in these plots. For example, the incidence of BYMV, SCRLV in treatments BYMV-Vv and SCRLV-Vv were 12.8% and 11.7% respectively, at the crop maturity (Table 2). Only 8.5% BYMV and 7.8% SCRLV spread was thus contributed from the central infector plants.

3.3.3.3 Rate of spread of BYMV and SCRLV in V.faba L. plots

The rate of spread was calculated from the following formula.

$$\text{Rate of spread (\%)} = \frac{X_2 - X_1}{n - X_1} \times 100$$

where n is the total number of plants in the plots, X_1 is the number of plants infected at the previous interval, and X_2 is the number of plants infected in the current interval.

Fig. 10 shows the relationship between time and rate of virus spread for each treatment in the experimental plots. In treatments BYMV-C and SCRLV-C, BYMV and SCRLV showed peak rates of spread at different times, and these also differed from treatments BYMV-Vv, SCRLV-Vv, BYMV-V and SCRLV-V where both viruses showed peak rates of spread at the same time.

The peaks in rate of spread coincided with periods of vector activity and the times of spread estimated by the use of trap plants (Fig. 15).

3.3.3.4 Statistical analysis

Observed gradients were fitted to a binomial model using a logit transformation (Nelder and Wedderburn, 1972). For the 1979 trial, for treatment BYMV-Vv the regression was negative and

Table 3. Regressions for gradients of BYMV and SCRLV incidence at indicated survey time in treatments BYMV-Vv, -V, -C; SCRLV-Vv, -V and -C.

Year	Number of Surveys	Regressions	Deviance	d.f	F
1979 trial	B*(4)** & B (5)	$n_{Vv} = -0.93-1.48d$	20.28	13 N.S.	$F_{1,13} = 15.33$ ($p < .01$)
	S (4)	$n_{Vv} = -1.0-0.5d$	1.85	9 N.S.	$F_{2,9} = 52.71$ ($p < .001$)
		$n_{Vv} = -1.73-0.23d$			
		$n_{C} = -7.85+1.23d$			
	S (5)	$n_{Vv} = -0.50-0.54d$	2.519	9 N.S.	$F_{2,9} = 44.12$ ($p < .001$)
		$n_{V} = -1.73-0.23d$			
		$n_{C} = -7.33+1.14d$			
1980 trial	B (3)	$n_{Vv} = 0.76-1.13d+0.07d^2$	16.26	21 N.S.	$F_{4,21} = 11.3$ ($p < .001$)
		$n_{V} = 1.98-1.83d+0.13d^2$			
		$n_{C} = -8.18+0.98d-0.05d^2$			
	B (4)	$n_{Vv} = 0.77-0.83d+0.05d^2$	18.66	21 N.S.	$F_{4,21} = 15.21$($p < .001$)
		$n_{V} = 2.21-1.18d+0.07d^2$			
		$n_{C} = 5.54+0.42d-0.01d^2$			

(Contd..)

Continuation of table 3.

Year	Number of surveys	Regressions	Deviance	d.f.	F
1980 trial	B (5)**	$n_{Vv} = 1.14-0.91d+0.06d^2$	15.08	21 N.S.	$F_{4,21} = 21.12$ ($p < .001$)
		$n_V = 2.43-1.24d+0.08d^2$			
		$n_C = -6.05+0.68d-0.04d^2$			
	S (3)	$n_{Vv} = -0.65-0.47d$	44.57	21 N.S.	$F_{2,26} = 18.47$ ($p < .001$)
		$n_V = -3.39-0.47d$			
		$n_C = -3.15-0.47d$			
	S (4)	$n_{Vv} = 0.19-0.39d$	14.73	24 N.S.	$F_{2,24} = 63.85$ ($p < .001$)
		$n_V = -7.64+0.43d$			
		$n_C = -6.77+0.34d$			
	S (5)	$n_{Vv} = 0.27-0.38d$	9.43	24 N.S.	$F_{2,24} = 138.98$ ($p < .001$)
		$n_V = -6.50+0.36d$			
		$n_C = -5.34+0.27d$			

B* = BYMV: S= SCRLV

Vv = virus + vector; V = virus only; C = no virus & vector provided (control)

** Numbers in parentheses indicate number of surveys.

no values were obtained for treatments BYMV-V and BYMV-C. For treatments SCRLV-Vv and SCRLV-V, gradients were negative and, for treatment SCRLV-C, the gradient was positive (Table 3.). The results showed for the 1980 trial (Table 3) that the final patterns of BYMV incidence in all treatments fitted quadratic regressions of either positive (BYMV-C) or negative gradient (BYMV-Vv and BYMV-V). The SCRLV patterns all fitted separate linear regressions, where SCRLV-V and SCRLV-C were positive, SCRLV-Vv was a negative gradient.

From the regression it is clear that treatments BYMV-Vv, BYMV-V and SCRLV-Vv differ from the treatments BYMV-C, SCRLV-V and SCRLV-C.

Figs.7,8 shows the positions of BYMV and SCRLV in experimental plots. Principal components analysis showed (Table 4) that virus spread was nondirectional, except for BYMV-Vv where spread was predominantly in an east-west direction. Prevailing winds were from the north-west to west during the cropping season.

Madden et al. (1982) reported that the ordinary runs test was most suitable for the determination of random or nonrandom distribution of virus infected plants. Therefore ordinary run analysis was carried out (Madden et al., 1982) for the treatments BYMV-Vv, -V, -C; SCRLV-Vv, -V, and -C, the results showed (Table 5) that except for SCRLV-V, and SCRLV-C, the distribution of SCRLV and BYMV was nonrandom.

If diseased plants are distributed nonrandomly the pathogen is assumed to be spreading from plant to plant within

Table 4. Spread of BYMV and SCRLV in V.faba L. in 1980 trial

Treatment	Number of infected plants **		Latent root		X_2^2 *	
	BYMV	SCRLV	BYMV	SCRLV	BYMV	SCRLV
Vv"	160	148	5440.61	3140.37	14.75	2.43
			2938.88	2425.46	(S)	(N.S)
V	182	29	4441.63	1522.47	0.03	0.48
			4328.57	1165.12	(N.S)	(N.S)
C	53	49	2776.56	2679.59	2.06	1.48
			1854.46	1876.62	(N.S)	(N.S)

Vv" = virus + vector; V = virus only; C = no virus & vector provided (control)

* The X^2 value is a test of equality of the latent roots, which is, in effect, a test of equality of the lengths of the principal components. (Principal components identifies any direction in the spread of the observation).

**All infected plants were included regardless of time of infection.

Table 5. The distribution pattern of BYMV and SCRLV in V.faba L. in 1980

Treatment	Replicate	Total number of diseased plants	Expected number of runs	Direction	S.D.for number of run	Test statistic "Z"	Type of spread*	Overall spread
BYMV+vector (Vv)	R_1	70	118.78	SE - NW	5.59	- 1.302	R	N
				SW - NE		- 3.806	N	
				E - W		- 1.302	R	
	R_2	79	130.70	SE - NW	6.16	- 3.928	N	
				SW - NE		- 6.526	N	
				E - W		- 4.253	N	
	R_3	15	29.98	SE - NW	1.36	- 4.773	N	
				SW - NE		- 1.827	N	
				E - W		- 4.773	N	
SCRLV + vector (Vv)	R_1	55	97.28	SE - NW	4.57	- 1.704	N	
				SW - NE		- 0.828	R	N
				E - W		- 0.390	R	
	R_2	48	86.55	SE - NW	4.05	- 0.752	R	
				SW - NE		- 5.685	N	
				E - W		- 0.752	R	
	R_3	48	86.55	SE - NW	4.05	- 7.165	N	
				SW - NE		- 6.672	N	
				E - W		- 6.672	N	

Contd..

Continuation of Table 5.

Treatment	Replicate	Total number of diseased plants	Expected number of runs	Direction	S.D. for number of run	Test statistic "Z"	Type of spread*	Overall spread
BYMV only (V)	R_1	78	129.41	SE - NW	6.10	- 4.577	N	N
				SW - NE		- 2.281	N	
				E - W		- 3.265	N	
	R_2	25	48.17	SE - NW	2.22	- 3.895	N	
				SW - NE		- 2.097	N	
				E - W		- 2.996	N	
	R_3	82	134.51	SE - NW	6.34	- 2.524	N	
				SW - NE		- 1.893	N	
				E - W		- 3.155	N	
SCRLV only (V)	R_1	10	20.55	SE - NW	0.91	1.050	R	R
				SW - NE		1.050	R	
				E - W		1.050	R	
	R_2	11	22.45	SE - NW	1.00	1.050	R	
				SW - NE		1.050	R	
				E - W		1.050	R	
	R_3	11	22.45	SE - NW	1.00	- 0.953	R	
				SW - NE		1.050	R	
				E - W		- 0.953	R	

Contd..

Continuation of Table 5

Treatment	Replicate	Total number of diseased plants	Expected number of runs	Direction	S.D. for number of run	Test statistic "Z"	Type of spread*	Overall spread
BYMV control (C)	R_1	23	44.60	SE - NW	2.05	- 2.483	N	N
				SW - NE		- 0.536	R	
				E - W		- 1.509	R	
	R_2	15	29.98	SE - NW	1.36	- 0.353	R	
				SW - NE		- 0.353	R	
				E - W		- 0.353	R	
	R_3	15	29.98	SE - NW	1.36	- 1.090	R	
				SW - NE		- 2.563	N	
				E - W		- 4.037	N	
SCRLV control (C)	R_1	19	37.36	SE - NW	1.71	1.250	R	
				SW - NE		- 1.090	R	
				E - W		0.080	R	
	R_2	16	31.84	SE - NW	1.45	- 0.234	R	
				SW - NE		1.149	R	R
				E - W		1.149	R	
	R_3	14	28.11	SE - NW	1.27	1.095	R	
				SW - NE		1.095	R	
				E - W		- 0.482	R	

*Designated random (R) or nonrandom (N) distribution of plants infected with BYMV and SCRLV.
"The asymptotic sampling distribution of Z is the standard normal distribution. Z will be a large negative number if there is clustering, so test for nan-randomness is one-sided.

the field. Conversely, random patterns of spread of infected plants suggest that pathogens are not spreading from plant to plant and that the pathogen source was outside (Hill et al., 1980; Madden et al., 1982).

Application of the test of Madden et al. (1982) to field map data provides evidence for plant to plant spread of BYMV and SCRLV in treatments BYMV-Vv, BYMV-V and SCRLV-Vv by vectors from a primary inoculum source within the experimental plots.

3.3.3.5 Aphid behaviour deduced from the pattern of spread

The clumping of diseased plants has been determined for each survey by examining the clump sizes in all 3 replicates for each treatment separately using the data in diagrams(Figs. 7,8). Two adjacent plants showing diseased symptoms have been counted as clumps of two, whereas isolated diseased plants are recorded as clumps of one.

The results of the clumping study are shown in Table 6. In treatments BYMV-Vv and BYMV-V, BYMV infected plants occurred both singly and in clumps at each time when active spread was observed (Table 6). This pattern of clumping in treatment BYMV-V appeared to be independent of the artificial introduction of vectors (treatment BYMV-Vv). The probable explanation for this, is that alate migrant vectors entered the crop from outside, acquired BYMV from sources within the crop and transmitted it in a non-persistent manner to adjacent or more distant new hosts. This sort of aphid behaviour has been reported by Kennedy et al. (1959); Van Hoof (1979); and Harrewijn et al.(1981).

Table 6: Frequency distribution of the size of clumps of adjacent infected plants tabulated according to either the date when clumps were first observed, (primary clumps), or independently of the time of formation (final).

		BYMV							SCRLV						
		Size of Clump							Size of Clump						
	Date	1	2	3	4	5	6	7+	1	2	3	4	5	6	7+
	28/8	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	10/9	8	2	1	0	0	0	0	2	0	0	0	0	0	0
*Vv	23/9	26	7	2	0	0	1	0	18	5	1	1	0	0	0
	9/10	44	9	2	0	0	0	0	43	11	1	2	1	0	1
	22/10	17	4	0	0	0	0	0	13	3	0	0	0	0	0
	†Final	38	11	4	2	3	1	6	35	9	10	2	2	0	3
	28/8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10/9	8	0	0	2	0	0	0	0	0	0	0	0	0	0
V	23/9	18	5	0	2	0	0	0	3	0	0	0	0	0	0
	9/10	37	11	0	2	2	1	2	13	1	0	0	0	0	0
	22/10	14	3	0	0	0	0	0	11	0	0	0	0	0	0
	Final	23	15	2	2	0	0	6	27	1	0	0	0	0	0

contd..

Continuation of Table 6.

		BYMV Size of Clump							SCRLV Size of Clump						
	Date	1	2	3	4	5	6	7+	1	2	3	4	5	6	7
	28/8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10/9	4	0	0	0	0	0	0	1	0	0	0	0	0	0
C	23/9	12	4	1	0	0	0	0	3	0	0	0	0	0	0
	9/10	17	1	0	0	0	0	0	17	0	0	0	0	0	0
	22/10	9	0	0	0	0	0	0	19	3	0	0	0	0	0
	Final	14	4	1	3	2	0	0	33	4	1	0	0	0	0

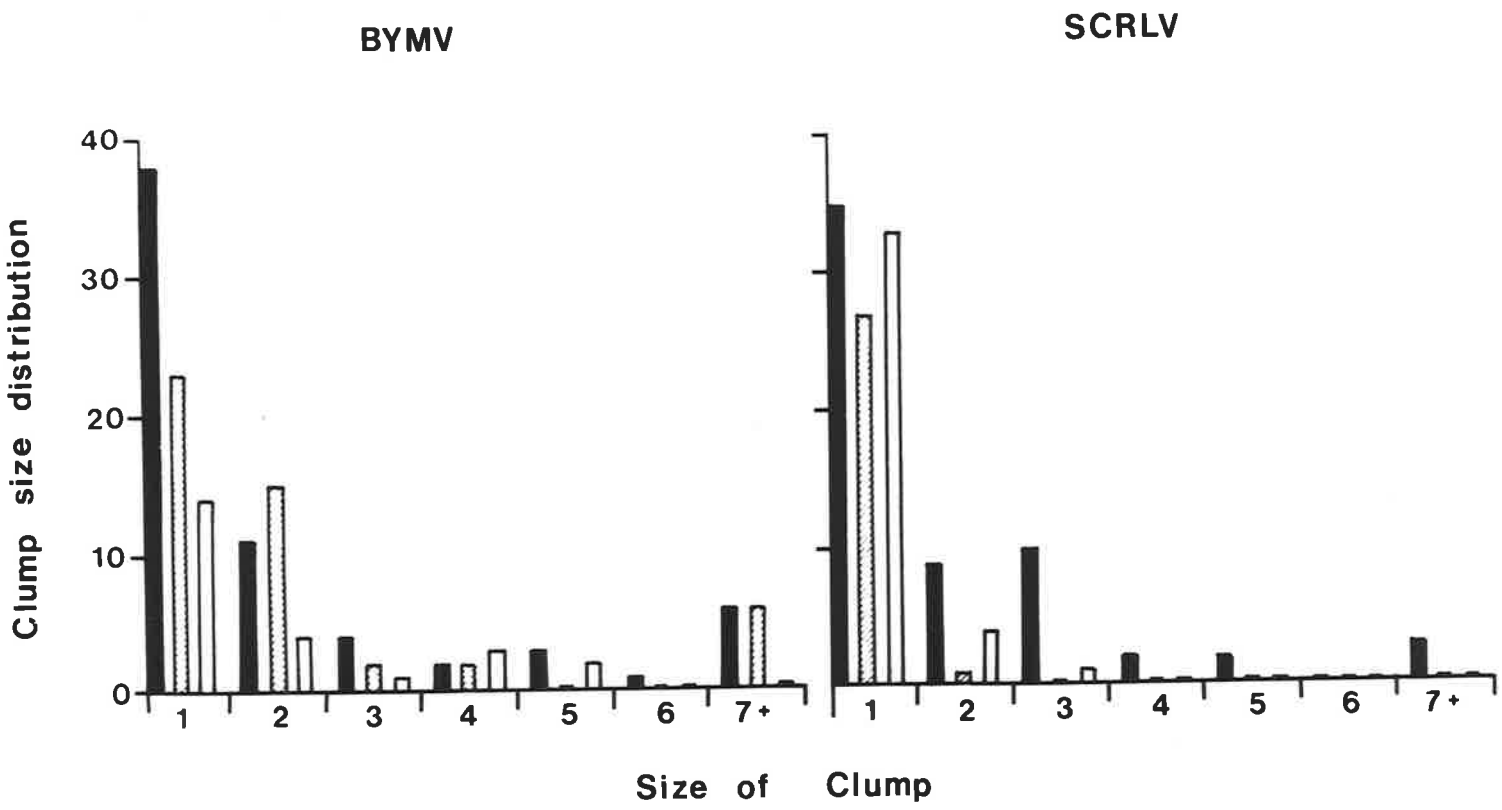
* Vv = virus + vector

V = virus only

C = no virus, no vector

† Clump size distribution at crop maturity

Fig. 11 Frequency distribution of the size of clumps of infected plants with BYMV (left), SCRLV (right) in treatments Vv (■); V (▒) and C (□) at crop maturity. Data obtained from distribution shown in Figs. 7 and 8.


BYMV
SCRLV
Clump size distribution
40
30
20
10
0
1
2
3
4
5
6
7+
Size of Clump

In treatment BYMV-C the number of clumps was less. This is probably due to the absence of initial virus source plants and to the requirement for alates to carry virus from an adjacent plot with source plants.

An examination of the clumping of diseased plants at the end of the trial (Fig. 11) showed for treatments BYMV-Vv, -V, and BYMV-C, similar frequency distributions of clump size. The final frequency distribution of clumps of BYMV infected plants (Fig.11) showed larger clumps than were observed at any of the survey times. This is probably due to colonization of infected plants and subsequent secondary spread to adjacent plants by apterous or alate vectors.

The results for treatments BYMV-Vv, -V and BYMV-C (Table 6) were analysed with Likelihood Ratio Statistic (X^2_{LR}) to further test whether there is any difference in clumps for the final clump size distribution at crop maturity. The X^2_{LR} value given in Table 7 indicated that each treatment has the same proportion of infected plants occurring in clumps.

Significant clumping of SCRLV occurred only in treatment SCRLV-Vv (Table 7) but not in treatments SCRLV-V and SCRLV-C. This indicates that early aphid colonization of the crop was necessary for foci to develop. When the time of clump formation was determined by tabulating the frequency distribution of new clumps each fortnight (according to the time of survey) it was found (Table 6) that most of the clumps were formed at the time of maximum rate of spread of SCRLV (9 October). This suggests that alate migrants developing on the artificially colonized infected plants alighted on and

Table 7: Comparison between clump size distribution at crop maturity (†) in different treatments in the 1980 winter-spring trial.

Treatment	X^2_{LR}	X^2(table)	Proportion of clumps
BYMV-Vv*	16.43	$< 18.307(X^2_{.95}(10))$	Same proportion
-V			
-C			
SCRLV-Vv	28.264	$> 12.592(X^2_{.95}(6))$	Different proportion
-V			
-C			
BYMV-Vv	4.55	$< 11.07\ (X^2_{.95}(5))$	Same proportion
SCRLV-Vv			
BYMV-V	23.56	$> 7.815(X^2_{.95}(3))$	Different proportion
SCRLV-V			
BYMV-C	8.67	$> 5.991(X^2_{.95}(2))$	Different proportion

* Vv = Virus + vector

V = Virus only

C = No virus or vector (control)

† see Table 6.

Fig. 12 *T.subterraneum* L. cv. Mt.Barker plants infected with SCRLV (a) and BYMV (b) around the central point source (arrow).

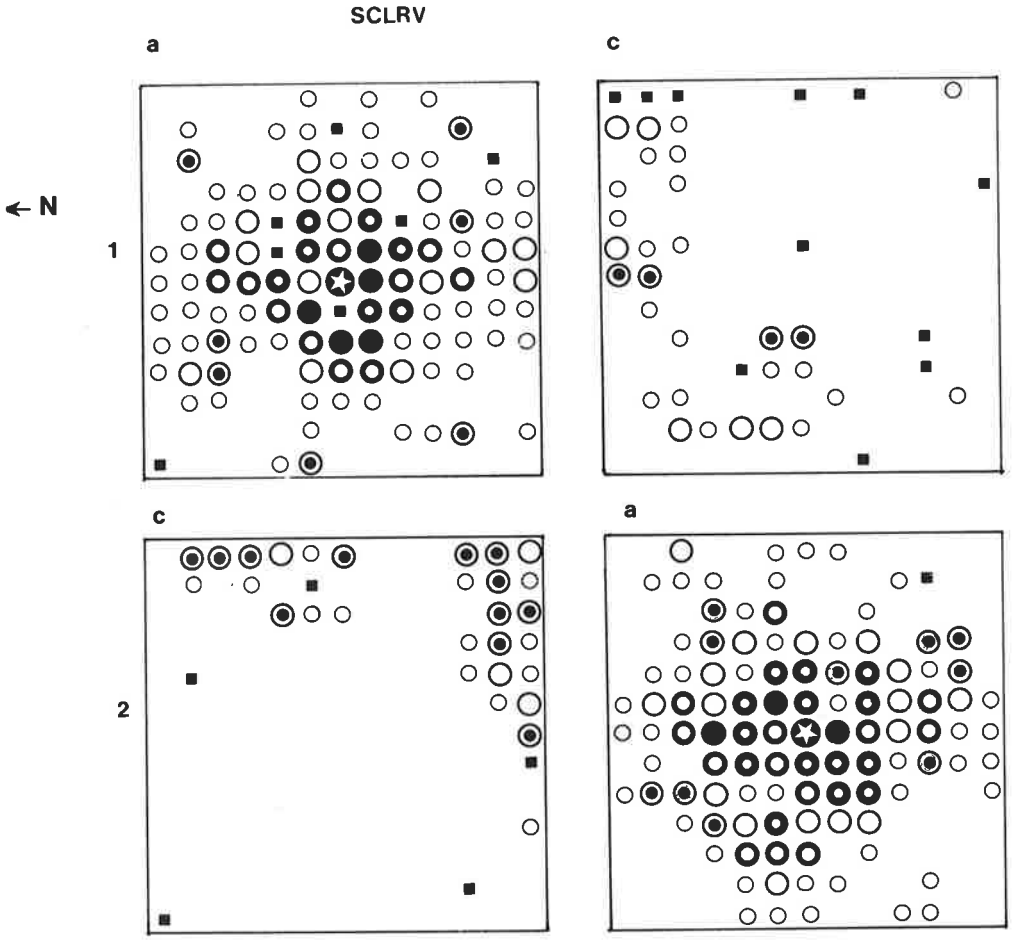

a
b

infected one or several adjacent plants before settling. The other possible explanation for this, since A.solani (kltb.) apterae have a high degree of mobility (Ashby et al., 1982) is that their free movement in V.faba L. crops (Johnstone and Rapley, 1981) could have resulted in them infecting many adjacent plants before settling.

The small difference between the pattern at the survey time, and the final pattern shows that minor secondary clumping occurred and therefore that secondary spread occurred from these initial foci, as a result of colonization followed by short range migration.

When the vectors were not introduced (treatments SCRLV-V and SCRLV-C) SCRLV showed no clumps larger than 3 (Table 6). The infrequent clumping in treatments SCRLV-V and SCRLV-C is consistent with viruliferous vectors entering the plots and inoculating one or two plants before either settling or leaving the plot. The frequency distribution of clump size (Table 6) for the three treatments SCRLV-Vv, -V and SCRLV-C analysed by the X^2_{LR} test (Table 7) showed that SCRLV-Vv had a different frequency distribution compared with treatments SCRLV-V and SCRLV-C. The observation of a high proportion of clumps in treatment SCRLV-Vv suggested that viruliferous apterae leaving the infected central foci would have contributed to the secondary spread of SCRLV. Analysis of final frequency distribution of clumping by X^2_{LR} tests showed (Table 7) that there was no difference in the proportion of clumping for treatments BYMV-Vv and SCRLV-Vv, but there was a difference in the clumping for the other two treatments for both viruses.

Fig. 13A The distribution at six survey times of SCRLV infected T.subterraneum L. plants around the point source (✪) where SCRLV and A.solani (Kltb.) were provided at the centre (1a, 2a), and control plots (no virus, no vector 1c, 2c). First data is shown at 4 week from the day the virus source was provided artificially (●) and then at 2 weekly intervals in the series ● , ✪ , ○ , ○, ◉.(■) No plants.


SCLRV
a
c
← N
1
c
a
2

3.3.4 Pattern of spread of BYMV and SCRLV in T.subterraneum L.

In the summer-autumn 1980 trial both viruses spread from the central infector source to neighbouring plants (Fig. 12). Appendix Table 1 and Figs.13A, B and 14, shows the pattern of spread of both viruses in the experimental plots.

In treatment Vv, BYMV and SCRLV showed a gradient extending from the centre of the plot towards the outside of the plots (Fig. 14). In treatment BYMV-Vv, all plants were infected in the row adjacent (1st row) to the central infector plants, whereas in the plots with treatment SCRLV-Vv the first two rows adjacent to the central infector plants had 100% infection. Furthermore in each row the number of plants infected with SCRLV in treatment SCRLV-Vv was higher than that of plants infected with BYMV in treatment BYMV-Vv (Fig. 14).

For both viruses in control treatments (BYMV-C and SCRLV-C) when virus and vector were not introduced into the plot the observed spread was from outside the plots towards the inside. It can be seen that both viruses spread from the adjacent plots (Fig.13A, B). BYMV spread as far as the third row in from the outer row, in the control plot (BYMV-C). SCRLV spread to the 4th row in from the outside of the control plots (SCRLV-C) (Fig.14). Furthermore in both treatments (BYMV-C, SCRLV-C) the observed gradients for both BYMV and SCRLV were only on sides proximal to Vv plots (Fig. 13 A, B).

The final patterns of distribution of BYMV in treatments BYMV-Vv and BYMV-C, fitted best to a quadratic regression (Table 8). The gradients were positive for

Fig. 13B The distribution of BYMV-infected T.subterraneum L. plants at six survey times around the point source (◉) where BYMV and A.craccivora Koch. were provided at the centre (1b, 2b), and in control plots (no virus, no vector 1c, 2c). First data is shown at 6 weeks (●) after the day the virus source was provided at the experimental plot and then at 2 weekly intervals in the series ⭘ , ○ , ○ , ◉ . Plants either died or seeds not germinated (■).

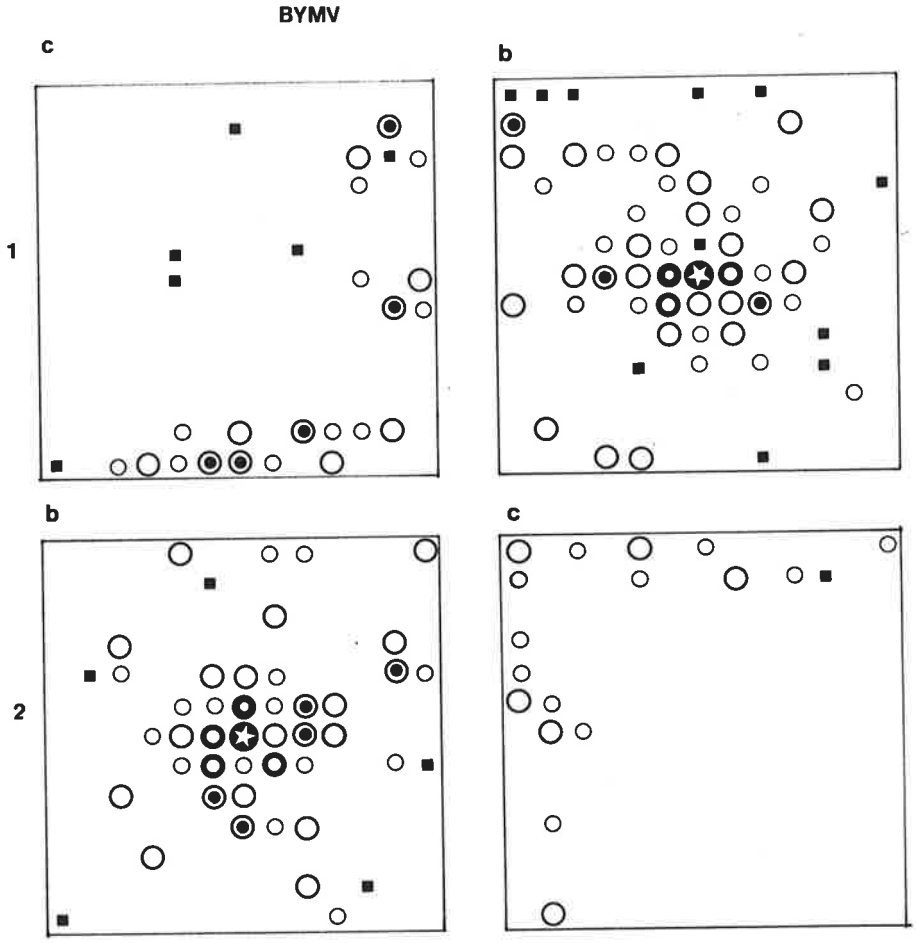
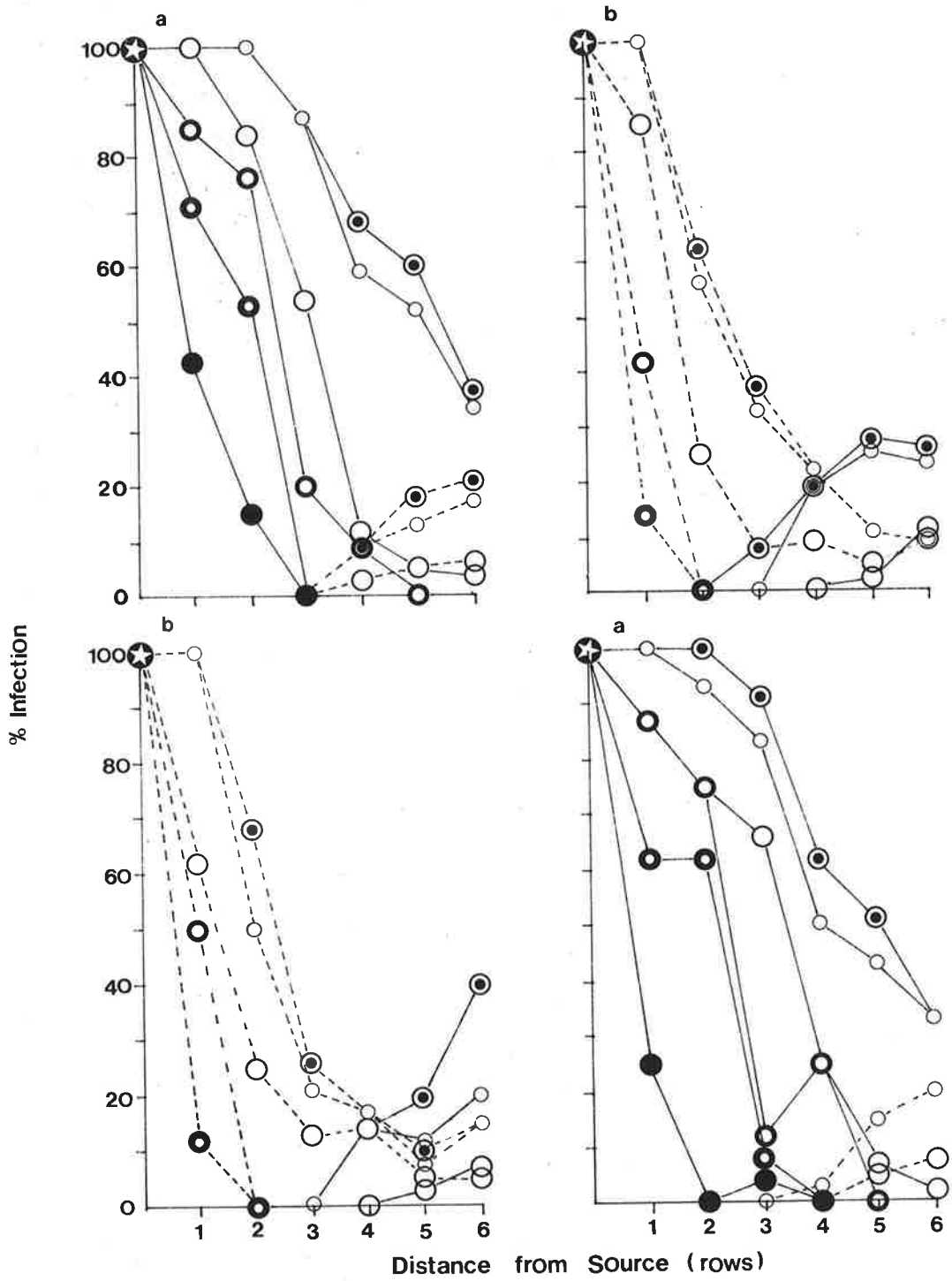

BYMV
c
b
1
b
c
2

Table 8: Regressions at last three survey times of gradients of BYMV and SCRLV incidence in treatments BYMV-Vv, -C; SCRLV-Vv, -C.

Survey time	Virus	Regression	Deviance (6 df)	F (2,6)
4th	BYMV	$n_C = -21.3+6.5d-0.56d^2$	2.79 NS	26.41 **
		$n_{Vv} = 2.61 - 2.61d+0.21d^2$		
5th		$n_C = -18+5.8d-0.5d^2$	6.10 NS	34.38 **
		$n_{Vv} = 4.6-2.6d+0.2d^2$		
6th		$n_C = -20+6.6d-0.6d^2$	4.11 NS	65.46 **
		$n_{Vv} = 5.4-2.6d+0.24d^2$		
4th	SCRLV	$n_C = -12.56+1.73d$	$3.04 \sim X^2_8$ NS	190.78 ***
		$n_{Vv} = 4.16-1.34d$		> F (1,8)
5th		$n_C = -15+5.15d-0.48d^2$	$6.99 \sim X^2_8$ NS	38.94 ***
		$n_{Vv} = 7.86-2.75d+0.22d^2$		> F (2,6)
6th		$n_C = -4.75+0.7d$	$12.38 \sim X^2_8$ NS	68.7 ***
		$n_{Vv} = 4.67-0.9d$		> F (1,8)

Fig. 14 Spread of BYMV (broken line) and SCRLV (solid line) in treatments Vv (virus + vector) and C (no virus, no vector). Diagonally opposite plots (a, a) received SCRLV + A.solani (Kltb.) at the centre. Similarly (b, b) plots received BYMV + A.craccivora Koch. at the centre. Thus b is the control for a, a is the control for b. Results are expressed for each plot in rows from the central source plants (✪), and plotted with increasing time after providing the infected plants at the centre of the experimental plots. The first data is shown at the 4th week (5/3/80) for SCRLV and at the 6th week for BYMV and then at 2 weekly intervals in the series ● , ● , O , ○ , ○ , ◉ . Row spacing was 0.5 m. Spacing between plots 1m.


a
b
b
a
100
80
60
40
20
0
% Infection
1
2
3
4
5
6
Distance from Source (rows)

treatment BYMV-C and negative for BYMV-Vv. The final patterns of SCRLV spread fitted best to linear regressions and the gradient was positive for SCRLV-C and negative for SCRLV-Vv.

The patterns of BYMV and SCRLV spread are graphed in Fig. 14. The rates of spread of BYMV and SCRLV in treatments, BYMV-Vv and SCRLV-Vv were much higher than in treatments BYMV-C and SCRLV-C at all times as would be expected because of the overall higher incidence of infected plants in the Vv plots. The rate of SCRLV spread in treatments SCRLV-Vv and BYMV-Vv was higher than that of BYMV in SCRLV-Vv and BYMV-Vv (Fig. 15). The peak rate of spread for both viruses occurred at the same time (16th April to 30th April). The analysis of aphid trapping records at that site (Table 9) revealed that flight of vectors peaked two to three weeks before the peak rate of spread. This interval is probably equivalent to the time required for symptoms to develop in plants inoculated with BYMV and SCRLV in the field at that time of the season. Furthermore it was observed that there was a drop in the spread of both viruses when number of aphids trapped was falling (Table 9).

An analysis of the development of clumps of adjacent infected plants according to time of appearance is shown in Table 10. In treatments BYMV-Vv and SCRLV-Vv more clumps were observed compared to BYMV-C and SCRLV-C. Peak clumping occurred at time of peak rate of spread. The chi/square (X^2) values given in Table 11 indicate that for both viruses the clump size distribution at crop maturity for the two treatments (-Vv, -C) was not significantly different.

Fig. 15 Mean fortnightly percentage rate of increase in incidence of BYMV and SCRLV in treatments Vv (●) and C (○) for the trial shown in Fig.14.

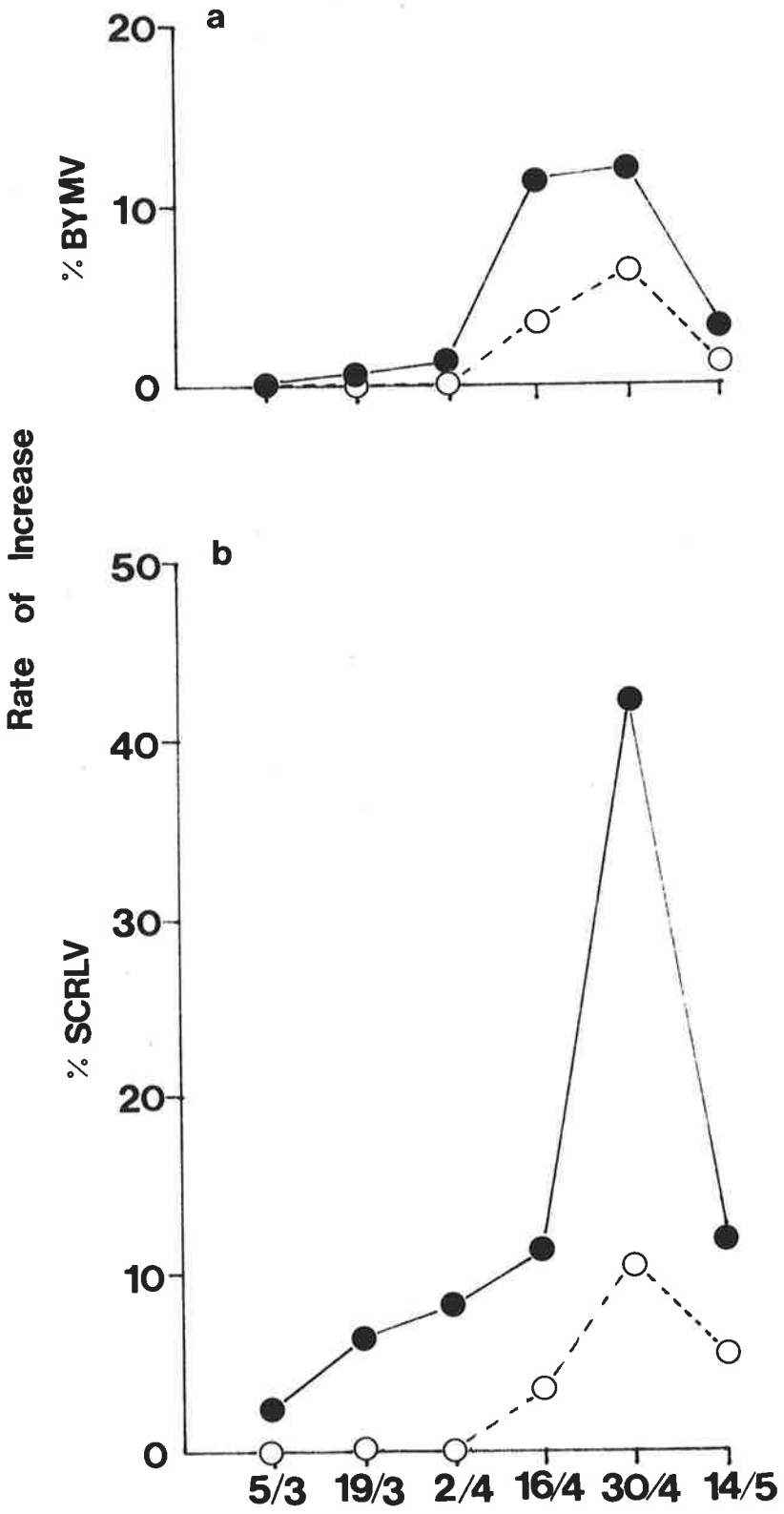

a
20
10
0
% BYMV
Rate of Increase
b
50
40
30
20
10
0
% SCRLV
5/3
19/3
2/4
16/4
30/4
14/5

Table 9: Number of 4 aphid species and other species caught weekly in a yellow water trap adjacent to the T.subterraneum L. trial in 1980. Collections were made weekly.

Date of collection	Aphid species M.persicae	M.euphorbiae	A.solani	A.craccivora	Other species	Total aphid
29/1/80	0	0	0	0	0	0
5/2	2	0	0	0	1	3
12/2	1	0	0	0	0	1
19/2	1	0	0	0	0	1
26/2	0	0	0	0	0	0
4/3	5	0	0	0	2	7
11/3	17	2	0	7	7	33
18/3	77	10	4	30	32	153
25/3	32	2	2	20	32	88
1/4	35	16	3	52	17	123
8/4	25	3	3	68	63	162
15/4	44	5	5	38	175	267
22/4	81	19	2	29	161	292
29/4	5	1	0	0	17	23
6/5	5	1	0	1	27	34
13/5	0	0	0	0	35	35
20/5	0	0	0	0	2	2
27/5	0	0	0	0	4	4
3/6	0	1	0	0	6	7

Table 10: Frequency distribution of the size of clumps of adjacent infected plants (T.subterraneum L.) in 1980 summer-autumn trial at Alverstoke Orchard, Waite Institute.

Treatment	Date	BYMV Size of clump							SCRLV Size of clump						
		1	2	3	4	5	6	7+	1	2	3	4	5	6	7+
Vv*	5/3	0**	0	0	0	0	0	0	3	1	1	0	0	0	0
	19/3	2	0	0	0	0	0	0	1	3	1	0	1	1	0
	2/4	3	1	0	0	0	0	0	3	2	4	0	0	0	0
	16/4	19	4	0	2	0	0	0	11	2	3	2	0	0	0
	30/4	12	6	0	1	0	1	0	4	2	2	0	0	0	7
	14/5	4	2	0	0	0	0	0	7	2	2	0	0	0	0
	† Final	10	4	1	0	0	0	2	1	0	1	0	0	0	2
C	5/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	19/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2/4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	16/4	10	1	0	0	0	0	0	4	3	0	0	0	0	0
	30/4	13	2	0	1	0	0	0	8	2	1	1	1	0	1
	14/5	3	1	0	0	0	0	0	3	2	1	0	0	1	0
	Final	5	3	0	2	0	1	1	3	0	0	0	0	0	4

* Vv = virus + vector
C = control
** = Number of clumps in each size class
† = Clumps size distribution at crop maturity

Table 11: Comparison between clump size distribution at crop (T.subterraneum L.) maturity (†) in different treatments in summer-autumn at Alverstoke Orchard in 1980.

Treatment	comparison		Chi square (X^2)	Proportion of clumps
BYMV-Vv*	VS	BYMV-C	$6.25 \sim X^2_6$ NS	Same
BYMV-Vv	VS	SCRLV-Vv	$5.35 \sim X^2_6$ NS	Same
BYMV-C	VS	SCRLV-C	$6.69 \sim X^2_6$ NS	Same
SCRLV-Vv	VS	SCRLV-C	$1.98 \sim X^2_6$ NS	Same

* Vv = Virus + vector
C = Control
† see table 10.

In this Chapter it has been demonstrated that in both T.subterraneum L. and V.faba L. the pattern of spread of BYMV and SCRLV in treatments where virus and vector were provided (BYMV-Vv, SCRLV-Vv) follows a similar trend. However, when compared with the number of plants infected by both BYMV and SCRLV at each row from the infector point source for both crops, it was observed that more plants of T.subterraneum L. were infected (Fig. 14) than V.faba L. (Fig.9). This may be due to any of the following. Firstly, procumbent habit of growth of the T.subterraneum L. would enhance the movement of the vectors of BYMV and SCRLV by interplant movement across the leaf bridges, whereas this would not apply to V.faba L. which has an erect habit of growth. Secondly, Mohamed (1979) found that A.craccivora Koch. dispersed earliest from T.subterraneum L. than from V.faba L. The explanation he gave for this was that V.faba L. plants provided a larger leaf surface area for the apterae to settle and larviposit than the same aged T.subterraneum L., thereby delaying the apterous dispersal. Since the introduced vector of BYMV (A.craccivora Koch.) has a high reproduction rate and a very short maturation time (Gutierrez et al., 1974) if they do not disperse early, over crowding would occur sooner on T.subterraneum L. plants than on V.faba L. On the other hand only little is known of the behaviour of the polyphagous A.solani (kltb.) and whether its apterae have a high degree if mobility (Ashby et al., 1982). Working with M.persicae (Sulz.), Ootake (1954), found that just before the apterae reach maturity they become active and leave their host (radish) plant even at low population density.

For both species the observed gradient was from the outside extending towards the inside (Fig. 7 and Fig. 14) in the control plots (BYMV-C, SCRLV-C) and this indicated the importance of effectively separating diseased from healthy plants. For example, in T.subterraneum L. trial, when control plots were separated by one meter from the plots with introduced virus and vector, virus spread was observed between the adjacent plots.

In the V.faba L. trial, the results indicate that BYMV spread was independent of and SCRLV was dependent on early colonization by vectors at the trial site.

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CHAPTER 4

APHID ACTIVITY AND TIME OF SPREAD OF BYMV AND SCRLV

4.1 TRAPPING OF APHIDS IN THE EXPERIMENTAL AREA

4.1.1 INTRODUCTION

Trapping is one of the methods of estimating numbers of flying insects and is particularly useful when insects are collected continously.

Flying aphids are very important as vectors of plant viruses. The timing and intensity of aphid flights in the field is of considerable interest for epidemiological reasons because many species not only damage plants through their feeding activities but are also responsible for transmission of a number of plant viruses. Relationships between trap catches of winged aphids and virus spread have been observed by Broadbent (1950), Watson and Healy (1953), Hollings (1955), and Randles and Crowley (1970). Barley yellow dwarf virus (BYDV) infects autumn sown cereals in south west Wales and it has been suggested that this is as result of BYDV infective aphids migrating into the emerging cereal crops in late autumn (A'Brook, 1974; A'Brook and Dewar, 1980). Therefore to study the spread of plant viruses in relation to aphid activity, a method that allows the number and species to be measured is essential. In the past many types of traps have been used to monitor aphid activity. The commonly used traps are sticky traps (Broadbent, 1948), suction traps (Johnson, 1950) and yellow water traps (Moericke, 1951). They may be made selective by applying colour (Broadbent, 1948; Eastop, 1955, Hughes et al., 1964; Irwin, 1980).

All these traps have advantages and disadvantages. Suction traps are most efficient because they are non-selective in capturing various species of alatae and give precise measurements of moving populations. The disadvantage is that this type of trap is costly and needs electric power (Gonzales and Rawlins, 1968). Sticky traps collect few aphids but have the advantage of requiring less attention (Heathcote, 1957; O'Loughlin, 1963), the number collected on the trap is a function of wind velocity (Taylor and Palmer, 1972). Yellow water pan traps have the advantage of ease of handling (Zettler et al., 1967) but there are disadvantages because some aphid species are not attracted to the yellow colour (Eastop, 1955, 1957; Heathcote, 1957; Robertson and Klostermeyer, 1958; O'Loughlin, 1963) and specimens must be recovered frequently to prevent spoilage (Gonzales and Rawlins, 1968). Water traps have been used extensively to trap alate aphids (Broadbent, 1948; Eastop, 1955; Lamb, 1958; Hughes et al., 1964; Evans and Medler, 1966; Landis, 1972; Sandvol and Cunningham, 1975; Bacon et al., 1976; Byrne and Bishop, 1979; Hill et al., 1980). They are simple plastic or metal bowls or trays filled with water which contain small amounts of detergent to trap and drown the insects, and a preservative (Hughes et al., 1964; Southwood, 1966). It has been observed that omission of the detergent results in a reduction in the total catch (Harper and Story, 1962).

In comparing the suitability of flat sticky traps, suction traps and yellow water traps for quantitative studies

related to aphids trapped, Heathcote (1957) reported that water traps were more effective for collecting aphids which are attracted to yellow surfaces than sticky traps of the same colour. Suction traps were the most efficient.

The efficiency of water traps in catching flying aphids depends upon several factors such as height of traps above ground (Heathcote, 1958; Landis, 1972), trap background (Moericke, 1957; Landis, 1972) and area of trap surface (Costa and Lewis, 1968).

The main purpose for setting up water traps in the experimental plot site was to obtain information on (i) the flight pattern and time of aphid migration in the field; it was thought the data obtained from 2½ years aphid trapping would be useful for forecasting the timing of flights of aphids in the field. Such data have been useful in predicting aphid population trends, e.g. Byrne and Bishop (1979) found that the number of alate Myzus persicae (Sulz.) caught in water traps in potato fields was correlated with adjacent field populations because the aphids collected were migrating out of the field rather than into the field; (ii) whether there is any relationship between the numbers of different aphid species (vectors of BYMV and SCRLV) trapped and the time of spread of BYMV and SCRLV at the experimental site.

4.1.2 MATERIAL AND METHODS

Two yellow water pan traps similar to those described by Moericke (1951) were used at the experimental site. Each yellow plastic pan (35x31x14 cm deep) was suspended

in a squaee steel framed mounted on a steel fence post. Pans were partly filled with water (c. 9 cm deep) containing a few drops of detergent and formalin. One pan was placed over bare ground to the N' of the central plot (see map Fig. 4a) in the experimental site and the other was adjacent to the trap plants over bare ground and separated from crop plants (Fig. 4a). Both were positioned 40 cm above the ground. This height was chosen because water traps placed at 80 cm or lower, and level with the plant canopy, consistently catch more aphids than those at ground level (Heathcote, 1958). Further, Heathcote (1958) recommended that water traps over bare ground shoud be as low as possible. The selected height was, therefore, a compromise and it further avoided the necessity of having to adjust the trap height as the plants grew. Collections of alatae were made each week from the two traps separately into vials containing 70% ethyl alcohol, stored, and counted. Traps were cleaned before refilling.

Trapping began on 27 July 1979 and ceased on 4 December 1981.

4.1.3 Identification of vectors of BYMV and SCRLV

As reported previously (Hughes et al., 1964) alate aphids have a characteristic way of floating with wings spread and legs and antennae extended and this helps to separate aphids from Diptera and Hemiptera of the same size.

Four of the vectors of BYMV, Myzus persicae (Sulz.), Macrosiphum euphorbiae (Thomas), Aphis craccivora Koch., Aulacorthum solani (Kltb.) (Kennedy et al. 1962; Johnstone, 1980)

and the vector of SCRLV, A.solani (Kltb.) (Kellock, 1971) were identified using the reference collection of the Dept. of Entomology, Waite Agricultural Research Institute, and the descriptions of Cottier, (1953). Identifications were confirmed by sending samples to Dr.M.Carver, CSIRO Division of Entomology, Canberra. The key for the identification of four aphid species is shown in Appendix 6.

4.1.4 Sub-sampling of large aphid populations

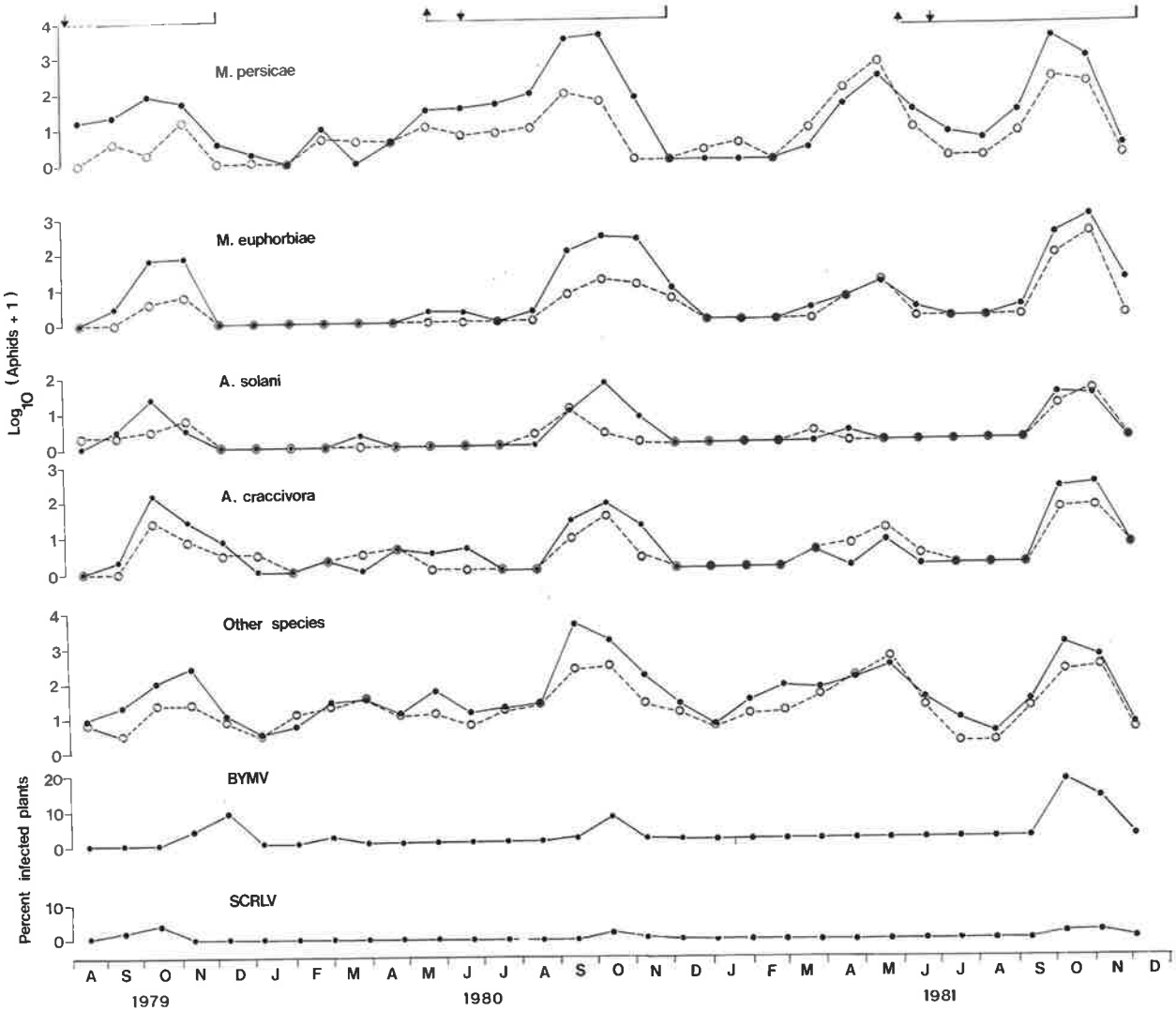
For collection with more than 1000 aphids the total number trapped and the numbers of the 4 species of interest were determined from a sub-sample of 50% of the aphids. The procedure adapted was similar to described by Mohamed (1980). The aphid collections were transferred to a 9 cm diameter petri dish marked into 8 equal sectors and stirred with a brush to spread them evenly. After the aphids settled in the dish excess fluid was removed with a pipette. Aphids in four alternate sectors were removed to another petri dish for counting and identification.

4.1.5 RESULTS AND DISCUSSION:

Numbers of four of the aphid species trapped each week at the experimental plot site and adjacent to the trap plants between 27 July 1979 and 4 December 1981 are presented in Appendix 7. Of the total of 22,081 aphids trapped (from both traps) for this period of 29 months, 62% belonged to these 4 species (Appendix 7).

The catch for 28 day periods of total numbers and of each of the above species is shown in Fig. 16. Trap

Fig. 16 Four weekly catches of alate aphids in traps in the experimental plot area (●——●); and adjacent to the trap plants (O---O) compared with the number of trap plants infected with BYMV or SCRLV during the same period. The 4 weekly catch is compiled from weekly trap counts. The times of planting V.faba L. line 383A (↑); inoculation of source plant (↓) and of harvest (I) in adjacent experimental plots are included, to show the times when artificially inoculated sources of BYMV and SCRLV were available in the area.


M. persicae
M. euphorbiae
A. solani
A. craccivora
Other species
BYMV
SCRLV
Log10 (Aphids + 1)
Percent infected plants
A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1979
1980
1981

catches were transformed to $\log_{10}$ (N + 1), where N = Number of aphids. The patterns of migratory activity of aphids were similar to sites, but generally fewer were trapped adjacent to the trap plants (Fig. 16). The highest numbers of aphids were trapped during spring (mid-September to early November for the year 1979, mid-August to early November for 1980, and mid-September to early November for 1981). Activity was also observed between February and July. The minimum activity was in mid-summer (December - January) and mid-winter (June - July).

In South Australia peak numbers of aphids generally occur during the spring and autumn, presumably in response to flushes of plant growth and suitable weather conditions (Maelzer, 1981). Trapping records for 2½ years at my experimental site also show that there is a small autumn peak and a major spring peak in close similarity to Hughes et al. (1964) trapping records for the Adelaide hills, South Australia. These studies were limited to the known vectors of BYMV and SCRLV which occur frequently in trap catches from South Australia (Hughes et al., 1965; Kennedy et al., 1962). Of these species (Fig. 16) M.persicae (Sulz.) was the most numerous aphid species trapped for the 2½ year period, followed by M.euphorbiae (Thomas) and A.craccivora Koch.. A.solani (Kltb.) was trapped infrequently during this period, in agreement with trapping records for 1961-62 in the Adelaide hills (Hughes et al., 1964; 1965). Each of these species showed peak of activity at about the same time (Fig.16) except that A.solani (Kltb.) was trapped rarely in the autumn (March - May) when the other species showed a low peak of flight activity.

4.2 EFFECT OF CLIMATE ON THE ACTIVITY OF VECTORS OF BYMV AND SCRLV

4.2.1 INTRODUCTION

A complex of biotic and physical factors affect migration, and dispersal of aphids. The number of migrants is determined by the physiology of the aphid, host plants, the meteorological conditions controlling vector populations, and the weather during their migration to the crop (A'Brook, 1980; Maelzer, 1981).

Meteorological variates, particularly temperature, are known to influence aphid development and migration (van Emden et al., 1969). The optimum conditions for aphid development are a moderate rainfall and temperature in the range 18 - 23°C, Hughes et al. (1964). Dean (1974) showed that the reproduction of Rhopalosiphum padi L. almost ceased at 10°C and below. Taylor (1957) reported that Aphis fabae (Scop.) flight activity might be restricted at temperatures below 15°C.

Alate forms are produced mainly in response to crowding and a deterioration in food supply (O'Loughlin, 1963). These aphids fly to new feeding areas. The main restrictions on flight are due to low light intensity, low temperature and high wind speed (Taylor, 1965; Dean, 1978). When light and temperature are suitable for flight, a windspeed of more than 10 knots would inhibit take off (Cochrane, 1980).

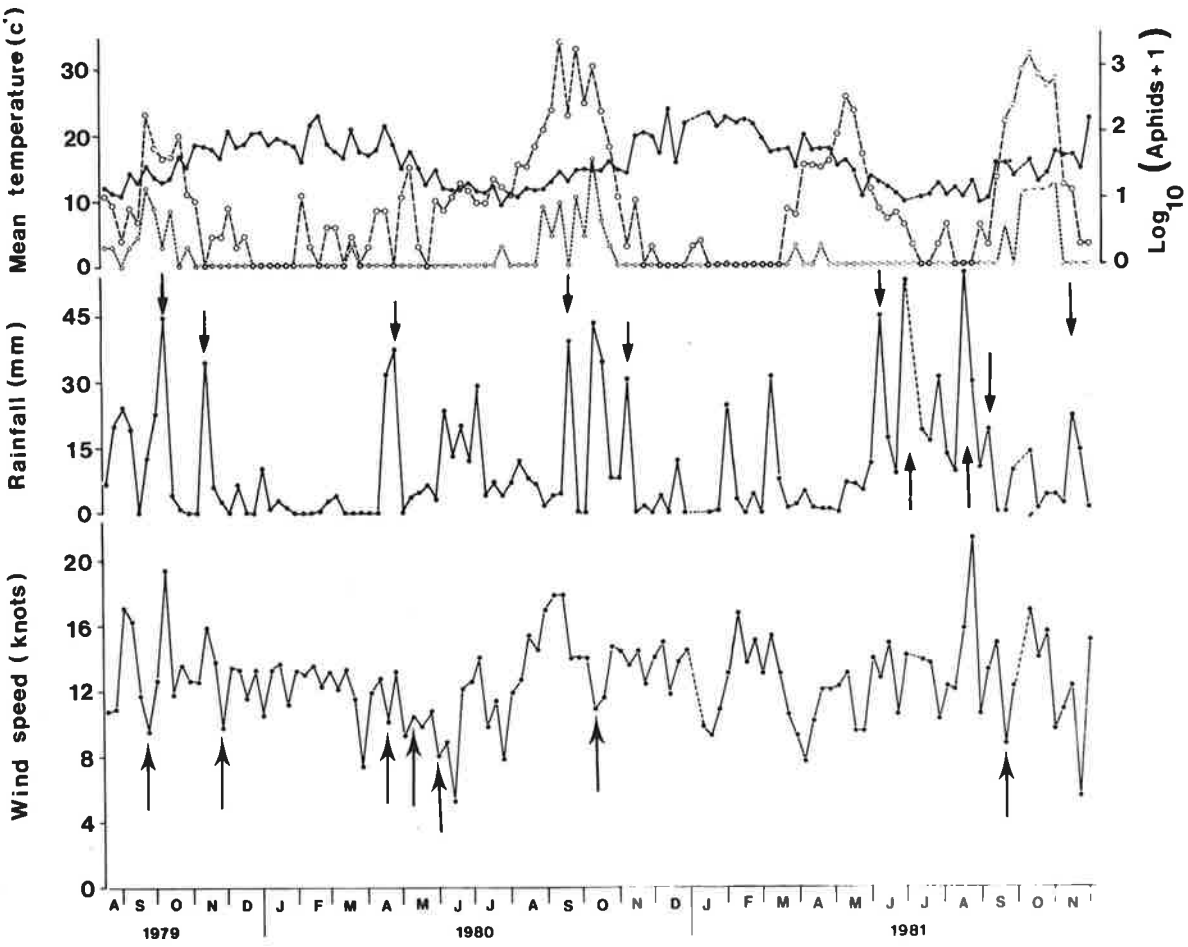
When average maximum temperatures were higher than 26°C or lower than 15°C, O'Loughlin (1963) reported little aphid activity in Victoria. Further, he pointed out that this may be

indirectly related to the season and the host plants. Randles and Crowley (1967) reported that reductions in flight activity of aphids coincided with heavy rainfall in some cases, and increased activity was coincided sometimes with rising temperature.

Robert and Rouze-Jouan (1976) showed during both summer and autumn migrations that peak numbers of the BYDV vector aphids could be related with combinations of preceding 10 day total rainfall and accumulated day degree temperature. Watson et al. (1975) has shown that the incidence of sugar beet yellowing viruses in late August is associated with the number of days with frost in January to March, and with April temperatures. Fewer frost days and warmer April temperatures resulted in a higher incidence of yellows in August due to more of the vectors Aphis fabae (Scop.) and Myzus persicae (Sulz.) reproducing and flying earlier in the year. A'Brook (1981) showed a similar association for Metopolophium dirhodum (Walker) and Sitobion avenae (Fabr.) caught in June. The high temperatures during January to February were associated with high numbers of alatae trapped in June.

The objective of this study was to investigate (i) the relationship between number of aphids trapped and the local weather data, and to determine the conditions favourable to and unfavourable for aphid migration; and (ii) whether meteorological data could be used for defining the periods when vectors of BYMV and SCRLV are likely to fly.

Fig. 17 Number of alate M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. (○---○) and A.solani (Kltb.) (○-----○) trapped each week in relation to the mean weekly temperature, weekly rainfall and mean weekly wind speed measured at 10m height. Dashed lines for the weather data indicate that no data were available for that period. Arrows in the weekly rainfall graph indicate those peaks of rainfall which coincide with reduced numbers of aphids trapped. Arrows in the mean weekly wind speed graph indicate when mean weekly wind speed below 11 knots coincided with more aphids trapped.


Mean temperature (c')
Rainfall (mm)
Wind speed (knots)
Log10 (Aphids+1)
A S O N D J F M A M J J A S O N D J F M A M J J A S O N
1979
1980
1981

4.2.2 MATERIALS AND METHODS

Meteorological records were obtained from the Strathalbyn Post Office which was 9 km from the experimental site where the traps were kept.

Mean weekly temperatures were calculated from daily maximum and minimum temperatures.

Mean weekly windspeed =

$$\frac{\text{Sum of daily mean windspeed for 7 days}}{7}$$

4.2.3 RESULTS AND DISCUSSION

Weather records are presented in Appendix 8. Figure 17 shows the mean weekly temperature, weekly rainfall and mean weekly windspeed from August 1979 to December 1981. To compare the aphid numbers trapped with each weather component the total catch (from both traps) of M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. and A.solani (Kltb.) were also plotted on the same figure after transforming the counts to $\log_{10}(N + 1)$ for the same period of time.

Temperature

M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. and A.solani (kltb.) were active ~~across the range of~~ at mean weekly temperatures ~~experienced~~ of 9.2 - 22.2°C (Fig.17). Greatest numbers were trapped in weeks when mean weekly temperatures lay between 13° and 17°C (Table 12).

Rainfall

During periods when the temperature was favourable aphid activity was sometimes terminated or the numbers were reduced, when the weekly rainfall increased (Fig.17). This suggested that flights could be retarded by high rainfall. Such periods are shown by the arrows in Fig. 17. A similar situation has been observed with the number of H.lactucae (L.) trapped compared with weekly rainfall by Randles and Crowley (1970).

Further analysis of the relationship between aphid flights and weekly rainfall showed that in weeks when the mean weekly temperature was in the range most favourable for aphid flight (13 - 17°C) rainfall had no effect on the number trapped (Table 12). Further when mean weekly temperature was below 13°C and above 17°C, fewer aphids of all 4 spp. were trapped when the weekly rainfall was above 7 mm.

Wind

No correlation was found between mean weekly windspeed (measured at 10 m above ground level and 9 km from the trapping site) and the number of aphids trapped. However, in some instances peaks in the population of winged aphids appeared to be highest in those weeks when mean weekly windspeed was below 11 knots (Fig. 17).

Randles and Crowley (1967) reported that rainfall, windspeed and temperature affected the number of aphids trapped. Similar relationships were observed here between the weekly rainfall, mean weekly temperature and mean weekly windspeed to

Table 12: Number of alate aphids trapped in relation to weekly rainfall and mean weekly temperature (July 1979 - December 1981)

Weekly rainfall (mm)	Mean weekly temperature (°C)	No. of weeks	Mean number of aphids trapped per week			
			M.persicae	M.euphorbiae	A.solani	A.craccivora
0-7	9-13	13	71.2	19.1	2.0	5.0
	13-17	20	301.4	31.0	2.2	8.3
	17-19	13	18.0	29.0	1.3	10.0
	19-24	8	4.0	0	0	0
7-21	9-13	15	7.0	0.2	0.1	0.1
	13-17	10	157.1	26.0	3.0	26.4
	17-19	5	1.0	1.2	0	1.2
	19-24	12	1.0	1.0	0.1	0.3
21-35	9-13	5	3.2	0	0	0.2
	13-17	4	16.8	55.0	3.0	4.0
	17-19	3	0	0	0	0
	19-24	2	2.0	0	0	1.0
35-71	9-13	3	3.0	0	0	0
	*13-17	1	980.0	93.0	41.0	17.0
	17-19	0	0	0	0	0
	19-24	0	0	0	0	0

* Rain confined to two days

number of aphids trapped (Fig. 17).

In conclusion the results show (Fig. 17 and Table 12) that the vectors of BYMV and SCRLV (Kennedy et al., 1962; Kellock, 1971) were more frequently trapped in weeks when mean weekly temperature lay between 13 to 17°C, and when mean weekly windspeed less than 11 knots. The rainfall above 7 mm per week appeared to affect flights, only when mean weekly temperature were below 13°C and above 17°C.

4.3 TIME OF SPREAD OF BYMV AND SCRLV IN THE EXPERIMENTAL AREA

4.3.1 INTRODUCTION

To develop control measures and methods of forecasting the progress and prevalence of known diseases, a knowledge of the main periods of spread is important. The time of spread can be detected by exposing susceptible plants for short intervals in the field (Broadbent et al., 1950; Posnette and Cropley, 1954; Tamaki et al., 1979).

The main objective of exposing susceptible V.faba L. plants near the experimental site was to find out when BYMV and SCRLV spread in that area.

4.3.2 MATERIALS AND METHODS

Virus-free broad bean (V.faba L.) cv. Aquadulce seeds were planted in wooden boxes (25 seeds per box) and allowed to germinate in an insect free glasshouse; 100 plants (5 boxes) were exposed in the field (at a site 20 m S' of the closest experimental plot, Fig. 4a) at Charlick Experiment Station, Strathalbyn for intervals of 28 days then replaced with a new

batch of 2-week old seedlings. The plants brought back from the field were sprayed with "Metasystox" (Demeton-S-Methyl) and kept in a separate glasshouse for one month for symptoms to develop. Plants showing symptoms of BYMV and SCRLV (see Section 3.3.1) were indexed (see Section 2.1.1) on C.amaranticolor, Coste and Reyne, V.faba L. cv. Aquadulce, P.vulgaris L.cv. Hawkesbury Wonder and T.subterraneum L.cv. Mt.Barker.

The first batch of plants was first exposed on 27 July 1979 and the trial was continued until 4 December 1981.

4.3.3 RESULTS

The data from the trap plants are shown in Appendix 9. Except in 1979, BYMV and SCRLV spread at the same time of the year (Fig. 16). The incidence of SCRLV at the peaks of spread was lower (1 to 4%) than that for BYMV (1 to 16%).

BYMV spread was observed during the spring season (Sept. to Nov.) and except in 1979 the increase and decrease of percent infection coincided with an increase and decrease of aphid flight activity (Fig.16).

In the summer of 1979 (December to January) BYMV spread started after A.craccivora Koch., A.solani (kltb.), M.euphorbiae (Thomas),M.persicae (Sulz.) flights peaked, and continued after A.solani (Kltb.), M.euphorbiae (Thomas) flights were no longer detectable by trapping. The other spp. were also active at the time, but at a reduced level. These data do not implicate any particular aphid spp. in the spread of BYMV, although it may be suggested that M.euphorbiae (Thomas) and A.solani (Kltb.) are not involved in either the December 1979

spread of BYMV or the autumn 1980 spread. The small autumn peak of BYMV spread coincided with a peak of M.persicae (Sulz.) in 1980, but a similar peak in activity of M.persicae (Sulz.) in 1981 did not lead to detectable spread of BYMV.

Spread of SCRLV was also observed during the spring and an increase and decrease in percent infection coincided with an increase and decrease in flights of its vector Aulacorthum solani (Kltb.) (Fig.16). There was a small peak of autumn flight activity but none of the trap plants exposed in the field showed SCRLV symptoms.

4.3.4. DISCUSSION

All four aphid species studied commonly infest V.faba L. (Johnstone and Rapley, 1979). The peak flights of all 4 spp. (see Section 4.1.5) could be correlated with the time of spread of BYMV and SCRLV into trap plants. Randles and Crowley (1967) found that the epidemiology of cauliflower mosaic virus (CaMV) in South Australia was complicated by the presence of more than one vector, which differed in their peak flight times and colonizing behaviour. In contrast, four of the vectors of BYMV, M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. and A.solani (Kltb.) (Kennedy et al., 1962; Johnstone, 1980) showed peak activity which coincided with trap plant infection with BYMV. The results (Fig.16) also show A.solani (Kltb.), the species which had one of the highest levels of consistency of occurrence for aphids trapped in Australia (Hughes et al.,1965) had a greater regularity between flight peak and the incidence of SCRLV.

BYMV and SCRLV spread into trap plants only at the time of year when the source of inoculum was provided artificially in the adjacent experimental plot. This suggests that both viruses would have spread from the infected source to trap plants during peak vector activity.

It was not possible to assess from the data obtained here, whether winged aphids coming into the experimental plots or winged aphids produced on colonized infected plants or both, contributed more to the spread of BYMV and SCRLV from the infected source to the trap plants during peak aphid flight activity. Nevertheless, the relative importance of each can be inferred from a discussion of the modes of transmission of the two viruses.

Broadbent (1960) reported that aphids probing for a prolonged period on infected plants (with non-persistent viruses) were not infective, but could become infective by such a probe followed by a shorter one. Kennedy et al. (1959) found that most of the Aphis fabae (Scop.) that made prolonged probes on bean leaves tended to pause briefly before flying, so that aphids which develop on infected plants could become infective by such a short probe before leaving the infected plants. If other aphid spp. (vectors of BYMV) colonizing V.faba L. behave the same as A.fabae (Scop.), presumably these aphids flying away from infected plants are infective and capable of spreading BYMV from the infected source to trap plants.

Diseased V.faba L. plants infected with BYMV and SCRLV are yellower than healthy plants. Some species of flying aphids are attracted more to yellow or yellowish green colours (Moericke, 1950; Muller, 1964; Hille Ris Lambers, 1972) than to

green colour. These flying aphids would be infective after a short feed on plants infected with a non-persistently transmitted virus (Bradley, 1954; Broadbent, 1960; Harrewijn et al., 1981) before the beginning of the main flight (Kennedy and Booth, 1963) or early flight (Van Hoof, 1980). Therefore it is possible that some of the migrating aphids, if they were vectors of BYMV, would have picked up the BYMV from the artificial inoculum source after being attracted to them and these contributed to the spread of BYMV. Conversely for persistently transmitted viruses, the aphids have to feed on the disease plants for a considerable period (a day or more) and pass the latent period after the acquisition feed before becoming infective (Broadbent, 1952). Therefore the migrating aphids alighting on the infected plants for a short period are unlikely to become infective. On the other hand the alate aphids produced from the colonized infected plants are viruliferous and presumably some of these aphids would have spread the SCRLV to trap plants.

4.4 RELATIONSHIP OF APHIDS TRAPPED TO TIME OF PLANT GROWTH

Under the climatic conditions prevailing at the experimental site, all non-irrigated plants dry off in the period November to April. Following the first heavy and consistent rains (March, 1980, May, 1981 - Fig. 17) pasture, weed and cereal crop species germinated in the area.

M.persicae (Sulz.), A.solani (Kltb.) and A.craccivora Koch. were trapped in 1980 before the opening rains. This suggests that the source of aphids was distant from the traps,

such as irrigated pastures and higher rainfall areas several kilometres from the experimental site. One of the major problems of aphid strategy in South Australia is survival over summer (Maelzer, 1981). The hot dry season is the most hazardous period for the survival of these four species ~~in~~ because high temperature and scarcity of suitable host plants ~~which~~ prevent development of aphids above mean daily temperatures of 28°C (Barlow, 1962). The mean maximum temperature (over 123 years) for Adelaide for the summer months (December to February) is above 28°C (South Australian Year Book 1981) which probably accounts for the negligible aphid flight activity in summer.

The period of major flight activity (September to November in all years) coincided with the later stages of growth of the artificially infected experimental plots adjacent to the trap. It was not possible from the data obtained to assess the contribution of the aphids in the crop to the number trapped because during the field experiments (1979 to 1981) aphid populations on the crop were not determined.

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CHAPTER 5

EFFECT OF CONTROLLING APHID POPULATIONS AND MOVEMENT ON SPREAD OF BYMV AND SCRLV

5.1 INTRODUCTION

One of the main objectives of the field studies described in Chapters 3 and 4 was to gain information to allow control of the spread of BYMV and SCRLV.

It was evident from the results of the field studies (Chapter 3) that BYMV spread was dependent on the presence of infected plants but independent of vector colonization whereas SCRLV spread was dependent on vector colonization of infected plants on the crop. Therefore to control BYMV, prevention of early infection of plants in the crop would be more important than preventing colonization by aphids, and conversely control of SCRLV may be achieved simply by preventing colonization of the crop with its vector A.solani (Kltb.)

The aphid trapping results for 2½ years revealed (Chapter 4) that there are two peaks of aphid flight activity. The major peak of flight occurs in the spring (September to November) and a smaller peak of flight occurs during autumn (March to May). This behaviour has been exploited in the past to control virus diseases transmitted by seasonally active aerial vectors (Stubbs, 1948; Harpaz, 1961; Booker, 1963; Shands et al., 1972; Abu Salih et al., 1973; Johnstone and Rapley, 1979). The area where the experiments described in this chapter were carried out (Chapter 3) had a winter-spring growing season and sowing commenced after the first rains. At present this method of control of virus spread cannot be used because sowing the V.faba L. seeds

after the spring peak flight of aphids is not possible unless irrigation is practised. Since the crop under irrigation is not economical at this region, the other possible methods were considered to control aphid population and movement of spread of BYMV and SCRLV.

Insecticide and other treatments to control plant virus diseases are directed towards preventing the migration of alatae and thus the spread of viruses from source plants to healthy plants. Such treatments would be effective only if the aphids were prevented from feeding on treated plants or alatae were killed before they transmitted the virus (Randles, 1961). Generally, therefore, insecticides are not effective in controlling non-persistently transmitted viruses because acquisition and inoculation occur before insecticides are effective. Nevertheless, the economic control of persistent viruses in some crops (e.g. potato, sugarbeet, cereals) with insecticides has been reported (Broadbent, 1957; Smith et al., 1964; Close, 1967; Rochow and Duffus, 1981; Matthews, 1981; Johnstone and Rapley, 1981). Instances of failure were also reported (Broadbent, 1957; 1965) and success appears to depend on whether the infection results from the activity of alate or apterae. Insecticides are often effective in decreasing spread by apterae within crops but do not affect movement of viruliferous alate into the crop from outside sources (Broadbent et al., 1960; Till, 1971; Johnstone and Rapley, 1981). Prevention of spread of non-persistent viruses through vector control using insecticides was not effective (Kuhn et al., 1975) because with these viruses transmission is almost immediate (Broadbent, 1957; Burt et al., 1964; Webley and Stone 1972; Loebenstein and Raccah, 1980; Boiteau and Wood, 1982).

Using barriers to prevent vectors from approaching their target plants has been practised in the past. Broadbent (1969); Loebenstein and Raccah (1980), pointed out that barriers are more effective in protecting plants from non-persistent viruses than persistent viruses because aphids will lose the non-persistent virus when they probe the barrier plants.

To determine the effectiveness of the treatments in each plot, field sampling of the crop was necessary.

Sampling of insect populations may be either extensive or intensive. Extensive sampling is used to survey large areas, whereas intensive sampling stresses the continued sampling of a population through time within a smaller area or plot (Morris, 1960; Strickland, 1961). In this study I was only concerned with intensive sampling.

Several methods have been described for estimating aphid abundance on plants. The techniques for sampling aphid populations on plants can be divided into five categories (Heathcote, 1972). They are direct observations, plant clippings, sweep nets, suction net samplers and ground cloths. Heathcote, (1972) reported that suction samples are not suitable for extracting aphids from broadleaf plants. The sweep net is not efficient because samples give low estimates of aphid population (Fention and Howell, 1957) and several factors influence catches (Saugstad et al., 1967). Irwin (1980) reported that aphids colonizing soybean can be sampled more satisfactorily by plant clipping and extracting or by direct observation. Further he also stated that the plant parts selected for sampling depend mainly upon the colonizing behaviour of the aphid species. Tanaka (1957) found that M.persicae (Sulz.) green race was most

Fig. 18 The plot layout for the virus control trial showing the five blocks (each 52.5m long, 5.5m wide) of V.faba L. plants. (a) Space between plants was 55 cm and between blocks 5m. The tall dark green plants along the infected row are the barley barriers, (b) shows the infector row in one of the blocks (arrow).


a
b

numerous on the old leaves of cabbage, whereas the pink form was most numerous on young leaves, indicating that different races of the same species of aphid are distributed differently in the same plant.

Way and Heathcote (1966) studied the population of Aphis fabae (Scop.) on V.faba L. by categorizing infestations on stems (extremely light, very light, light, medium or heavy), where the method used by Banks (1954); Gutierrez et al. (1971) and Mohammed (1980) used plant terminals and whole plants to study Aphis craccivora Koch. populations in the pasture legumes and V.faba L. respectively. Johnstone and Rapley (1979, 1981) studied the degree of infestation of Aulacorthum solani (Kltb.) on V.faba L. by brushing them from each plant and clipping whole plants.

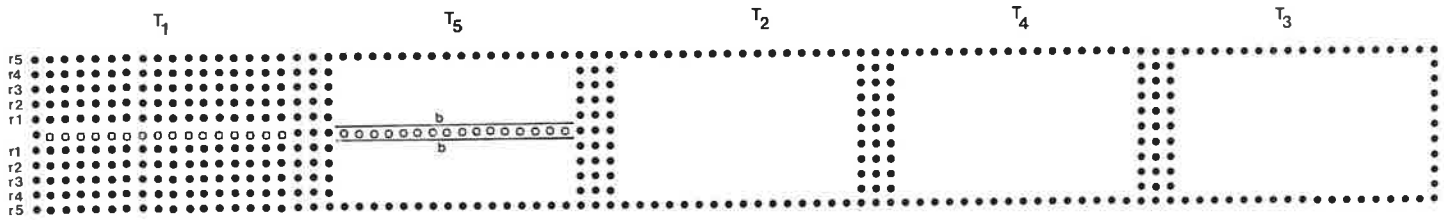
The purpose of the experiment, discussed in this chapter, was to observe the effects of several insecticides and other treatments on aphid activity and the comparative spread of BYMV and SCRLV.

5.2 MATERIALS AND METHODS

5.2.1 Establishment of V.faba L. plots

The experiment was conducted in the winter spring main crop growing season in South Australia during 1981 at the Charlick Experiment Station, Strathalbyn. Prior to planting V.faba L. line 383 the seeds were treated with Nodulaid group E and Benlate as described in Section 3.2.1. The seeds were planted by hand (at 2 seeds per hill) on 9 June 1981, in 5 blocks which were 5m. apart (Fig. 18a). The spacing between plants was

Fig. 19 Detailed sketch of one block divided into five plots which received 5 treatments (T_1 to T_5). The plots were separated from each other by 2 rows of V.faba L. Open circles indicate the position of plants doubly infected with BYMV and SCRLV and both of their vectors (A.craccivora Koch; A.solani (Kltb.) respectively); b shows the position of the two barley barrier rows placed between the infector row and the adjacent row. Solid circles indicate the positions of healthy plants; r and T represents row and treatment. Only one plot (T_1) is drawn completely.


T1
T5
T2
T4
T3
r5
r4
r3
r2
r1
b
b
r1
r2
r3
r4
r5

55 cm. Each block was 52.5m. long and 5.5m wide (Fig. 18a). There were 11 rows (spaced 55 cm. apart) in each block and each block was divided into 5 plots, end to end in one line. Each plot in each block was separated from adjacent plots by 2 guard rows (Fig. 19).

No fertilizer was applied. To control winter weeds Glyphosate was used once on 13 July (for this procedure see Section 3.2.1). The plots were furrow irrigated on 16 September and 10 October when some plants started to show slight wilting. Plants in the 6th row (centre row from either side) were inoculated at the two leaf stage (3 July) (Fig.18b). In each plot, 17 plants at the 6th row, leaving one plant each from both ends (so plots were separated from each other by two rows of V.faba L. plants) were inoculated with BYMV and SCRLV by placing infected stem pieces (V.faba L.) containing viruliferous A.craccivora Koch. and A.solani (Kltb.) (approximately 50 of each species per plant) as described in Section 3.2.3. After placing the aphid infested stem pieces on the plants, plants were covered with plastic pots in which the top was covered with muslin cloth for one week (10 July) and later removed to allow aphids to infect and infest the plants.

5.2.2 Treatments

The 5 treatments were replicated 5 times and allotted at random to 10.45 m. x 5.5 m. plots. The treatments consisted of one granular insecticide, two foliar insecticides, one barrier crop and one untreated plot.

Table 13 shows the 3 insecticide treatments applied. Two properties were considered when selecting the insecticides, firstly their known ability to control some species of aphids and secondly their degree of persistence on foliage.

The granular formulation (Disyston) was applied (28 kg/ha) 2 cm below the seeds at planting time. The other two foliar insecticides (Malathion and Metasystox) were first applied when there was 90 percent emergence (26 June) using a "Solo hand jet-445E" knapsack sprayer and then every 14 or 28 days respectively. Throughout the spray programme the manufacturers recommendations were followed. For both Malathion and Metasystox this was 1.1 l per ha (Table 13). All the insecticides were applied as simple emulsions, in water with a wetter (Table 13). The plants were sprayed to run-off and the undersides of the leaves were covered as thoroughly as possible. The central infector plants were not sprayed and Disyston granules were not applied. All spraying was done at early morning to minimize drift to other plots. Hordeum vulgare L.cv. Clipper was grown as a dense barrier crop in two rows, 25 cm away from either side of the infector row. Seeds were soaked for 24 hours and were sown 2 weeks before the V.faba L. seeds were planted so that they emerged before the V.faba L. seedlings.

5.2.3 Sampling of aphids in the V.faba L. trial plots

M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. and A.solani (Kltb.) colonized V.faba L. at different sites. For example A.craccivora Koch. and A.solani (Kltb.) preferred to colonize the terminal part

Table 13: Active constituent of the insecticides in the 1981 experiment, rate of applicable, and the date of application.

Insecticide	Active constituent	Source	Rate	Date of application
Disyston (R)5	50 g/kg Disulfoton	Bayer, Aust.	29 kg/ha	9/6
Metasystox(R)(I)	250 g/l.(25% w.v.) demeton-s-methyl	Bayer, Aust.	1.1 /ha	26/6, 24/7, 21/8, 18/9, 16/10
Malathion	Maldison 50	Chemical Recovery Co., South Aust.	1.1 /ha	26/6, 10/7, 24/7, 7/8, 21/8, 4/9, 18/9, 2/10, 16/10
Wetting Agent				
Agral (R)60	600g/ phenol ethylene oxide	ICI Aust.	10ml/100	every spraying

(growing point) and the under surface of lower leaves of the plant respectively. Since different species of aphids differed in their distribution on the plant, to obtain a better estimate of the population it was appropriate for the whole plant to be clipped from ground level.

In this study aphid populations were assessed through direct observation and removing parts of V.faba L. For plants with low aphid numbers whole plant counts were made (10 plants per plot) in situ; but at higher infestation rates, leaves with stems (shoots) were sampled at random (using random numbers) for each plot. Samples were placed in brown paper bags and transported back to the laboratory. These samples were immediately placed in the cold room ($5^{o}C$) and counting was done within 2 to 3 days.

Each time samples were taken the day before insecticide was sprayed.

5.2.4 Extraction of aphids from plant shoots

Heathcote (1972) described several ways of extracting aphids and small insects from leaves, stems, soil plants and surface trash by using slow acting toxicants or anaesthetics, gradients of light, and heat or brushing. Hussein (1982) showed that 60 min. exposure at $50^{o}C$ is the best to extract aphids from potato leaves. I have used Hussein's (1982) method to extract the aphid from V.faba L.shoots. Shoots in paper bags, were placed in a drying cabinet at $50^{o}C$ for 1 hr. Later the shoots were shaken on to a white sheet of paper and aphids were collected into a tube containing 70% ethyl alcohol for

identification.

5.2.5 Counting and identification of aphids

Aphids from stem samples were classified into adult apterae and alatae and identified as to species (M.persicae (Sulz.), M.euphorbiae (Thomas), A.craccivora Koch. and A.solani (Kltb.)

The separation of adult apterous aphids from nymphal instars was made visually, based on body length, number of antennal segments, shape of antennal tubercle, rostrum length, cornicle length and shape, caudal size and shape (Cottier,1953). The aphids were identified using the descriptions of Cottier (1953) and the pictorial field key of MacGillivray (1979). Large aphid populations were subsampled by the procedure described in Section 4.1.4.

5.2.6 Survey procedure

All the plants in the 5 blocks were inspected for visible BYMV and SCRLV symptoms 6 weeks after the plastic pots were removed from the centre infector row, then at bi-weekly intervals. The first observation was done on 21 August 1981. The observations were terminated when the symptoms could not be recognized because of senescence of the plants. The mean percentage of infection in each row was determined (see Section 3.2.5)

5.2.7 Indexing of diseased plants

Cuttings (3 to 4 per plot) were taken from diseased plants in untreated plots, brought back to the glasshouse for indexing in polythene bags chilled on ice.

These cuttings were indexed on indicator plants to confirm the identity of BYMV and SCRLV as described in section 3.2.6

5.3 RESULTS AND DISCUSSION

5.3.1 Confirmation of BYMV and SCRLV in diseased V.faba L. in the experimental plots in 1981

Cuttings brought back to the glasshouse showed typical symptoms of BYMV SCRLV when they were indexed on their respective indicator plants (see Section 3.3.1). No other virus diseases were found in the trial plots.

5.3.2 Effect of different treatments on aphid population

Table 14 shows the number of adult apterous and alate aphids per 50 shoots for each of the 5 sampling times and for each of the treatments. A.solani (Kltb.) and A.craccivora Koch. were the first apterous aphids observed in the plots on the 21.8.81 but alate M.persicae (Sulz.), M.euphorbiae (Thomas), A.solani (Kltb.) and A.craccivora Koch. were not present (Table 14). This suggests that the apterous A.solani (Kltb.) and A.craccivora Koch. have moved from the infector row where these two aphid species were introduced artificially and not from the outside area (Fig.15). Conversely observations on 3.9.81 showed that alate M.persicae (Sulz.) and M.euphorbiae (Thomas) were present before the apterae suggesting that alate may have migrated from outside the plots and that some of the alates could have colonized and produced apterae (Table 14).

The plots which received Disyston, Metasystox and Malathion had fewer total aphids than the untreated plots and the plots with barley barriers (Table 14). Initially the

Table 14: Number of aphids per 50 shoots on five occasions

Date and treatment	M.p adult * Ap.	Al.	M.e adult Ap.	Al.	A.s adult Ap.	Al.	A.c adult Ap.	Al.	Other species including young of Mp;Me;As;Ac;	Total aphids (all 5 replicates)	Mean total aphids	
21 August												
Control	0	0	0	0	6	0	3	0	0	9	1.8	
Disyston	0	0	0	0	1	0	0	0	0	1	0.2	
Metasystox	0	0	0	0	1	0	0	0	0	1	0.2	
Malathion	0	0	0	0	4	0	1	0	0	5	1.0	
Barley barrier	0	0	0	0	3	0	2	0	0	5	1.0	
3 September												
Control	0	5	0	1	4	0	0	4	0	14	2.8(1.757)+	a
Disyston	0	0	0	1	1	0	0	7	0	9	1.8(1.443)	ab
Metasystox	0	0	0	1	0	0	0	4	1	6	1.2(1.264)	abc
Malathion	0	2	0	0	4	0	0	5	5	16	3.2(1.887)	abd
Barley barrier	0	2	0	3	11	0	2	11	23	52	10.4(3.275)	e

Contd..

Continuation of Table 14.

Date and treatment	M.p+ adult		M.e adult		A.s adult		A.c adult		Other species including young of Mp;Me;As;Ac;	Total aphids (all 5 replicates)	Mean total aphids	
	Ap.*	Al.	Ap.	Al.	Ap.	Al.	Ap.	Al.				
17 September												
Control	0	60	14	8	24	0	12	104	308	530	106.0(10.28)+	a
Disyston	0	44	4	15	7	0	16	109	152	347	69.4(8.31)	ab
Metasystox	0	77	5	8	4	1	22	122	255	495	99.0(9.81)	abc
Malathion	0	42	15	15	55	0	3	56	70	256	51.2(7.03)	bd
Barley barrier	0	61	6	26	41	1	50	109	211	505	101.0(9.9)	abc
1 October												
Control	106	114	64	50	162	8	159	82	1763	2508	501.6(6.06)++	a
Disyston	76	64	4	64	50	4	66	61	997	1390	278.0(5.44)	ab
Metasystox	22	48	0	27	8	4	3	43	148	303	60.6(3.97)	c
Malathion	96	92	18	39	122	0	52	72	720	1211	242.2(5.48)	abd
Barley barrier	108	64	76	32	104	4	178	178	1704	2448	489.6(6.14)	ad

Continuation of Table 14.

Date and treatment	M.p+ adult		M.e. adult		A.s. adult		A.c adult		Other species including young of Mp;Me;As;Ac;	Total aphids (all 5 replicates)	Mean total aphids	
	Ap.*	Al.	Ap.	Al.	Ap.	Al.	Ap.	Al.				
15 October												
Control	172	120	71	48	50	2	166	10	2709	3348	669.6(6.20)++	a
Disyston	104	39	14	45	41	2	52	4	703	1004	200.8(5.16)	b
Metasystox	4	7	2	25	2	1	1	3	158	203	40.6(3.67)	c
Malathion	190	98	12	36	89	2	80	28	1633	2168	433.6(6.06)	ad
Barley barrier	72	24	24	82	72	4	75	10	1179	1542	308.4(5.61)	abd

Note: Numbers in the same column, and the same time of count group, followed by the same letter are not significantly different at P=0.05 according to L.S.D. test.

+Mp = Myzus persicae; Me = Macrosiphum euphorbiae
As = Aulacorthum solani; Ac = Aphis craccivora

*Ap = Apterae; Al = Alate.

† = Data in parenthesis are square root transformation values (sqrt (aphid+.5)).

†† = Data in parenthesis are natural log transformation values (log (aphid+1)).

	L.S.D. at 5%
3/9/81	0.6077
17/9/81	2.1653
1/10/81	0.6862
15/10/81	0.8281

Disyston granules controlled aphids as effectively as Metasystox but later (17.9.81 to 15.10.81) the number of apterae observed in Metasystox sprayed plots were less compared to other treatments followed by Disyston (Table 14). This suggests that Metasystox and Disyston have prevented aphid colonization to a greater extent than the other treatments used. Taso and Clark (1961) found that Disyston leached downward and to a lesser extent laterally from treated cotton seeds. Shorey (1963) found that Disyston was effective in controlling M.persicae (Sulz.) for up to 3 months after application to pepper plants. So it is possible that the initially reduced aphid population on plots treated with Disyston was due to absorption by roots of the insecticide released from granules. There was no difference (P = 0.05) in numbers of living aphids between Disyston and Metasystox at initial stages but later there were less aphids in Metasystox plots than in Disyston (P = .05). Metasystox was superior (P = .05) to Malathion, barrier and untreated plots in suppressing the aphid population in the plots.

Initially bi-weekly spraying with Malathion was effective in reducing the aphids compared to untreated and barrier plots but in the last two samplings Malathion did not reduce the aphid population below that of the untreated plots.

There was no significant difference between the numbers of aphids in untreated and barrier plots.

5.3.3 The effect of insecticide treatments and barrier rows on virus incidence

The incidence of BYMV and SCRLV on five occasions is shown in Table 15. Data in Table 15 show that on the first two occasions the incidence was less with a gradual build-up on the last three occasions in all five treatments.

The plots receiving Disyston and Metasystox insecticides had significantly (P = 10% and P = 5% level) less SCRLV incidence than untreated and barrier plots. This suggests that insecticides used in the plots reduced spread of viruliferous aphids from diseased to healthy plants. However, there was no significant difference between Metasystox and Disyston at P = 5% and 10% level. The greater incidence of virus in Disyston treated plots agrees with the greater number of aphids found in these plots in late samples (Table 14). Conversely 4 weekly Metasystox sprays ~~controlled aphids~~ had resulted in low virus incidence throughout the season. Metasystox was superior (P = 10% and 5% level) to Malathion, barrier and untreated plots in reducing the incidence of SCRLV. Johnstone and Rapley (1981) reported that Metasystox spraying efficiently reduced the incidence of SCRLV compared with unsprayed plots. However, the incidence of SCRLV infection in Malathion treated plots was not different from Disyston, barrier or untreated plots. Barriers did not reduce the incidence of SCRLV at P = 5% and P = 10% levels.

The results in Table 15 show that a barley barrier did not reduce the incidence of BYMV at P = 5% level, but it may have delayed introduction of BYMV (see 17/9 data). The pattern in

Table 15: Effect of different treatments on accumulated incidence of SCRLV and BYMV on five occasions

Virus	Treatment	Days				
		21/8	3/9	17/9	1/10	15/10
BYMV	Control	0	0.1	2.83(7.91)cf	14.7(20.6)a	47.0 ad
	Disyston	0	0	1.02(4.25)abde	13.6(19.0)a	42.3 ad
	Metasystox	0	0	0.60(2.78)ad	18.1(22.7)a	56.2 bd
	Malathion	0	0.3	2.13(6.0) bcef	13.1(17.8)a	49.5 ad
	Barley barrier	0	0	0.53(2.62)ad	10.6(17.8)a	33.7 ad
SCRLV	Control	0	4.6	35.3(36.0)ce	55.1(48.1)ce	69.8 cf
	Disyston	0	2.3	16.7(23.1)ad	39.4(38.8)bde	46.4 abde
	Metasystox	0	1.7	15.5(21.9)ad	26.6(30.7)ad	35.2 ad
	Malathion	0	4.8	27.9(30.4)bcde	49.8(44.9)bce	59.5bcef
	Barley barrier	0	3.4	24.4(27.9)abd	48.9(44.3)bce	61.1cef

Note: Data in parenthesis are angular transformed values. Numbers in the same column followed by the same letter are not significantly different at P = 0.1 (a to c) and at P = 0.05 (d to f) according to L.S.D. test.

BYMV	treatment variance		6.19	0.49	6.22
SCRLV	treatment variance		3.72	4.63	5.73
For BYMV:		5%	2.70		25.19
	LSD	10%	2.23		20.83
For SCRLV:		5%	8.88	9.49	16.75
	LSD	10%	7.31	7.82	13.85

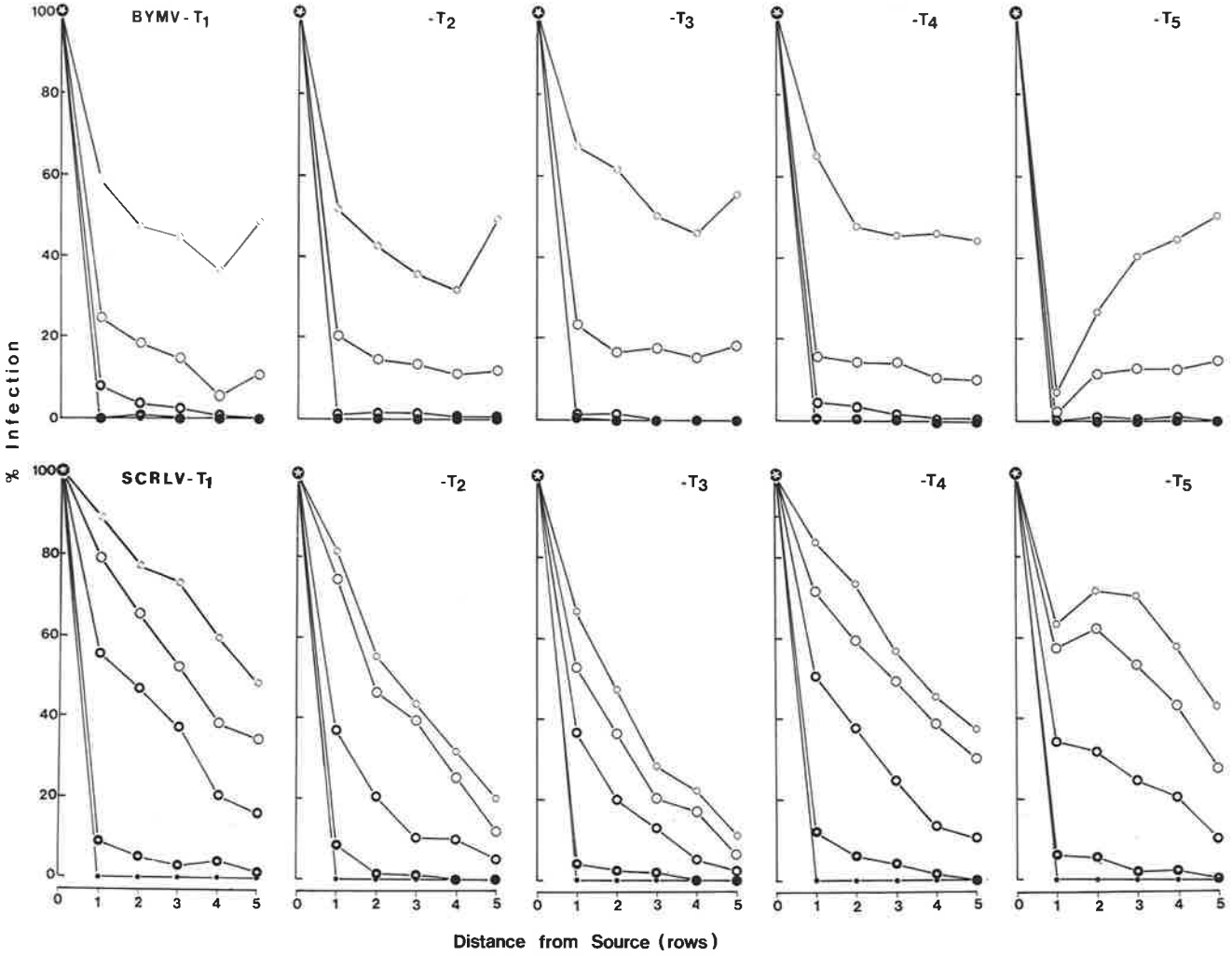
Fig.20 T_5 however shows that the barley barrier provided protection for the row adjacent to the central infector row, but it did not influence spread of BYMV into the outer rows.

Using Disyston at planting time or spraying Malathion at bi-weekly intervals was not effective in reducing the incidence of BYMV compared with untreated and barrier plots (Table 15) (P = 0.1) suggesting that aphids can acquire and transmit BYMV more quickly than they are killed by the insecticide . These results agree with the work done with other non-persistent viruses to control them with contact and systemic insecticides (Broadbent et al.,1956); Burt et al., 1960; Randles, 1961; Webley and Stone, 1972; Kuhn et al.,1975; Ferro et al., 1980; Gabriel et al., 1981). Table 15 shows that Metasystox sprayed plots had a higher incidence of BYMV compared with Disyston, Malathion, barrier and untreated plots, at P = 10% level, but not at P = 5% level. A similar situation was observed by Randles (1961) when he sprayed with insecticides to control cauliflower mosaic virus. Insecticides have been shown to increase incidence of virus infection for various reasons including: a wetting agent sprayed on leaves may lead to increased aphid probing (Heathcote, 1955), paration treated tobacco leaves attracted more aphids than untreated leaves (Shanks, 1960) and insecticide treatments may cause aphids to move from plant to plant more often (Broadbent et al., 1963).

5.3.4 Effect of treatments on the spread of SCRLV and BYMV

Fig. 20 shows the pattern of spread of BYMV and SCRLV

Fig. 20 Spread of BYMV and SCRLV in treatments T_1 (control); T_2 (Disyston granules); T_3 (Metasystox); T_4 (Malathion) and T_5 (barley barrier) (see Fig. 19). Results are expressed as the mean incidence of disease (for 5 replicates) in rows from the central infected row (✪) and are plotted with increasing time after inoculation of source plants. First data is shown at 6 weeks (●) after the time the central row was inoculated with both viruses and then at 2 weekly intervals in the series ● , ◉ , ○ , ○ .


BYMV - T1
-T2
-T3
-T4
-T5
SCRLV - T1
-T2
-T3
-T4
-T5
% Infection
100
80
60
40
20
0
0 1 2 3 4 5
Distance from Source (rows)

in plots subjected to five treatments. No appreciable decrease in the spread of BYMV and SCRLV was observed following the use of the insecticides (Fig. 20). A possible explanation for this is that the insecticides sprayed on the test plants were not quick enough to kill the aphids which were moving away from the unsprayed infector rows, 55 cm away from the 1st row of test plants.

There was a well defined gradient of the incidence of SCRLV away from the source (Fig. 20). SCRLV spread was observed in all five treatments two weeks before the initial BYMV spread occurred. At all times (last 4 observations) SCRLV spread was high from row 1 (centre) to row 5 (outside) in control and Malathion treated plots when compared to Disyston and Metasystox treated plots. This is possibly due to more apterous A.solani (Kltb.) being present in the control and their survival in the Malathion treated plots (Table 14).

At the second and third observations, Disyston and Metasystox treated plots had essentially identical patterns of virus spread. However, at the 4th and 5th observation times virus spread was higher in Disyston treated plots compared to the Metasystox treated plots. Aphids counts in the Disyston treated plots (Table 14) showed gradual increase of A.solani (Kltb.) apterae, compared to the Metasystox treated plots. This suggests that Disyston granules were effective in suppressing aphid build-up and thereby reducing virus spread for a limited period from the time of its application to the soil (see Section 5.3.2).

In plots with a barley barrier, SCRLV spread was very

similar to SCRLV spread in the plots receiving the other treatments. Less disease was observed in the first row (adjacent to barley barrier) compared to the 2nd row. A possible explanation for this is that the 1st row (close to infector row) was overgrown by the spreading habit of growth of the barley such that the barley protected the 1st row. Consequently, winged aphids leaving the infector row would alight on the first row less frequently than on the other rows.

None of the treatments except the barrier influenced BYMV spread from the central infected row to nearby healthy plants (Fig. 20). Disyston, Metasystox and control plots had very similar patterns of spread of BYMV. All the insecticide treated plots, as well as the control plots, showed more BYMV spread at the 4th and 5th observations compared to the first three observations (Fig. 20). Further, the last two observations for the Disyston, Metasystox and control plots had a gradual reduction in spread of BYMV from the infector row towards the outside of the plots up to the 4th row and then an increase in disease on the 5th row (outer row). This indicates that BYMV was spreading from the central infector row towards the outer rows, as well as from outside the plot (probably from neighbouring plots) towards inside the plots. Apart from BYMV spreading from the centre row of every plot, in all 5 treatments there was a tendency for more plants to be infected with BYMV in the outside rows. This may have resulted from factors such as a tendency for a certain distance of flight (Maiden flight) by aphids (Harrewijn et al., 1981) which are leaving from infected plants or the attraction of bare ground between plots for aphids

(A'Brook, 1968) some of which are viruliferous.

Fig. 20 shows that at the 5 observation times in the plot with barrier rows, infected plants were in low incidence close to the central infector row compared to those in control or insecticide treated plots. This suggests that, thickly sown barley barrier (nonsusceptible to the BYMV) rows effectively prevent BYMV spread from the central infector row.

The different pattern of spread of SCRLV from BYMV could have been due to low frequency of winged forms of A.solani (Kltb.) during the test (Table 14).

5.4 CONCLUSION

It was apparent from this experiment that using Disyston granules alone at the planting time is not sufficient to suppress the aphid population beyond four months, but Metasystox sprays at 4 weekly intervals have suppressed the aphids more effectively than any other insecticide used in this experiment. During the early stages there was no significant difference ($P = 0.05$) between Disyston and Metasystox (Table 14). Therefore, either insecticide can be used for early aphid control after considering the cost of the chemical. However, none of the insecticides used were able to prevent the virus (BYMV, SCRLV) spread to nearby healthy plants from unsprayed infector row. Perhaps SCRLV spread could have been reduced further if systemic insecticides had been sprayed on the virus source (infector row) to prevent colonization of vector aphids. ~~It is interesting to note that Metasystox spray delay in the spread of SCRLV and losses can be reduced if spread is delayed until plants are older.~~

Insecticides are not effective in controlling the BYMV spread under the conditions tested.

Nonsusceptible barrier crops are useful to control V.faba L. in small plots. An indirect approach to control both these viruses by controlling aphids using parasites and predators during peak aphid activity time is worth investigating.

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CHAPTER 6

CYTOLOGICAL STUDIES

6.1 INTRODUCTION

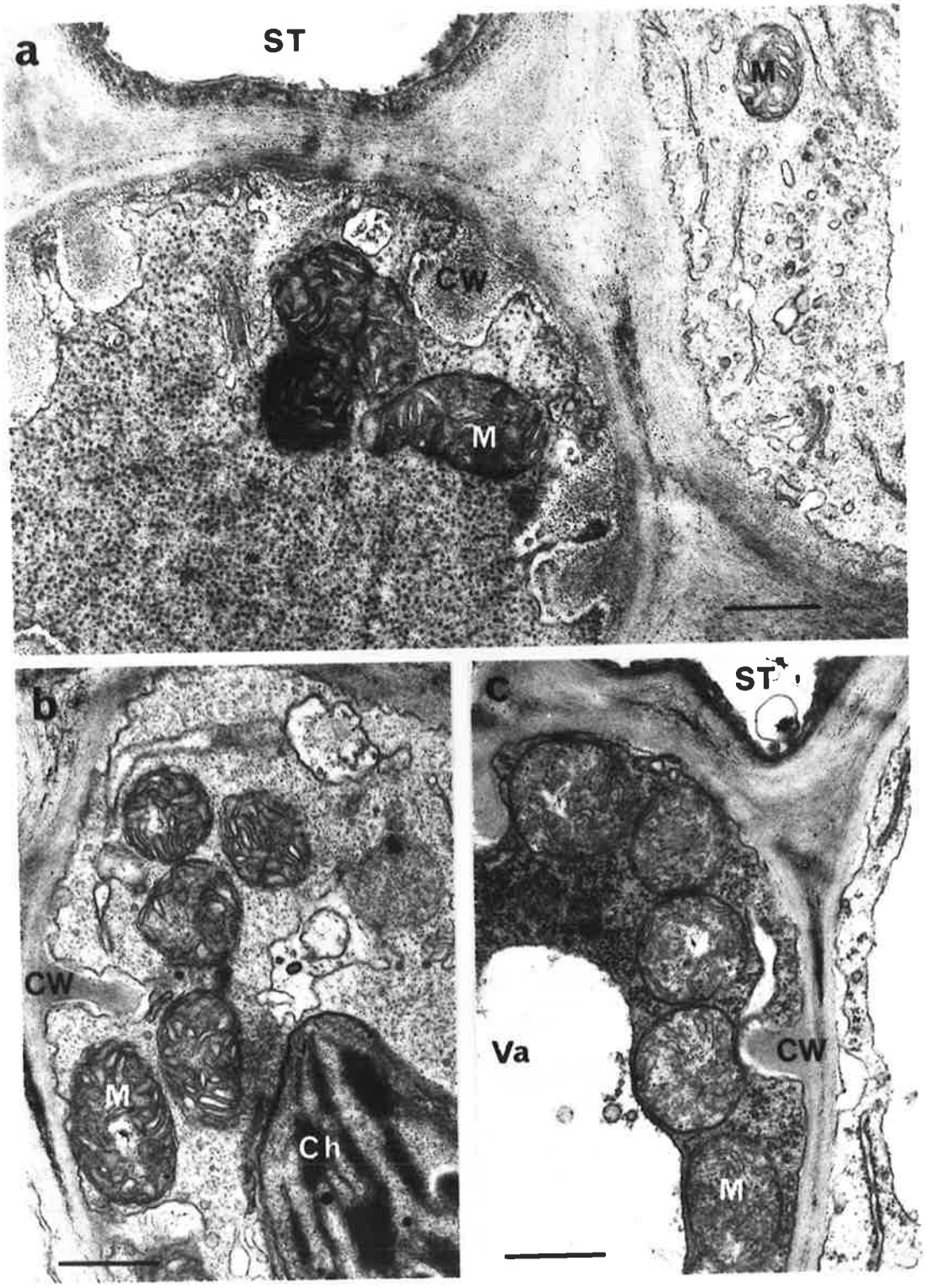
Luteoviruses are apparently confined to the vascular tissue of infected plants (Rochow and Duffus, 1981). Only host range (Teh, 1978) and vector relationships (Kellock, 1971) are known for SCRLV. Virus particles have not been previously observed in SCRLV infected tissue and the main objective of the work described in this chapter was to identify and localize virus particles in tissue.

6.2 MATERIALS AND METHODS

6.2.1 Fixing and embedding the section of leaf tissue for electron microscopy

Leaf tissue pieces about 5 mm square from infected and healthy T.subterraneum L. were fixed for 16 hrs. at 4°C in a mixture of 4% paraformaldehyde and 3% glutaraldehyde in 0.1M phosphate buffer, pH 6.8 (Hatta and Francki, 1981). The fixed pieces of tissue were then cut into 1 mm square pieces placed in SSC buffer (0.15M sodium chloride and 0.015M sodium citrate pH 7) amd washed in SSC buffer 6-7 times at room temprature, changing the buffer at hourly intervals. The sections were divided into two batches and one batch was incubated for 16 hrs. at 25°C in SSC buffer containing pancreatic ribonuclease (Type IIIA, Sigma Chemical Co., St.Louis, Mo.) (concentration 2 µg/ml RNase in SSC buffer) and the other incubated for the same time at 25°C without ribonuclease. They were then postfixed in 1% osmic acid at room

Fig. 21 Thin sections of leaf phloem transfer cells from SCRLV infected (a) and healthy (b, c) subterranean clover plants. Cells in (a) and (b) are from tissues which had been treated with RNase after aldehyde fixation, whereas those in (c) are from untreated tissue. The cell on the left in (a) shows numerous densely stained virus-like particles scattered throughout the cytoplasm, whereas the cell on the right is devoid of such particles. The cell shown in (b) has no darkly stained particles due to RNase treatment, whereas that in (c) contains numerous darkly stained ribosomes in the cytoplasm. CW, Ingrowth of cell wall; M.mitochondrion; ST, sieve tube; Ch. chloroplast; Va, vacuole. Bar markers represent 0.5µm.


a
ST
M
CW
M
b
CW
M
Ch
c
ST
Va
CW
M

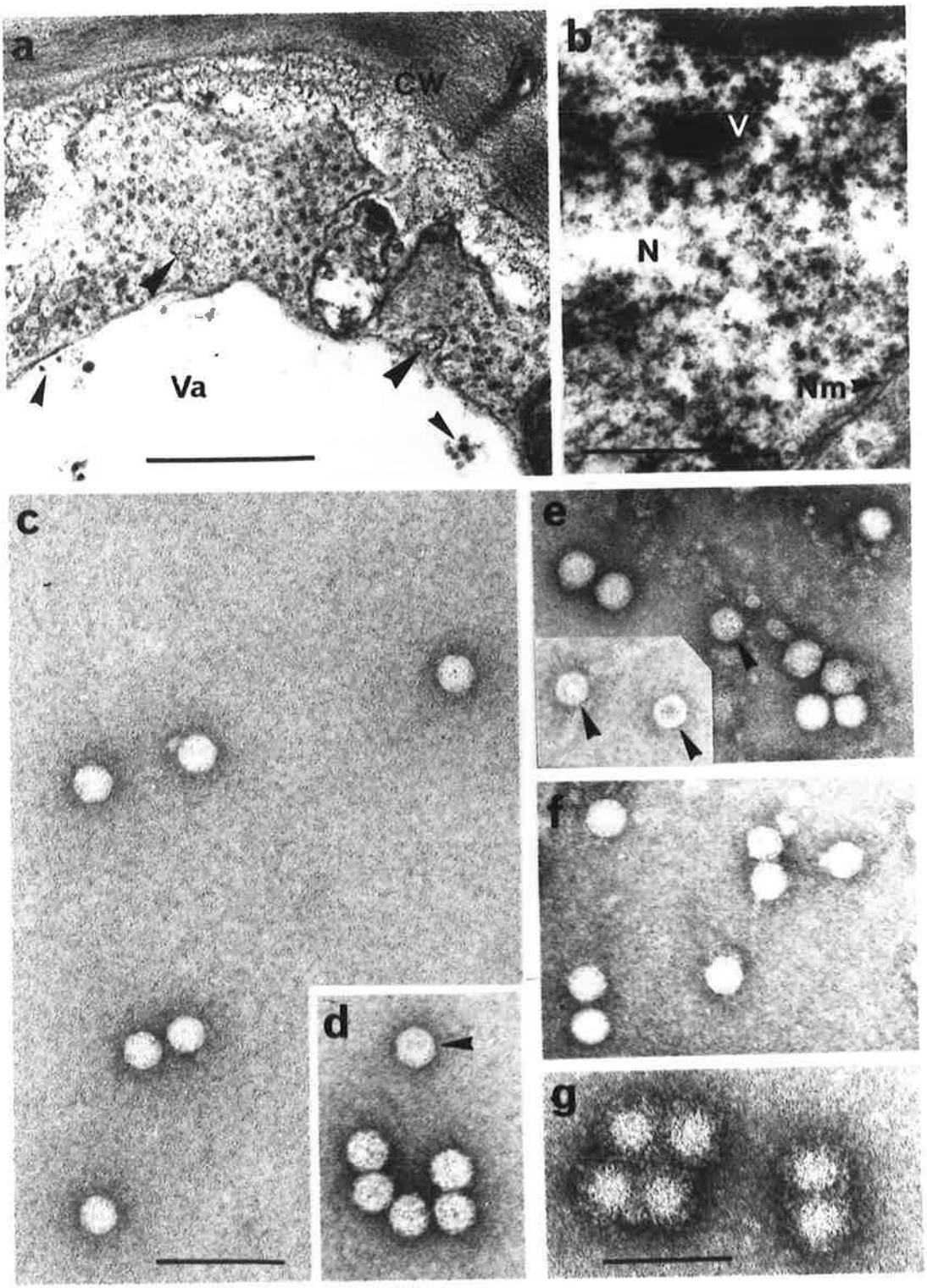
temperature for 3 hours. Dehydration was carried out through a graded ethanol series (Sections were transferred to 70%, then 95% and then 100% ethanol for 5,10 and 10 min. respectively). Sections were then transferred to small tubes containing 100% ethanol and propylene oxide (equal volume) for 5 mins. and then transferred through propylene oxide (3 times) at intervals of 10 mins. They were left in solution containing propylene oxide and epoxy resin (equal volume) for 30 mins. after which they were transferred to epoxy resin solution for 1 hr. at 45°C. They were then transferred to an embedding mould which contained epoxy resin for 16 hrs. at 25°C, followed by 3 days at 60°C (Hayat,1970).

Thin sections were cut from tissue embedded in epoxy resin with an LKB ultratome equipped with a diamond knife. Sections were collected on formvar-coated 400 mesh grids and stained with 2% uranyl acetate for 3 min. then in lead citrate for 30 sec. These grids were examined in a JEM 100 CX electron microscope.

6.3 RESULTS AND DISCUSSION

Examination of thin sections of RNase treated tissue of SCRLV infected plants (Fig. 21a) showed densely stained particles in many of the phloem transfer cells. Cells containing these particles were often located next to cells devoid of densely stained particles (Fig. 21a) indicating that the enzyme treatment was effective in digesting RNA of the ribosomes, but not the densely stained structures which were presumed to be virus particles. Isometric virus particles

Fig. 22 Thin sections of leaf phloem transfer cells from SCRLV-infected subterranean clover plants are shown in (a, b). The cells are from RNase-treated tissue showing virus-like particles scattered in the cytoplasm (a), the vacuole (small arrow in a) and the nucleoplasm (b). CW, Cell wall; Va, vacuole; Nm, nuclear membrane; N, nucleoplasm; V, virus-like particles. Bar markers represent 0.5 μm. Virus-like particles isolated from SCRLV-infected subterranean clover plants are shown in (c) and after mixing with a preparation of RCNMV in (d) (arrow points to a particle from SCRLV-infected plants in d, whereas the remaining particles are those of RCNMV). Bar marker represents 100nm. Virus-like particles isolated from PLRV-infected P.floridana L. plants are shown in (e to g). A partially purified preparation in (e) shows two types of particles, some similar to those in (c) and some smaller and rounder ones (arrows). The same preparation as in (e) is shown in (f) after having been trapped on grids with anti-PLRV serum, and in (g) after having been trapped and decorated with the same antiserum. Bar marker represents 100 nm.


a
CW
Va
b
V
N
Nm
c
d
e
f
g

20 - 24 nm in diameter in infected cells are indistinguishable from cytoplasmic ribosomes (Crowley et al., 1969; Hatta, 1976) when viewed by electron microscopy. Hatta and Francki (1979; 1981); and Randles et al.(1981) demonstrated that virus particles could be distinguished from cytoplasmic ribosomes by digesting ribosomal RNA with RNase. No ribosomes were observed in transfer cells, or the healthy tissue incubated with SSC buffer containing RNase (Fig. 21b). However for healthy tissue not incubated with RNase, ribosomes were observed in the transfer cells (Fig. 21c). The densely stained particles resembling virus were never detected in the cytoplasm of cells from healthy T.subterraneum L. plants.

Similar particles were observed in partially purified preparations from SCRLV infected plants showing red leaf symptoms (see Chapter 7). In infected cells virus like particles were scattered throughout the cytoplasm (Fig. 21a, 22a). In some of the cells, particles were also detected in the nucleus (Fig. 22b) and in vacuoles (Fig. 22a). Transfer cells containing virus-like particles often contained small vesicles with stranded material (larger arrows in Fig. 22a) similar to those observed in barley yellow dwarf virus (BYDV) infected oat leaf cells (Gill and Chong, 1975).

Plants infected with the luteoviruses for example, potato leaf roll virus (Kojima et al., 1969), beet western yellow virus (Esau and Hoefert, 1972; D'Arcy and de Zoetein, 1979) barley yellow dwarf virus (Gill and Chong, 1975) and soybean dwarf virus (Tamada, 1975) have been found to contain virus

particles in the vascular tissues, principally in the phloem. Hatta and Francki (1981) observed RNase resistant particles with morphology and cellular distribution similar to that observed in SCRLV-infected plants for plants infected with potato leaf roll virus.

The observation of virus-like particles in vascular tissues (phloem transfer cells) but not in mesophyll or epidermal cells, together with observations on symptomatology and vector relationships (Matthews, 1979) ~~show~~ show that SCRLV is a member of the luteovirus group.

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CHAPTER 7

PURIFICATION OF SCRLV AND SCRLV-RNA

7.1 INTRODUCTION

Viruses in the luteovirus group can be identified by their host range and transmission tests with aphid vectors. For some viruses e.g. beet western yellows, this procedure is simple, because only one vector species (M.persicae (Sulz.)) is required (Duffus, 1960) but for other viruses such as barley yellow dwarf virus (BYDV) (Rochow, 1967, 1979; Gill,1967, 1969) a single vector species may not suffice. These aphid transmission tests are tedious, require special facilities, take considerable time to complete, and comparative studies between viruses are usually not done. Since viruses belonging to this group occur in low concentration in the infected plant, studies of the relationships among members of this group by serology cannot be readily done because the production of antiserum requires large amounts of pure virus and many serological techniques will not detect cross reactions between the viruses unless they have been concentrated. The location of luteoviruses in the plant and the low yield of virus from plant extracts explains why little is known of the physical and chemical properties of most of the viruses belonging to this group (Takanami and Kubo, 1979a). Therefore success in purification of these viruses depends mainly on which procedure is used to extract the virus particles from the vascular tissue (Rochow and Duffus, 1981) the type of extracting buffer and the use of cellulolytic enzymes (Takanami and Kubo, 1979a).

The mode of transmission (Kellock, 1971; Teh, 1978; Johnstone 1978), types of symptoms produced (Wilson and Close, 1973; Teh, 1978; Ashby et al., 1979) and the nature of the virus-like particles located in the infected plant (see Chapter 6, and Jayasena et al., 1981) have suggested that SCRLV (Johnstone et al., 1982; Ashby and Kyriakou, 1982) is a member of the luteovirus group.

RNA has been obtained from some of the viruses belonging to the luteovirus group e.g. barley yellow dwarf virus (Brakke and Rochow, 1974), potato leaf roll virus (Rowhani and Stace-Smith 1979; Takanami and Kubo, 1979b; Mayo et al., 1982), tobacco necrotic dwarf virus (Takanami and Kubo, 1979b), and pea leaf roll virus (Ashby and Huttinga, 1979). The RNA is single stranded of molecular weight 2.0×10^6 (Rochow and Duffus, 1981). Falk et al (1977) reported the presence of two RNA species for some isolates of beet western yellow virus.

The molecular hybridization essay (MHA) using DNA complementary to viral RNA (cDNA) allows the identification and comparison of viruses on the basis of their RNA nucleotide sequence (Palukaitis and Symons, 1980; Palukaitis et al., 1981a, 1981b; Abu Samah and Randles, 1981). To synthesize cDNA only small amounts of RNA are required and very low concentrations of RNA are detectable (Palukaitis et al., 1981a).

The main objective of this study was to purify SCRLV, and to obtain its RNA for use in systhesizing a cDNA probe (see Chapter 8).

7.2 MATERIALS AND METHODS

7.2.1 Virus propagation

In the present study, infected T.subterraneum L. cv. Mt.Barker or P.sativum L. cv. Puget were used for purifying SCRLV.

Two week old T.subterraneum L. cv. Mt.Barker or P.sativum L. cv. Puget seedlings were inoculated with SCRLV-T by placing viruliferous A.solani (Kltb.) on the plants and allowing them to feed as described in Section 2.3. These plants were kept in a growth cabinet at 20 ± 2°C, (Teh, 1978) and illuminated at 23,000 lux for 16 hrs. per day (Rowhani and Stace-Smith, 1979). Four days after the inoculation, plants were fumigated with Mafu 50 (a.i. 500 g/l (50% w/v) Dichlorvos). Infected whole plants were harvested 4 weeks after inoculation Johnstone et al., (1982) and stored at -70°C for later use.

7.2.2 Partial purification of SCRLV

The purification procedure used by Kojima et al. (1969), Kojima and Tamada (1976), Ashby and Huttings (1979), and Rowhani and Stace-Smith (1979) to purify PLRV, PeLRV, SDV and PLRV respectively, were unsatisfactory for purifying SCRLV because few particles were isolated and detected by electron microscopy. The method of Takanami and Kubo (1979a) was encouraging and was modified as follows. Since Ashby and Kyriakou (1982) found that the use of Cellulase led to equal or better yields than for Driselase in comparative experiments, Driselase was replaced by Cellulase (Onozuka R-10, Yakult Biochemicals, Japan or Type I Sigma Chemical Co.). Preparations

clarified by treatment with chloroform and n-butanol, contained fewer particles than those clarified with Triton X 100 and so the latter was used routinely.

SCRLV was purified by either of two methods, both of which employed cellulase digestion of crude extracts. In method A frozen whole infected plants (500 gm) were ground into a powder in a pestle and mortar in the presence of liquid nitrogen (Brakke and Rochow, 1974). D'Arcy (1978), found that when tissue was frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle, much higher concentrations of purified beet western Yellow virus could be obtained than when the tissue was processed similarly but without the use of liquid nitrogen. The powdered tissue was partially thawed and blended in a Virtis homogeniser for 2 min. with 0.1M citrate buffer (2ml/gm material) pH 6.0 containing 1.5% cellulase (Onozuka R-10) and 0.1% thioglycollic acid. The blender container was immersed in an ice bath. The extract was then incubated at 25°C for 16 hrs. with continuous shaking at 80 oscillations per min. Na N_3 was added to a concentration of 0.02% to prevent bacteria growing. The incubated crude extract was again homogenized using the Virtis homogenizer and then sonicated. The pH was then adjusted to 7 by adding 0.2M Na_2HPO_4 because Tamada and Harrison (1980) found that at a pH below 6.5 much of PLRV was sedimentable at low centrifugal forces. Then Triton X 100 was added to 1% and the extract was kept overnight at 4°C. The extract was then filtered through a muslin cloth and centrifuged at 10,000 rpm for 10 min. in a GSA rotor at 15°C. The supernatant was subjected to two cycles of differential centrifugation to concentrate the virus particles.

The first high speed centrifugation was done in a Spinco 30 rotor at 27,000 rpm for 3 hrs. at 15°C and the pellet was taken up in 0.01M phosphate buffer pH 7.6 and stirred gently overnight in the cold room (4°C). This buffer was used to suspend the pellets in all subsequent steps in the purification. The preparation was then kept at room temperature for 1 hr. before clarification by centrifugation at 10,000 rpm for 10 min in the SS 34 rotor. All centrifugations were done at 15°C to avoid the aggregation of particles that can occur at low temperatures (Takanami and Kubo, 1979a). The supernatant fluid was then layered over 20% (w/v) sucrose (20% sucrose occupied one quarter of the tube) and centrifuged in the Ti 70 rotor for 2½ hrs. at 60,000 rpm at 15°C.

(10-40%) Sucrose gradients in 0.01M phosphate buffer pH 7.6 were prepared in SW 50 tubes using a gradient former and kept overnight at 4°C before use, to allow diffusion to take place. Samples (0.5 ml) of partially purified virus were layered on top of the gradients which were then centrifuged for 3½ hrs. at 28,000 rpm at 15°C. Gradients were fractionated using an ISCO density gradient fractionator and ultraviolet (UV) scanner at 254 nm wave length. The virus-containing fractions from the gradients were collected and diluted with 0.01M phosphate buffer 7.6 and centrifuged at 50,000 rpm for 2 hrs. in the SW 50 rotor to remove sucrose and to pellet virus. For further purification, pellets were resuspended in 0.7 ml 10mM phosphate buffer (pH 7.4) and mixed with 2 ml of buffered Cs_2SO_4 solution (10mM phosphate, pH 7.4) to give a final

density of about 1.26 gm/cm^3. The mixture (2.7 ml) was then placed in tubes ~~containing~~ over 1.8 ml buffered Cs_2SO_4 (density 1.46 gm/cm^3), overlaid with light mineral oil (0.6 ml) and subjected to isopycnic density gradient centrifugation at 42,000 rpm for 18 hrs. in a Spinco SW 50 rotor. Virus particles were collected from a band formed in the central zone (Fig.26a) and sedimented by centrifugation at 240,000 g for 90 min. in a Spinco 65 rotor.

Method B was a modification of method A, which used liquid nitrogen and a pestle and mortar for the initial shearing of P.sativum L. tissue followed by blending in 0.1M sodium citrate pH 4.7, containing 10 mM EDTA, 0.1% thioglycollic acid and 0.5% cellulase (Type I, Sigma Chemical Co.). Extracts were incubated for 3½ hrs. and the mixture was then clarified at 1000 rpm for 15 min. in the Sorvall HG 4L rotor at 15°C before adding polyethylene glycol (PEG) 6000 to 8% and NaCl to 0.4M. The precipitate was collected by low-speed centrifugation (4000 rpm - HG 4L rotor), and resuspended in 10 mM phosphate buffer, pH 7.6 by stirring for 16 hrs. at 4°C. Triton X 100 was added to 1%, the mixture was stirred for a further 2 hrs and then clarified by centrifugation at 10,000 rpm (GSA rotor) for 15 min. at 15°C. A second PEG precipitation step was followed by resuspension, as above, and clarification by centrifugation at 10,000 rpm for 30 min. in the GSA rotor. After fractionation on a 10 - 40% sucrose gradient virus was sedimented by high speed centrifugation, then resuspended and centrifuged to equilibrium in a Cs_2SO_4 gradient and concentrated by high speed centrifugation as

described in method A.

7.2.3 Electron Microscopy

To estimate the virus concentration in different purification methods and for assaying fractions on sucrose gradients, fractions collected from sucrose gradients were placed on an electron microscope grid washed with 25 to 30 drops of distilled water and negatively stained with 2% UAc or PTA and examined in an electron microscope (see Section 2.4). The number of SCRLV particles per standard area (1000 μm^2) was counted, particle numbers were assessed by examining 10 electron microscope fields at 20,000 x magnification (Roberts, 1980).

7.2.4 Measurement of virus particle diameter

Negatively stained virus particles were examined in the electron microscope and photographs were taken at 50,000 magnification. Particle diameter was measured by the procedure described by Randles and Hatta (1979). Initially the instrument was calibrated at 10,000 with a carbon grating replica (2160 lines/mm; Ladd Research Industries Inc.). A small part of the replica was photographed at a magnification of 10,000 to obtain an accurate magnification. The same object was then photographed at an instrumental magnification of 50,000 to calibrate the instrument at this magnification. Particles were measured in photographic prints using a scale attached to a magnifier lens with a resolution of 0.1 mm.

7.2.5 Infectivity assay

The infectivity of the partially purified virus preparations from method A and method B was checked by allowing nonviruliferous A.solani (Klotb.) to feed theough a stretched

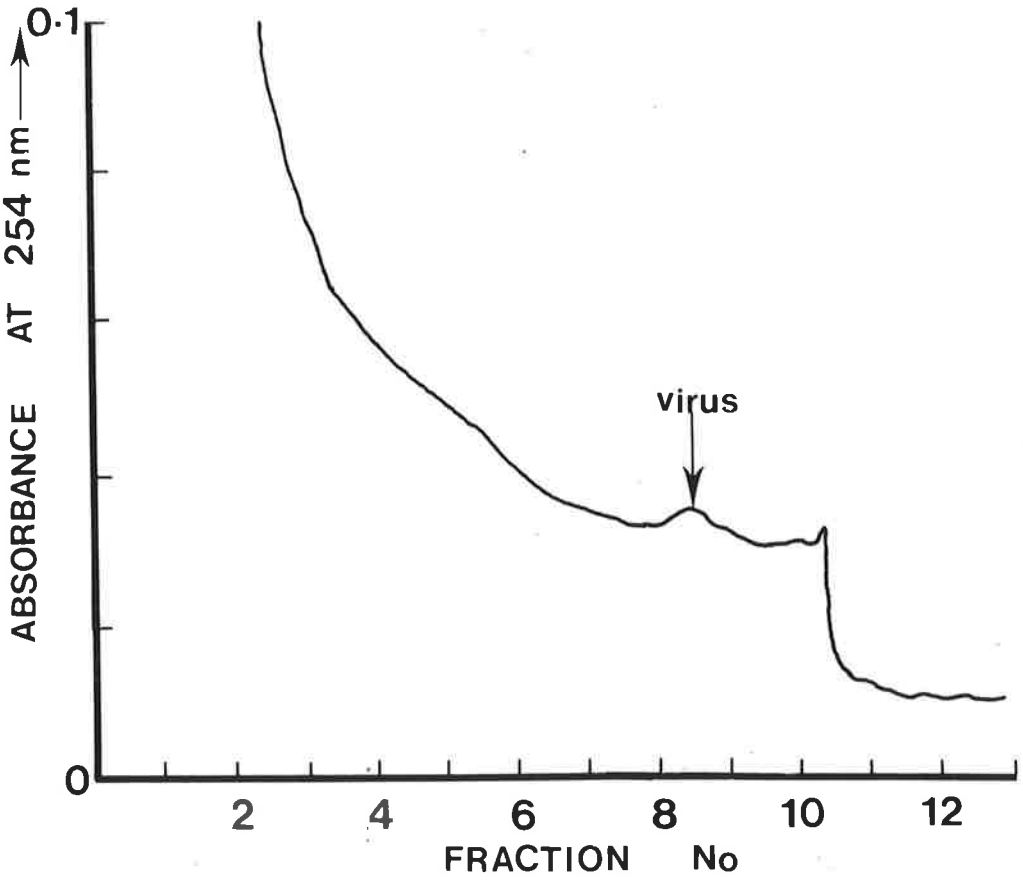
parafilm (Marathon Products) membrane on a drop of virus suspension in 25% sucrose. About 100 aphids were permitted to feed on the virus preparation at room temperature for 24-48 hours before they were placed on two week old T.subterraneum L. cv. Mt. Barker test seedlings in groups of five aphids per seedling. Control aphids were allowed to feed on 25% sucrose solution (without virus suspension) for the same period and then transferred to test plants for 3 days before they were killed by spraying with Malathion.

7.2.6 Immuno electron microscopy (IEM)

The IEM technique was used to determine whether the virus-like particles prepared by method A and B (see Section 7.2.2) were serologically related to SCRLV.

IEM was done by trapping the particles on antiserum treated grids and then using the antibody decoration procedure (Milne and Luisoni, 1977). SCRLV antiserum (Titer 1/512) donated by Dr. J.W. Ashby, Lincoln, New Zealand, was used. Trapping was done by diluting antiserum to 1/1000 in distilled water, placing a drop of diluted antiserum on an ionized carbon coated formvar grid and incubating it for 5 min. in a humid petri dish at room temperature. The grid was washed with distilled water three times at 1 min. intervals. After draining the water from the grid with a piece of filter paper a drop of the virus preparation purified from infected T.subterraneum L. or P.sativum L. was placed and incubated for 15 min. at room temperature. The excess liquid was drained off and trapped particles were decorated by incubating for 30 min. on a drop of SCRLV antiserum diluted 1/100 in distilled water at room

Fig. 23 U.V. absorption patterns of 10 - 40% sucrose gradient containing preparations from T.subterraneum L. infected with SCRLV. SCRLV was prepared from 500 g. of infected tissue. The tissue was homogenized with 0.1M. citrate buffer (2 mℓ/g material) pH 6.0 containing 0.1% thioglycollic acid and 1.5% Cellulase. Pellets resuspended in 0.1 M - phosphate buffer, pH 7.2. The gradients were centrifuged for 3.5 hrs. at 28000 rev/min in SW 50.1 rotor. Virus was detected by E.M.


0·1
ABSORBANCE AT 254 nm
0
virus
2
4
6
8
10
12
FRACTION No

temperature. The grid was then washed again with water and stained with 2% uranyl acetate for 30 sec. and examined in the electron microscope. The same procedure was followed to decorate PLRV with PLRV antiserum (from Dr.Stace-Smith, Agriculture Canada Research Station, Vancouver, British Columbia) and to test PLRV and SCRLV in reciprocal tests.

7.2.7 Nucleic Acid

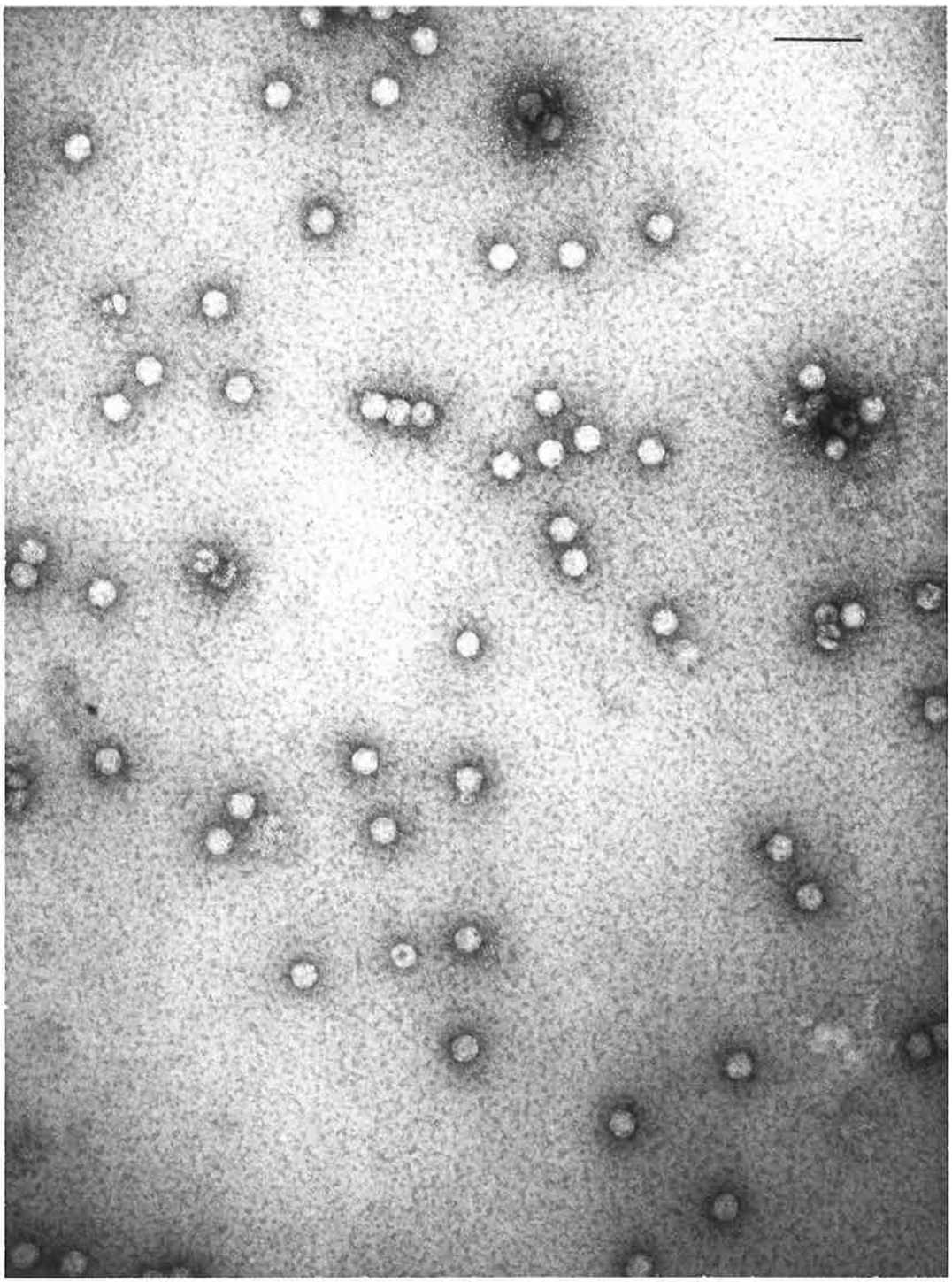
7.2.7.1 Precautions against ribonuclease

All the materials used to extract RNA were treated to eliminate contamination with ribonuclease. Single or double glass distilled water, buffers, solutions of inorganic salts, including SDS, were autoclaved at 120°C, 15 psi for 15 min. Glassware was kept at 130°C in an oven overnight. Heat labile acrylamide was made up in sterile water. Centrifuge tubes, plexiglass tubes and electrophoresis apparatus were sterilised by soaking for 2 min. in a solution containing 2N KOH in 90% ethanol followed by rinsing several times with sterile distilled water immediately before use. Sterilised materials were maintained RNase free by keeping them sealed at room temperature and RNA preparations were stored at - 20°C in small aliquots.

7.2.7.2 Extraction of total nucleic acids from healthy *Nicotiana clevelandii* Gray

N.clevelandii Gray nucleic acids were used as RNA markers. Total leaf nucleic acids from healthy tissues were extracted by the phenol method described by Loening and Ingle (1967). Leaf tissue (10 g) was homogenized with a pestle and

Fig. 24 An electron micrograph of a purified SCRLV preparation negatively stained with 2% uranyl acetate. The bar represents 100 nm.



mortar in equal volumes of 1% sodium dodecyl sulphate (SDS) and 90% phenol by shaking for 10 min. and centrifuging at 10,000 rpm for 10 min (2 times) in the SS 34 rotor. To the supernatant 2M sodium acetate was added to bring the solution to 0.1M, together with three volumes of redistilled ethanol, and it was allowed to stand at -20°C for more than one hour before the RNA precipitate was collected by centrifugation at 10,000 rpm for 15 min. in the SS 34 rotor. The precipitate was washed twice with 95% ethanol to remove phenol. The precipitate was drained, dried, dissolved in 1 ml of sterile distilled water and stored at -20°C.

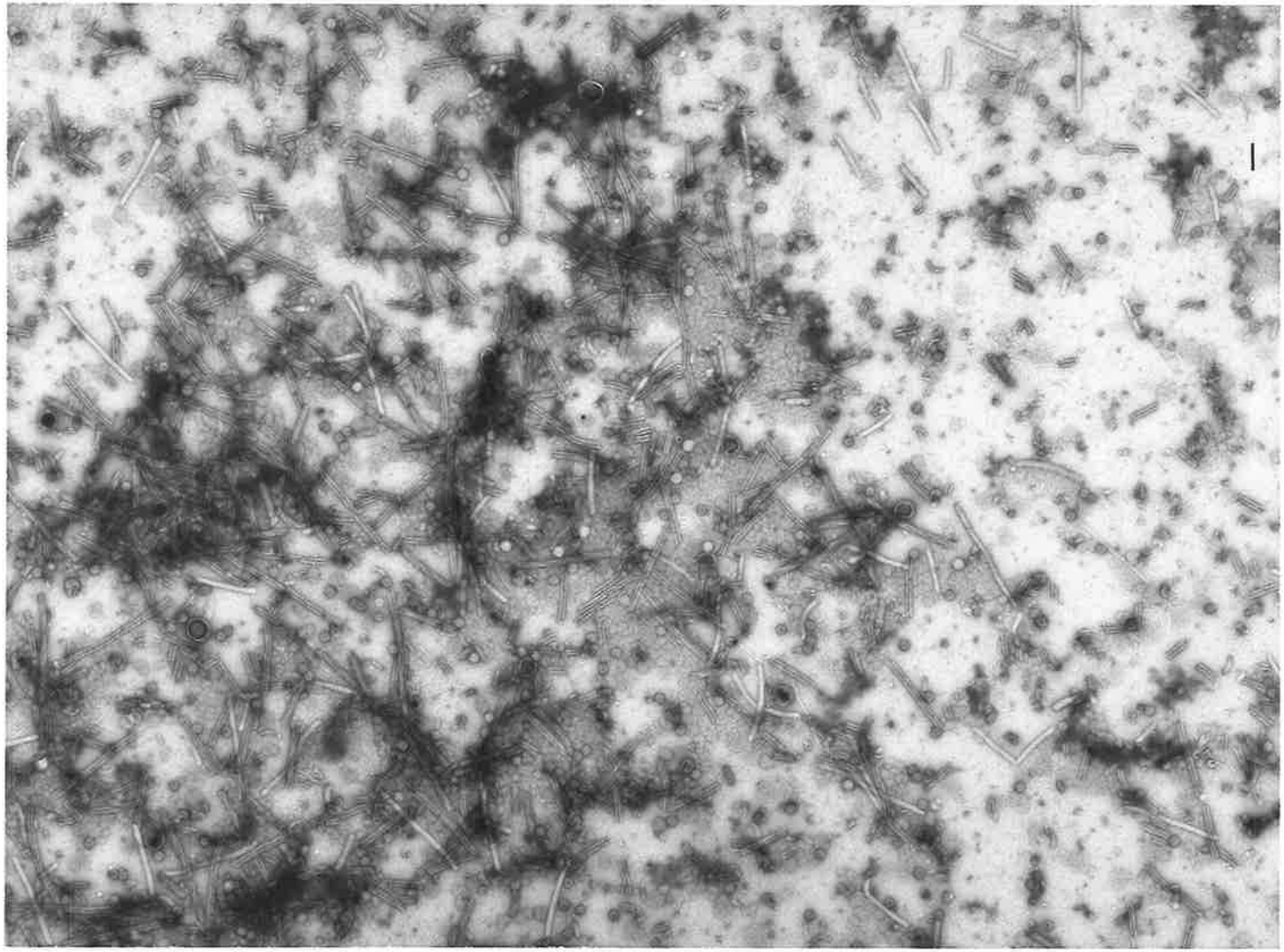
7.2.7.3 Extraction of velvet tobacco mottle virus (VTMoV) RNAs.

VTMoV provided by Dr. P.W.Chu, (Waite Agricultural Research Institute, South Australia) was used for extracting RNA to run as a marker with SCRLV-RNA. The method adopted to extract SCRLV-RNA was also used to extract VTMoV-RNA (see Section 7.2.7.4).

7.2.7.4 Extraction of subterranean clover red leaf virus (SCRLV) RNA

The procedure of Murant et al. (1972) was slightly modified for the extraction of RNA from purified SCRLV (Randles, 1975). The preparation of SCRLV in 0.01M phosphate buffer pH 7.6 was mixed with an equal volume of predigested 0.1% pronase containing 0.5% SDS and 0.1M sodium acetate and incubated for 16 hrs. at 37°C. Nucleic acid was precipitated by adding four volumes of ethanol and leaving for more than 4 hours at -20°C. The precipitated nucleic acid was sedimented by centrifugation at 10,000 rpm for 15 min. in a SS 34 rotor. The pellet was once again washed with ethanol and sedimented by centrifugation at 10,000 rpm for 15 min. and the nucleic acid

Fig. 25 An electron micrograph showing tubules often seen together with SCRLV particles in partially purified preparations negatively stained with 2% Uranyl acetate. The bar represents 100 nm.



pellet was drained, dried and stored at - 20°C.

7.2.7.5 Measurement of nucleic acid concentration

Concentration of purified nucleic acid was measured spectrophotometrically using a Unicam SP-1800 double beam spectrophotometer assuming $A_{260}^{0.1\%} = 25$.

7.2.7.6 Polyacrylamide gel electrophoresis

The nucleic acid isolated from SCRLV which was purified by method A, was analysed by electrophoresis under denaturing conditions in 3.3% polyacrylamide-8M urea slab gels (Air et al., 1976) using the Tris-borate-EDTA (TBE) pH 8.3, buffer system (Peacock and Dingman, 1968).

Electrophoresis in slab gels was done using the Bio-Rad model 220 vertical slab gel electrophoresis apparatus. The slab gel was 1.5 mm thick, 120 mm deep and 140 mm wide. The wells were loaded with the following:

(i) SCRLV RNA 40 µl + 20 µl 50% Glycerol + 20 µl 1% SDS

(ii) VTMoV RNA 2 µl + 20 µl 50% Glycerol + 20 µl 1% SDS

(iii) N.clevelandii RNA 5 µl + 20 µl 50% Glycerol + 20 µl 1% SDS.

The RNA samples were subjected to electrophoresis at 25 mA for 240 min. at room temperature. After electrophoresis the gels were stained with 0.01% toluidine blue (in 5% acetic acid) for 20 min. and destained in several changes of sterile distilled water.

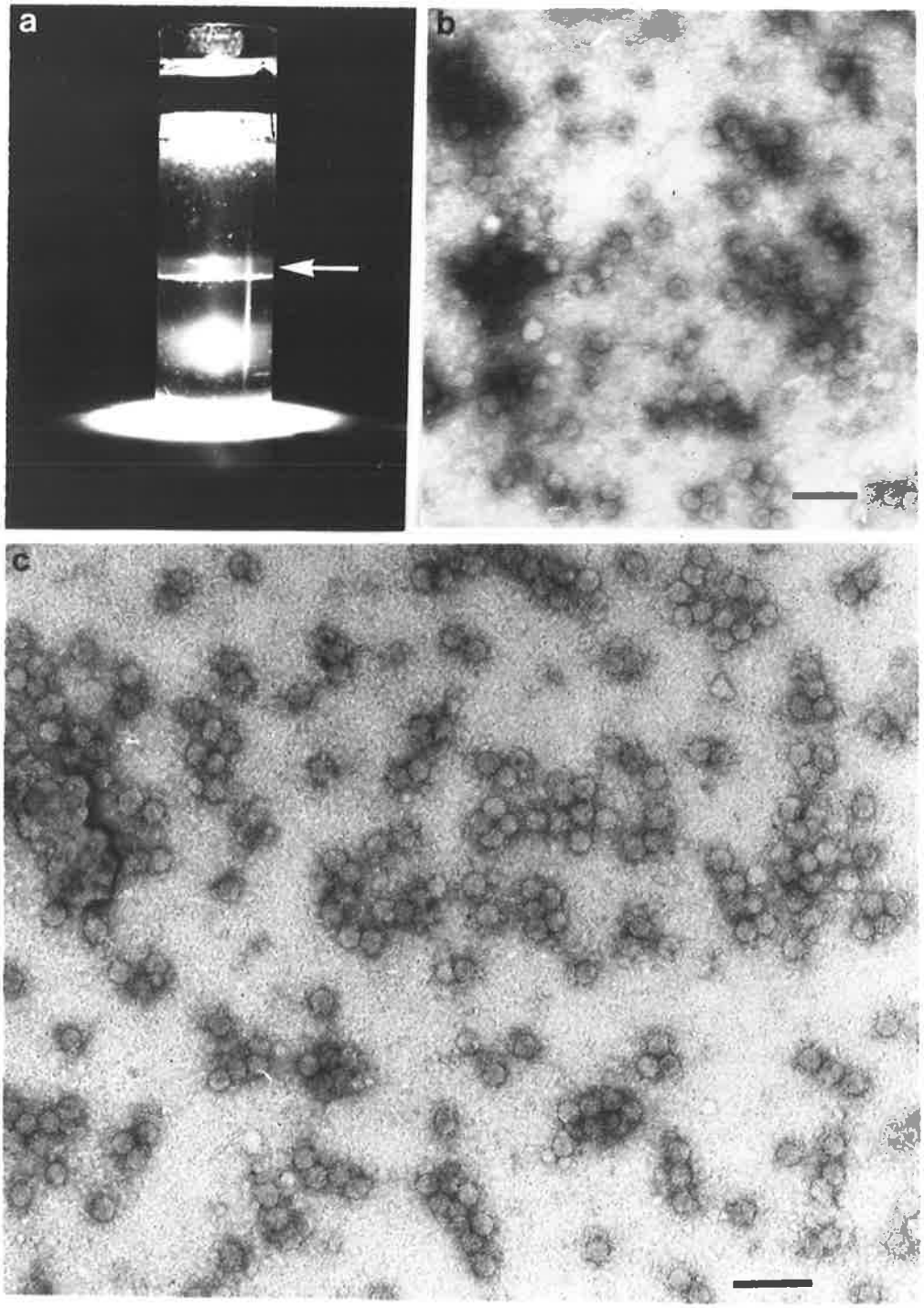
7.2.7.7 Estimation of molecular weight of SCRLV-RNA

Migration of the RNA bands including marker RNA in the gel was measured. The molecular weight of the SCRLV RNA was

Fig. 26 (a) Light-scattering zone (arrow) formed by SCRLV after centrifugation for 18 hrs. at 42000 rev/min in a Cs_2SO_4 gradient.

(b) Particles of purified preparations of SCRLV after centrifugation in Cs_2SO_4 gradient and stained with 2% UAc. Bar represent 100 nm.

(c) Purified preparation of SCRLV after Cs_2SO_4 gradient attached to a SCRLV antiserum (1/200 diluted) coated grid followed by negatively staining with 2% UAc. Bar represents 100 nm.


a
b
c

estimated from their relative mobilities with respect to VTMoV RNA -3, and cytoplasmic ribosomal RNA's. The molecular weights of the marker RNA are listed in Table 16.

Table 16: Molecular weights of marker RNA components

Reference	Marker RNA		Mol.wt(x 10^{-6})
Leaver and Key (1970)	Ribosomal	25 S	1.3
		18 S	0.7
Leaver (1973)	Ribosomal	23 S	1.1
		16 S	0.56
Randles et al. (1981)	VTMoV-RNA 3		0.12

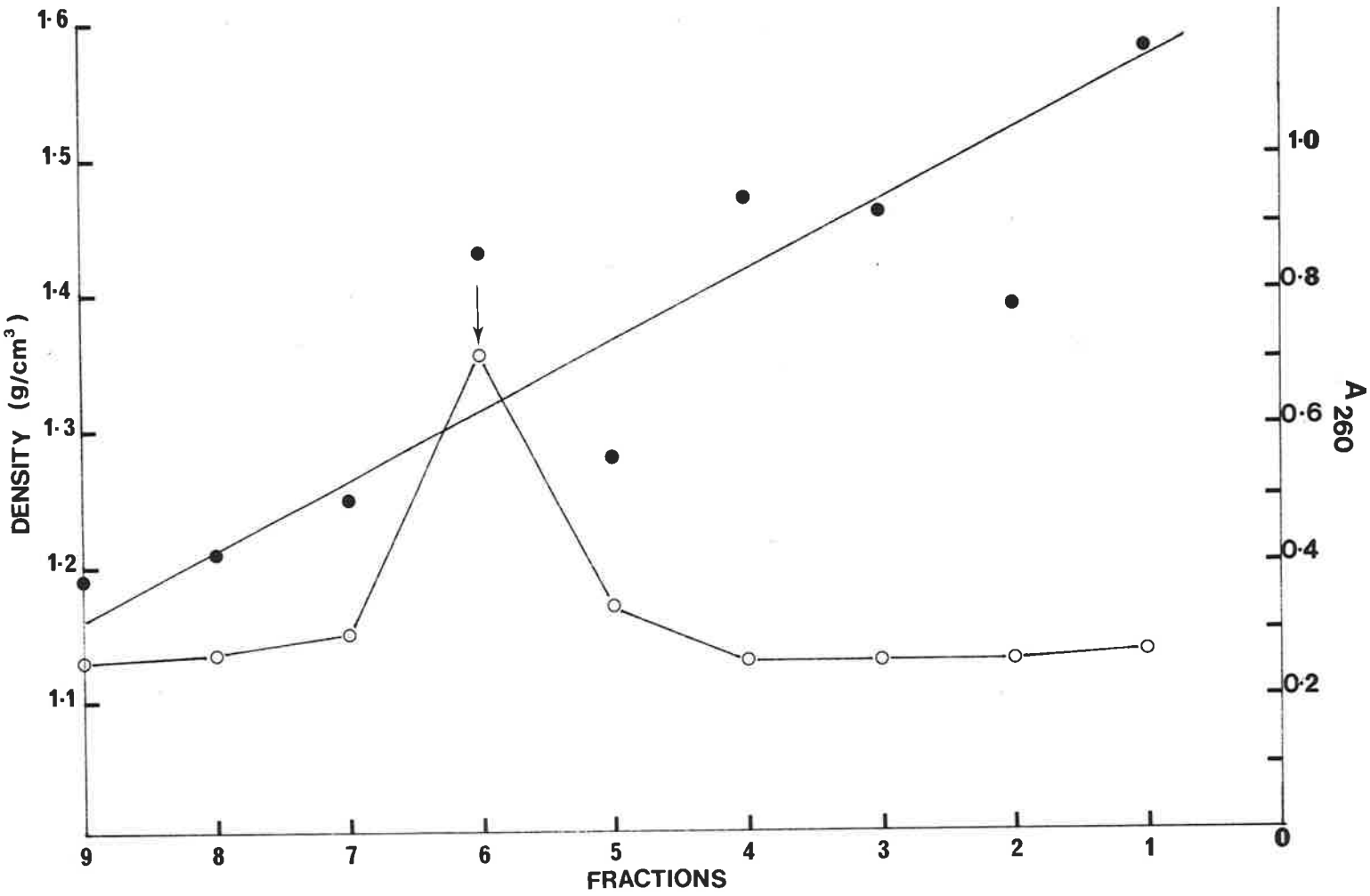
The molecular weights of the test RNAs were estimated from the regression of electrophoretic mobility on $\log_{10}$ molecular weight of the standards (Murant et al., 1981).

7.3 RESULTS

7.3.1 Virus purification

When T.subterraneum L.was used to purify SCRLV as described in method A, two UV absorbing zones (Fig. 23) were detected in sucrose gradients. Examination in the electron microscope showed two types of particles in the 2nd peak down the tube. One type of particle was isometric (Fig. 24) and the other rod-shaped (Fig. 25) with a central hollow core. Preparations from healthy T.subterraneum L. showed similar but fewer rods. Similar rod shaped particles were also seen by Teh (1978); Ashby and Kyriakou (1982), when infected T.subterraneum L. was used to purify SCRLV. Purification of SCRLV from P.sativum L. also gave a UV absorbing peak at the

Fig. 27 Bouyant density determination of SCRLV on isopycnic Cs_2SO_4 density gradient.


1·6
1·5
1·4
1·3
1·2
1·1
DENSITY (g/cm³)
1·0
0·8
0·6
0·4
0·2
0
A 260
9
8
7
6
5
4
3
2
1
FRACTIONS

same position in the sucrose gradient as obtained for T.subterraneum L. and this peak contained only isometric particles when examined in the electron microscope. When partially purified SCRLV was subjected to Cs_2SO_4 centrifugation, Fig. 26b shows the high degree of purity as determined by electron microscopy.

The yield from P.sativum L. was low for method A (24 µg/kg of tissue) and high for method B (1.34 mg/kg of tissue), when the same plant material was used as the source for purification. Only traces of virus were recovered from tissue of infected T.subterraneum L.

7.3.2 Density gradient centrifugation

In Cs_2SO_4 gradients a milky band was formed about 25 mm below the meniscus after centrifugation for 18 hrs. at 42,000 rev/min. (Fig. 26a). When fractions obtained after Cs_2SO_4 gradient centrifugation were converted to their corresponding densities, the fraction with the highest UV absorbance had a density of 1.31 gm/cm^3 (Fig. 27).

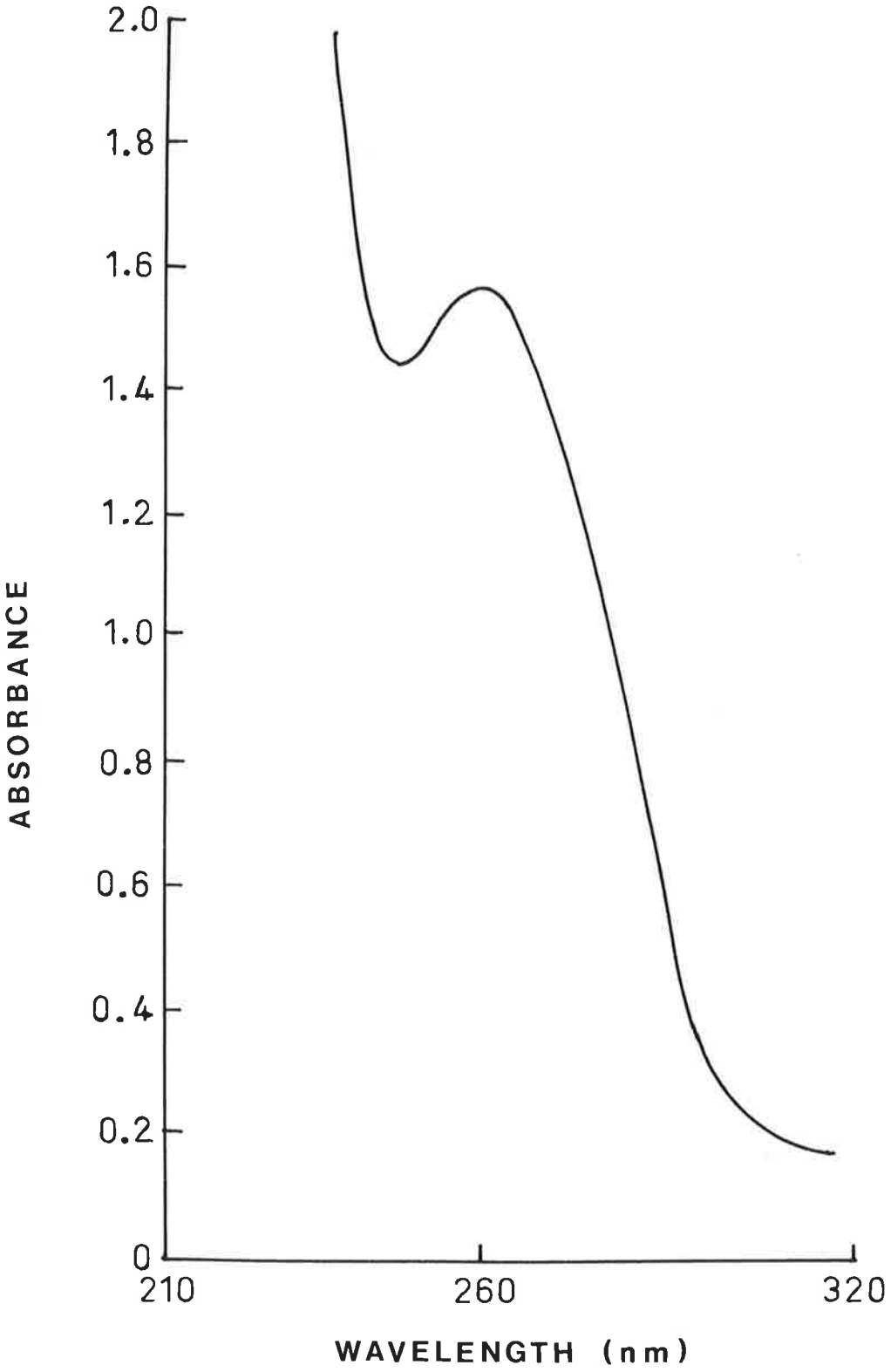
7.3.3 Absorption spectrum

Fig. 28 shows that purified preparations of SCRLV obtained after centrifugation in Cs_2SO_4 density gradients had a UV absorption spectrum typical of a nucleoprotein. The A_{260}/A_{280} ratio was about 1.5 and this is consistent with an RNA content of about 20% (Gibbs and Harrison, 1976).

7.3.4 Measurement of virus particle diameter

When SCRLV particles were compared with those of red clover necrotic mosaic virus (RCNMV) at the same magnification

Fig. 28 Ultraviolet absorption spectrum of fraction containing SCRLV from a Cs_2SO_4 isopycnic density gradient.


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCE
210
260
320
WAVELENGTH (nm)

and taking the diameter of RCNMV particles as 34.2 nm (Hatta and Francki, inpublished results) the diameter of particles from SCRLV infected plants was calculated to be 30.4 nm (see appendix Table 13).

7.3.5 Infectivity assay

None of the test plants produced symptoms when the virus preparation from method A was used but the virus preparation from method B caused red leaf symptoms on indicator Mt. Barker plants. Waterhouse and Murant (1981) observed no transmission when virus preparations (carrot red leaf virus) were made after treatment with Driselase for 16 hrs.

7.3.6 Immuno electron microscopy (IEM)

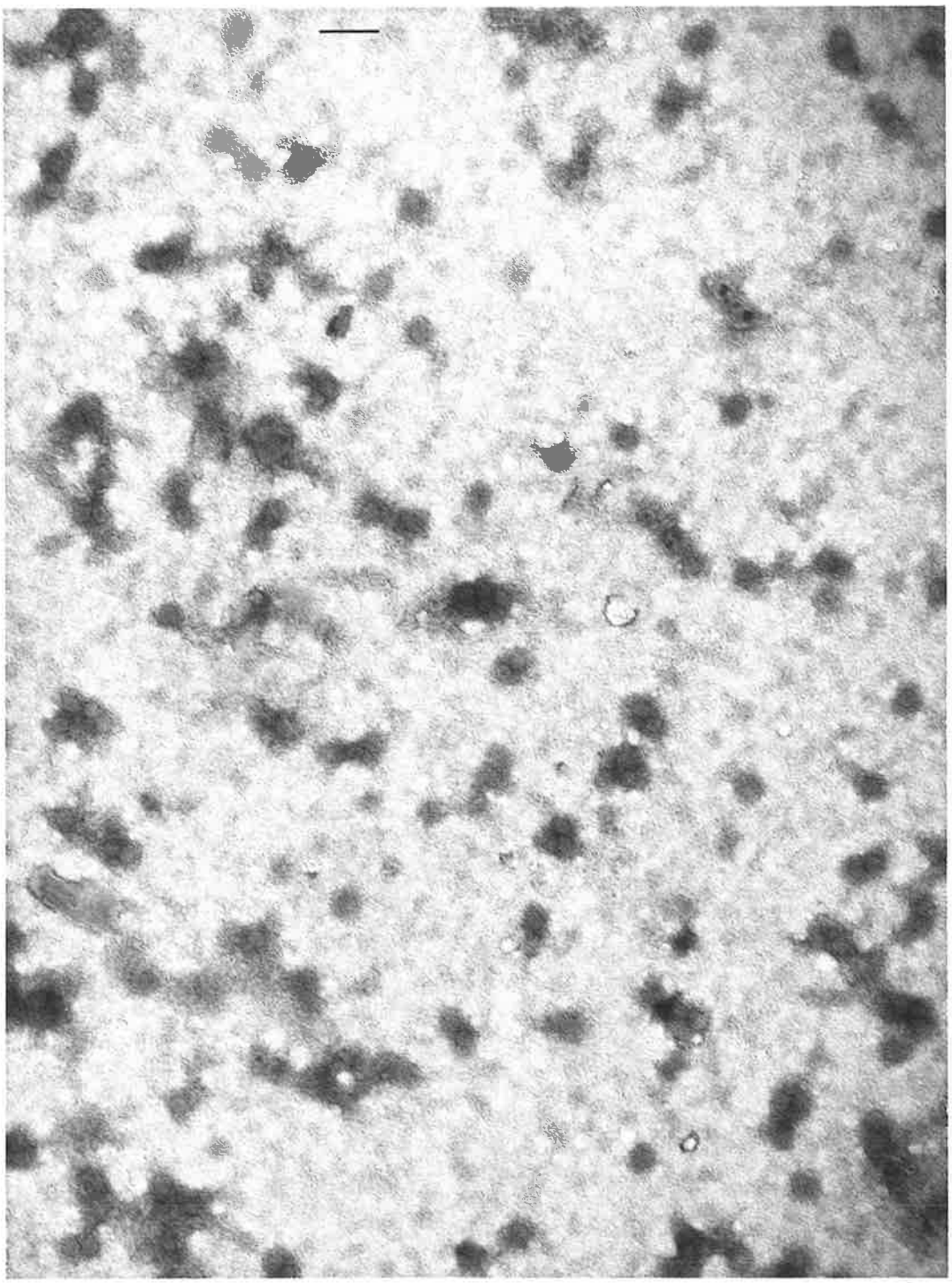
The results show that virus-like particles purified from SCRLV infected plants were trapped (Fig. 26c) and decorated only with SCRLV antiserum (Fig. 29) and not with PLRV antiserum. Conversely PLRV was decorated only with PLRV antiserum (Fig.21g) and not with SCRLV antiserum.

These results show that the virus-like particles partially purified from infected Mt.Barker plants and peas were closely related to the SCRLV used by Ashby and Kyriakou (1982) and not to PLRV.

7.3.7 Serology

When antiserum to SCRLV (1/512 titer) received from Dr.J.W.Ashby, New Zealand was tested against purified SCRLV, in gel - diffusion tests, a single line of precipitate occurred in these tests at dilutions up to 1/10 (Fig. 30a). No precipitation bands were seen when antiserum was tested against

Fig. 29 Particles of SCRLV after attachment to SCRLV antiserum coated grid (1/1000) dilution and then decorated by SCRLV antiserum diluted 1/100 for 30 min. at room temperature. Bar represents 100 nm.



crude sap from healthy P.sativum L. (Fig. 30a). Furthermore SCRLV antisera (1/2048 titer) received from Drs.P.M.Waterhouse and K.Helms from Canberra (Fig. 30b) also reacted with the virus preparation.

Fig. 31 shows that in serological tests with ELISA (for the ELISA method see Chapter 8), the optimal values were obtained when the microtiter plates were coated with SCRLV γ-globulin at a ~~dilution~~ concentration of 5 µg/ml and conjugate ~~dilution~~ was at 1.25 µg/ml. In these tests, purified SCRLV could be detected down to a dilution of 10^{-3} (Fig. 31).

The possible application of ELISA to the detection of SCRLV in infected T.subterraneum L., V.faba L. and aphids fed on infected plants was also investigated. Table 19 shows that virus was readily detected in infected plants in which extinction values ($E_{400\ nm}$) varied from 0.88 to 1.74 while values for the healthy control were less than .07. Further virus can be readily detected in extracts of aphids fed on infected T.subterraneum L. plants and the extinction value varies from 0.17 to 1.62 (Table 19). For nonviruliferous aphids, the value was less than 0.1.

7.3.8 Measurement of nucleic acid concentration

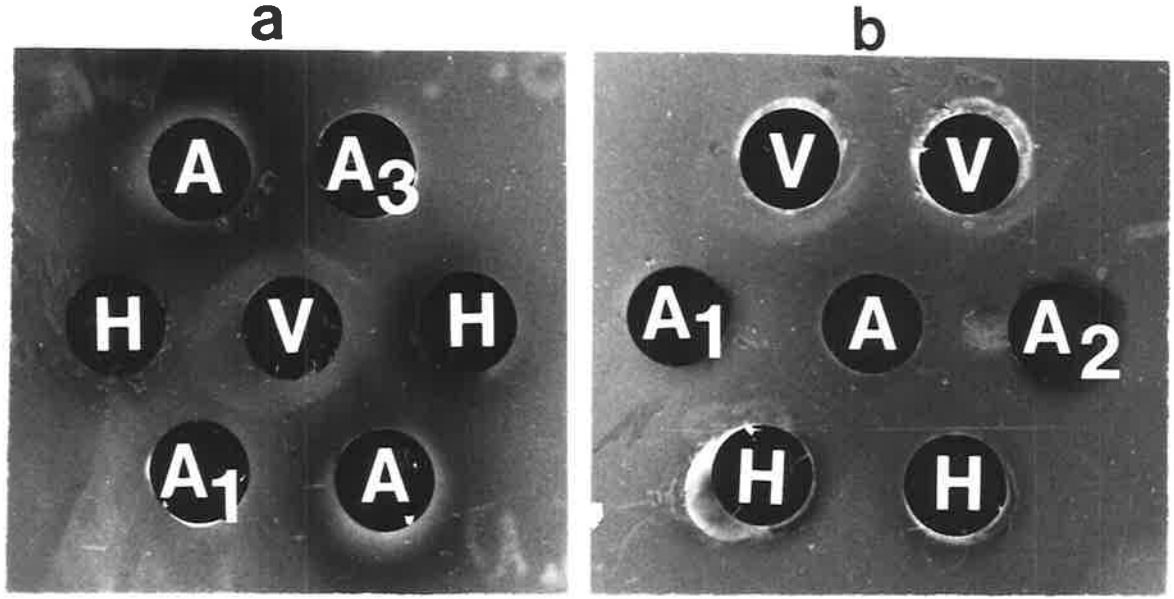
Absorption spectra were corrected for light scattering as described by Noordam (1973). The concentrations of RNA extracted from N.clevelandii GRAY. and VTMoV were 0.84 and 1 mg/ml respectively. Concentration of SCRLV - RNA was too low to measure.

7.3.9 Nucleic acid composition of SCRLV

Figures 32a, b, c show the nucleic acids of

Fig. 30 Serological reactions in a gel-diffusion test between a purified preparation of SCRLV from P.sativum L. cv. Puget and antisera to antigen (A). In a, well V contained purified preparation of SCRLV. Well A contained undiluted antiserum of SCRLV from New Zealand (1/512 Titer) provided by Dr. J.W.Ashby, well A_1 contained the same antiserum diluted 1/200, well A_3 contained the same antiserum diluted in 1/10. H, healthy P.sativum L. sap as control.

In b, well A SCRLV antiserum (titer 1/2048) undiluted from Dr.P.M.Waterhouse and Dr.K.Helms Canberra; A_1, antiserum at a dilution of 1/20; well A_2, antiserum diluted in 1/200; well H, healthy P.sativum L. sap as control. Precipitating reaction are seen between A and V only, with no reaction against H.


a
A
A3
H
V
H
A1
A
b
V
V
A1
A
A2
H
H

N.clevelandii GRAY, VTMoV and SCRLV fractionated on denaturing polyacrylamide gels. Nucleic acid from the SCRLV preparation yielded 3 bands (Fig. 32c - Nos.1, 2 and 3). To find out which band consisted of RNA, the SCRLV- NA solution was divided into 3 parts and these were untreated or treated with RNase A (final concentration 50 µg/ml) (bovine pancreatic, Sigma Chemical Company) or DNase I (final concentration 50 µg/ml) (DN - EP, Sigma Chemical Company) in 0.01M $MgCl_2$ and 0.01M Tris-HCl buffer, pH 7.4. The RNase was preheated at 100°C for 5 min. to destroy DNase. Each aliquot was incubated at 37°C for 30 min. The reaction was stopped by adding 1% SDS and keeping at - 20°C for more than one hour. Nucleic acids were precipitated by ethanol and sedimented by centrifugation as described before (see Section 7.2.7.4). The pellets were drained, dried and electrophoresed in denaturing 3.3% polyacrylamide gel (see Section 7.2.7.6).

Bands 1 and 3 were susceptible to RNAase (Fig. 33c) but were resistant to DNase. Similarly band 2 was not resistant to DNAase (Fig. 33b) and resistant to RNAase (Fig. 33b).

From these observations it was concluded that bands 1 and 3 are RNA while band 2 is DNA.

7.3.10 Molecular weight of SCRLV-RNA

Migration of the RNA bands including marker RNA in the gel was measured. The results show (Table 17, Fig. 34) estimated molecular weights for SCRLV RNA-1 and RNA-2 of 2.08×10^6 and 1.08×10^6 respectively when N.clevelandii GRAY

Fig. 31 Enzyme-linked immunosorbent assay (ELISA) absorbance values in relation to different concentration of SCRLV γ - globulin and conjugate.
O = control

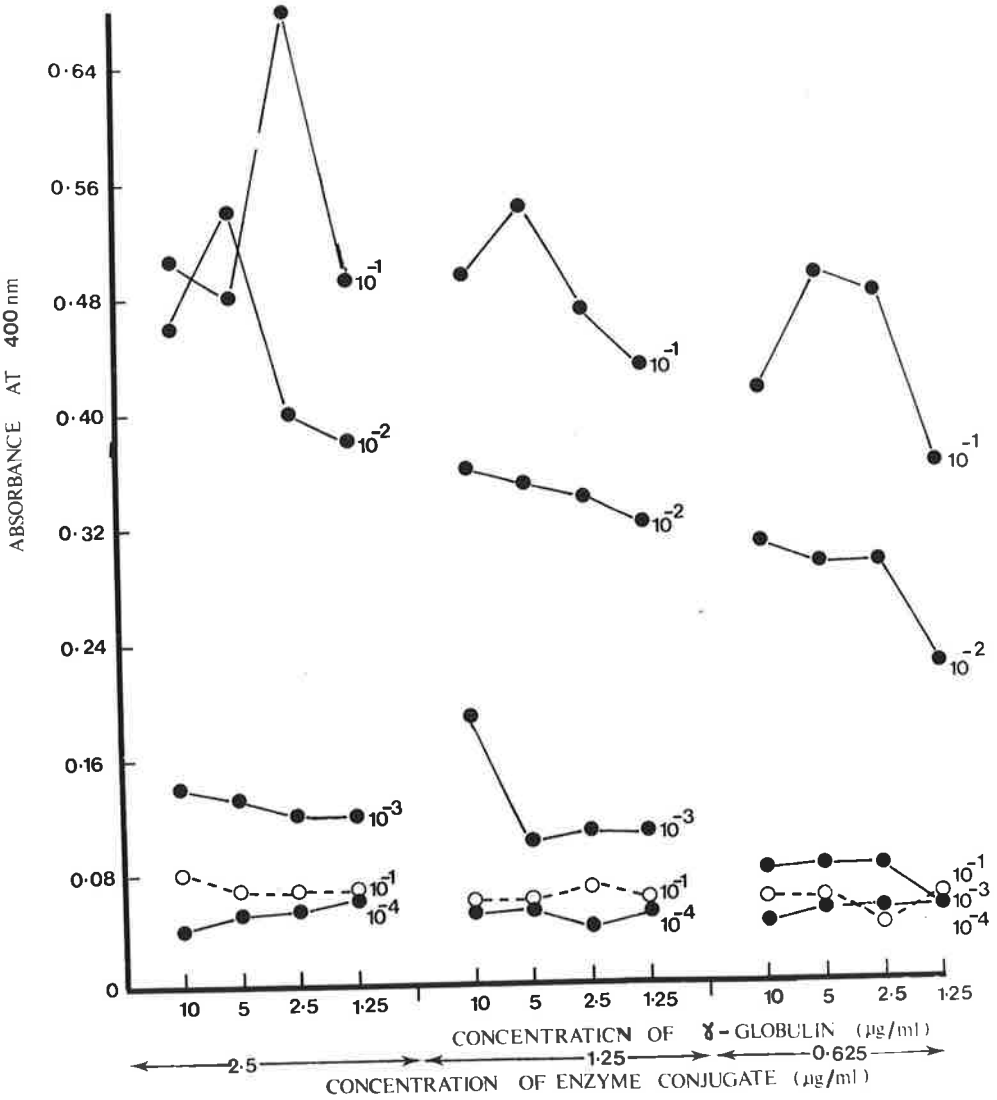

ABSORBANCE AT 400 nm
0·64
0·56
0·48
0·40
0·32
0·24
0·16
0·08
0
10
5
2·5
1·25
CONCENTRATION OF γ-GLOBULIN (μg/ml)
2·5
1·25
0·625
CONCENTRATION OF ENZYME CONJUGATE (μg/ml)

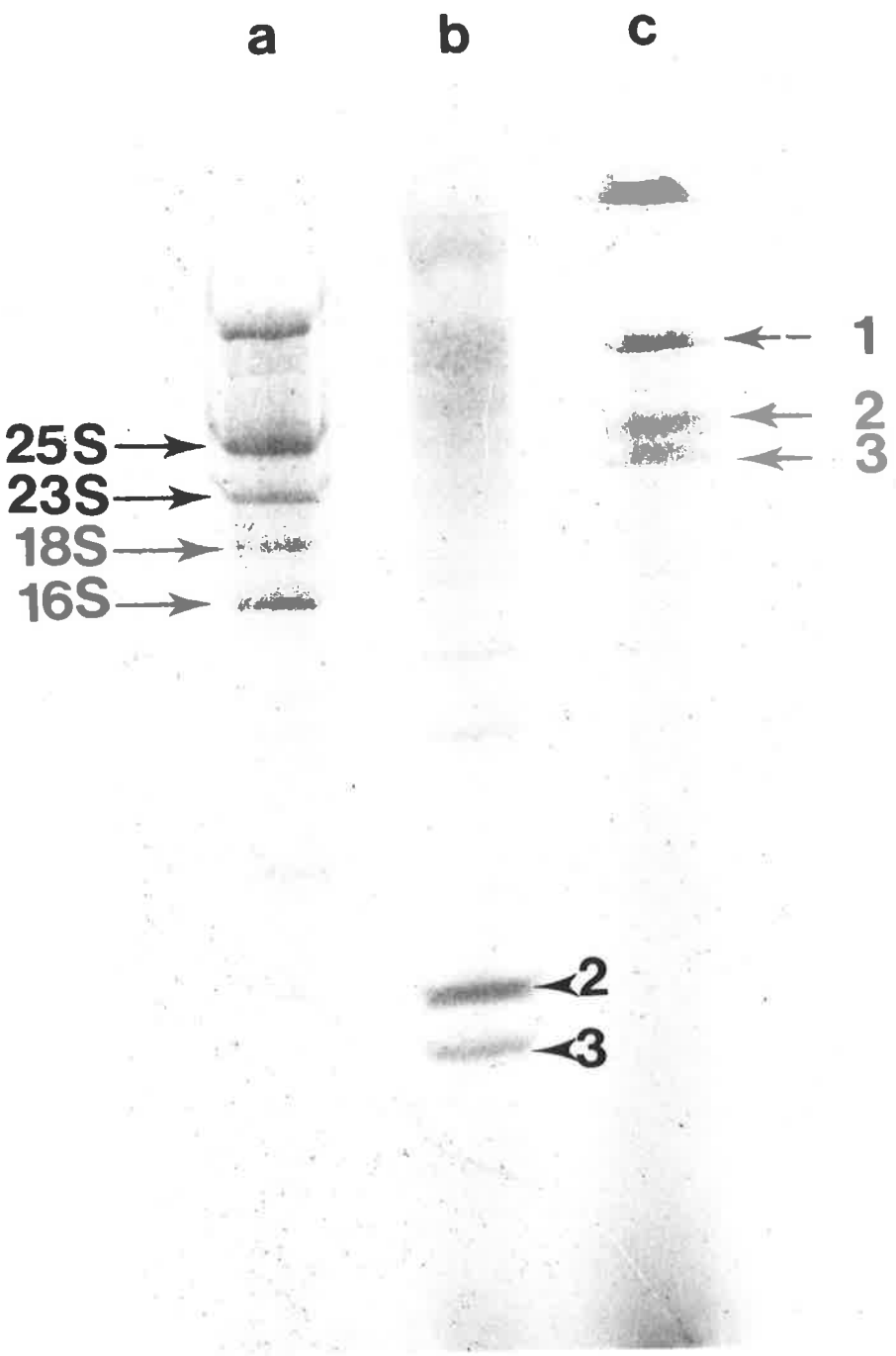
Table 17: Mobility of RNAs on 3.3% polyacrylamide-8M urea gel electrophoresis

Source	Mol.Wt. of RNA	Relative Mobility (mm)					Average Mobility (mm)
		R 1	R 2	R 3	R 4	R * 5	
N.clevelandii	1.3 x 10^6d	20	29	46	-a	-	31.6
	1.1 x 10^6d	24	32	55	-	-	37.0
	0.7 x 10^6d	28	37	67	-	-	44.0
	0.56 x 10^6d	33	42	74	-	-	49.6
VTMoV	1.2 x 10^5d	72	90	85	-	-	82.0
SCRLV	RNA1 2.08 x 10^6d	12.5	26	27	10.5	24	20.0
	RNA2 1.08 x 10^6d	22	45	47	22	-	34.0

R* = Replicate

a = No data

Fig. 32 3.3% polyacrylamide 8M urea gel electrophoresis of purified SCRLV nucleic acid and marker nucleic acids. The fractions of 16S, 18S, 23S and 25S ribosormal RNA markers are shown for N.clevelandii GRAY (a); RNA 2 and 3 for VTMoV (b); RNA (1 and 3) and DNA (2) for SCRLV (c).

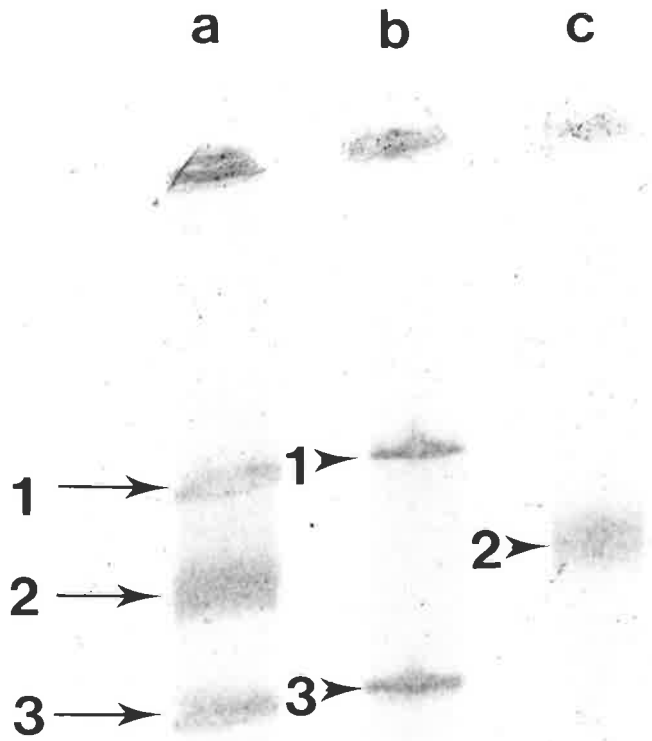

a
b
c
25S
23S
18S
16S
1
2
3
2
3

were used as internal markers.

7.4 DISCUSSION

The main aim of the SCRLV purification was to obtain sufficient highly purified viral RNA for molecular hybridization studies. Previous attempts to purify SCRLV from T.subterraneum L. using methods A and B showed that the virus was present in low concentration in this host. When Teh (1978) attempted to purify SCRLV from T.subterraneum L. it was unsuccessful. Ashby and Kyriakou (1982) reported that ~~for purification of SCRLV using infected~~ P.sativum L. cv. Puget was a better source than infected T.subterraneum L. cv. Mt.Barker. They obtained virus yields from infected P.sativum L. cv.Puget of 0.5 to 3.0 mg/Kg of tissue. Similarly Johnstone et al. (1982) obtained virus yields between 0.5 to 2.0 mg/Kg of tissue using the same host. In the present study the virus yield was very low (24 µg to 1.34 mg/Kg) of tissue compared with those of Ashby and Kyriakou (1982), and Johnstone et al. (1982). It was observed that when Cellulase (Onozuka R 10) was used to digest the plant tissue at the given buffering system and pH 6.0 at room temperature (method A), a minimum of 16 hrs. was needed. But with Cellulase (Sigma type I) with the same buffering system at pH 4.7 less than 4 hrs. was needed (method B). Since the method A procedure is very time consuming and yield is low when compared to method B, SCRLV purification was later attempted only by method B. Further it was observed that virus (SCLRV) purified from enzymatic digestion for 16 hrs. was not transmitted in infectivity assay tests using its efficient vector A.solani (Kltb.). The reason for this is not known. But Waterhouse and Murant (1981)

Fig. 33 3.3% Polyacrylamide 8M urea gel electrophoresis of SCRLV nucleic acid (a) and the same preparation after incubation with DNase (b) or RNase (c) at 37°C for 30 min.


a
b
c
1
2
3
1
2
3

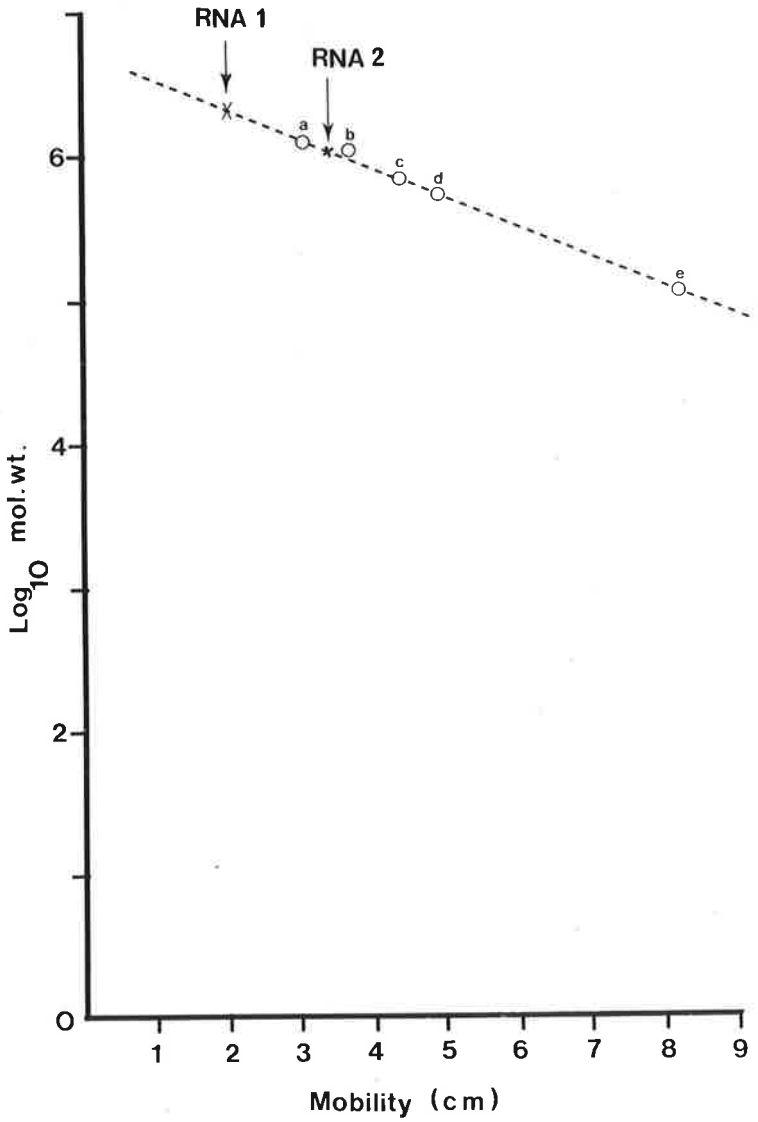
reported a similar situation when purifying carrot red leaf virus and suggested that prolonged incubation may cause damage to the nucleic acid and/or protein components of the virus. This needs further investigation.

Purified SCRLV has 30.4 nm isometric particles with a density of approximately 1.31 g/cm^3 in Cs_2SO_4. This value is close to the value of 1.32 g/cm^3 and 1.33 g/cm^3 reported for pea leafroll virus (Ashby and Huttinga, 1979) and an isolate of barley yellow dwarf virus (Hammond et al., 1983) respectively in Cs_2SO_4 gradient. The ratio A_{260}/A_{280} of 1.5 is lower than the value(1.85) obtained for SCRLV by Ashby and Kyriakou (1982) and lower than that found for other luteoviruses which range from 1.62 for carrot red leaf virus (Waterhouse and Murant, 1981) to 1.96 for soybean dwarf virus (Kojima and Tamada, 1976).

The nucleic acids isolated from SCRLV prepared by method A (Fig. 33) contained two RNA bands (RNase sensitive, DNase resistant) with estimated molecular weights of 2.08 x 10^6 (band 1) and 1.08 x 10^6 (band 3). The relationship between these two bands is not known and requires further investigation. Falk et al. (1977) has reported that beet western yellows virus which is also a member of the luteovirus group contains two RNA species. Higher molecular weight RNA is close to the molecular weight determined already for the RNA of other members of the luteovirus group (Rochow and Duffus, 1981). Ashby and Huttinga (1979) reported quoting Geelen's (1974) work that the condition (nondenaturing or denaturing) of which the polyacrylamide gel electrophoresis carried out can affect the molecular weight

Fig. 34 Determination of mol. wt. of SCRLV RNA by electrophoresis on 3.3% polyacrylamide 8M urea slab gels. The line was obtained by plotting the log of mol. wt. of marker RNA's against their distance of migration.

a) 25S mol. wt. 1.3×10^6; (b) 23S mol. wt. 1.1×10^6; (c) 18S mol.wt. 0.7×10^6; (d) 16S mol. wt. 0.56×10^6; (e) VTMoV-RNA3 mol. wt. 0.12×10^6. The arrow indicates the position of the two SCRLV-RNA species.


RNA 1
RNA 2
a
b
c
d
e
6
4
2
0
Log10 mol.wt.
1
2
3
4
5
6
7
8
9
Mobility (cm)

estimate. Since most of the molecular weight of RNA has been estimated on nondenaturing gels e.g. BYDV-RNA (Brakke and Rochow, 1974); PLRV-RNA (Rowhani and Stace-Smith, 1979; Takanami and Kubo, 1979b); PeLRV-RNA (Ashby and Huttinga, 1979) there is uncertainty about the true value of the slow migrating RNA extract from purified SCRLV preparations. A comparison of molecular weight from nondenaturing and denaturing gels (Murant et al., 1972) with a range of luteovirus RNAs should be done. The more diffuse band observed between the two RNA bands (Fig. 32) was shown to be DNA (band 2) by enzyme digestion. Copurification of DNA with the SCRLV-RNA may be a result of either, attachment to, or encapsidation in SCRLV particles. Its origin is unknown, but it may be analagous with the DNA which Sarkar (1976) found to be associated with preparations of the RNA containing potato leaf roll virus (Rowhani and Stace-Smith, 1979).

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CHAPTER 8

MOLECULAR HYBRIDIZATION ANALYSIS

8.1 INTRODUCTION

In this Chapter the feasibility of using the molecular hybridization assay (MHA) to identify SCRLV in plants and the aphid vectors is investigated. Identification of SCRLV-infected plants in field samples is time consuming (Jayasena and Randles, 1984) because of the need to use aphid vectors for virus transmission work. Moreover, because V.faba L. infected with SCRLV shows symptoms very much similar to subterranean clover stunt virus (SCSV) and bean leaf roll virus (BLRV) (Johnstone, 1978), the correct vector must be used to identify the virus. Therefore, a rapid and reliable means of detecting and identifying SCRLV would be an advantage.

ELISA would satisfy these requirements, but the need to prepare milligram amounts of virus for immunization together with possible difficulties of ensuring specificity and interpreting serological interrelationship between isolates by this method, led to attempts to develop MHA suitable for use with SCRLV. MHA using DNA complementary to RNA (cDNA) provides a very sensitive means of detecting viruses, and allows a comparison of their nucleotide sequences (Abu-Samah and Randles, 1981, 1983; Boccardo et al., 1981; Palukaitis et al., 1981b; Gould and Francki, 1981). Palukaitis et al. (1981b) reported that a cDNA probe can detect viroid specific RNA as low as 1×10^{-5}% by weight of total leaf RNA extracts and hybridization percentages can be used to study relationships between viruses and virus

strains (Palukaitis and Symons, 1980; Abu-Samah and Randles, 1981, 1983).

8.2 MATERIALS AND METHODS

8.2.1 Virus purification

For virus purification methods see Chapter 7.

8.2.2 Extraction of SCRLV-RNA

The method of Murant et al.,(1972) was slightly modified for the extraction of RNA from partially purified SCRLV (see Chapter 7). Since, highly purified RNA was required for the synthesis of complementary DNA and for hybridization studies (Abu-Samah, 1982) the RNA from partially purified virus preparations was subjected to sucrose density gradient centrifugation.

Partially purified virus prepared by polyethylene glycol precipitation (PEG) and centrifugation to equilibrium in a caesium sulphate gradient (see Chapter 7) was incubated for 16 hrs. at 37°C in predigested 0.1% pronase containing 0.5% sodium dodecyl sulphate (SDS) and 0.1M sodium acetate (Murant et al. 1972; Randles, 1975). After adding 0.25M Tris-HCl buffer pH 9.0 containing 0.1M ammonium bicarbonate, 1mM EDTA and 1% SDS to the digested preparation, the viral RNA was separated by centrifugation on 7.5 - 30% sucrose step gradients buffered in 0.5M Tris-HCl pH 9.0 (Reddick and Barnett, 1983) at 25,000 rpm for 16 hrs. at 14°C in the Spinco SW 41 rotor. The RNA zone was located using an ISCO density gradient fractionator and ultra violet scanner. Fractions from UV absorption peaks were collected and RNA was recovered by ethanol precipitation in the presence of

0.2 M sodium acetate. The dried pellet was then dissolved in sterile double distilled water (o.1 ml) and stored at -20°C.

8.2.3 Extraction of Nucleic Acid from SCRLV infected and healthy plant materials

Total nucleic acids were extracted from healthy or SCRLV-infected Pisum sativum L. Puget and Trifolium subterraneum L. cv. Mt. Barker plants. Physalis floridana L. plants either infected with potato leaf roll virus or healthy, were also used as a source of total nucleic acids.

Before extracting the total nucleic acids, plants were ground to a powder in the presence of liquid nitrogen using a pestle and mortar. Powdered plant material was transferred to a sterile beaker containing equal volumes of 1% SDS and water saturated phenol and stirred for 1 hr. at room temperature. The rest of the procedure was as described in Section 7.2.7.4. The pellets obtained after ethanol precipitation were dried and divided into two. One lot was used directly for hybridization studies and the other lot was first subjected to cetyl trimethyl ammonium bromide (CTAB) precipitation to remove non nucleic acid material (Ralph and Bellamy, 1964) before using for hybridization studies. The CTAB procedure was as follows. The dry pellets were dissolved in 180 µl of sterile double distilled water. 20µl of 2M NaCl and 100 µl of 1% CTAB were added while mixing gently. Mixtures were left at 0°C for 1 hr. before centrifugation at 10,000 rpm for 15 min. The pellets were washed twice with 0.1M sodium

acetate in 75% ethanol and once with redistilled ethanol. The pellets obtained after low speed centrifugation were dried and stored at -20°C prior to being used for hybridization analysis.

8.2.4 Extraction of total Nucleic Acid from Aphids

Total nucleic acid was extracted from viruliferous and nonviruliferous Aulacorthum solani (Kltb) and Myzus persicae (Sulz.), allowed to feed on healthy or SCRLV infected T.subterraneum L. plants as described above (Section 8.2.3) and subjected to CTAB purification.

8.2.5 Synthesis of complementary DNA for SCRLV-RNA

The method described by Taylor et al. (1976) and modified by Gould and Symons (1977); Abu-Samah and Randles (1981) was used to synthesize cDNA to SCRLV.

The DNA primer required for cDNA synthesis was prepared as follows. Salmon sperm DNA (25 mg) was dissolved in 5 ml of 1mM Tris-acetate buffer pH 7.4 containing 1mM magnesium acetate, then 350 µg DNase 1 was added followed by incubation at 37°C for 2 hrs. The solution was then heated at 121°C for 10 min. by autoclaving (Taylor et al., 1976) and stored frozen till use. To synthesize cDNA purified SCRLV-RNA was mixed with 70 µl of sterile double distilled water and 75 µl of ^{3}H-dCTP (Amersham) (75µCi (3 nmoles)) were added and frozen in liquid nitrogen, then lyophilised for 3 hrs. For a reaction mixture of 50µl, the dried dCTP and RNA was dissolved in a solution containing 25 µl salmon sperm DNA primer, 100 mM KCl, 8mM $MgCl_2$, 50mM Tris-HCl pH8.3, Actinomycin D (100 µg/ml), 0.67mM dATP, 0.67mM dGTP and 0.67mM TTP, 30mM dithiothreitol and 7 units of avian myeloblastosis virus reverse transcriptase. The mixture was

incubated at 37°C for 2 hrs. The reaction was stopped by the addition of 100 µl of 1% SDS and 300µl 0.5M NaOH. The RNA template was hydrolysed by incubation at 20°C for 16 hrs. The cDNA was separated from the rest of the unreacted deoxyribonucleotide triphosphates and small ribonucleotides by passage through a 1.5 x 15 cm Sephadex G-50 column equilibrated with freshly prepared 0.1M NH_4HCO_3 (Abu-Samah, 1982). The fractions (10µl each) were collected and radioactivity in each fraction was determined. The peak fractions of cDNA (Fig. 36) were combined and lyophilised after the addition of 1/10 the volume of redistilled triethylamine to remove ammonia and triethylammonium salts. The cDNA was then taken up in 800µl of 10mM EDTA pH8, ethanol was added to 10% and it was stored at - 15°C.

8.2.6 Determination of $R_o t$ value of SCRLV-RNA and nucleic acid extracted from viruliferous A.solani (K1tb.)

The reaction mixtures (containing nucleic acid, 3µl ^{3}H-cDNA and hybridization buffer) were heated at 100°C for 3 min. and then incubated at 65°C for 72 hrs. to give a maximum $R_o t$. The procedure is as described in Section 8.2.7. The $R_o t$ value was as

$$\text{Rot} = \frac{\text{RNA concentration x hybridization time}}{\text{molecular weight of ribonucleotide}}$$

$$= \frac{\mu g/ml \times t\ (sec.)}{320000} \quad \text{or} \quad \frac{mg/ml \times sec.}{320}$$

where RNA concentration expressed as mg per ml., hybridization time in seconds and molecular weight for the ribonucleotides is

assumed to be 320 (Abu-Samah, 1982).

8.2.7 Hybridization analysis of SCRLV-RNA, plant extracts and aphid extracts with ^{3}H-cDNA to SCRLV

Hybridization was done as described by Gould and Symons (1977) in hybridization buffer containing 0.01M Tris-HCl pH 7.0, 0.18M NaCl with 1mM EDTA and 0.05% SDS. Hybridizations were done in siliconized glass test tubes.

Reaction mixtures of 40 µl contained 3 µl of ^{3}H-cDNA (5000 cpm), nucleic acid and 8µl hybridization buffer. The mixtures were then heated at 100° C for 3 min. and incubated at 65°C for 72 hrs. respectively in a water bath. The hybridization was terminated by adding 500µl low salt S_1 assay buffer which contained 0.03M sodium acetate, 0.05M NaCl, 1mM $ZnSO_4$, 5% glycerol pH 4.6 and 40µg per ml of denatured calf thymus DNA. The extent of hybrid formation was determined by resistance of ^{3}H-cDNA digestion with the single strand specific nuclease S_1 of Aspergillus oryzae (Vogt, 1973). The hybridized mixture was divided into two, each 250µl, and to one portion 5µl (5 units) of S_1 nuclease was added. S_1 nuclease was not added to the other aliquot. The two samples were then incubated at 45°C for 30 min. The reaction was terminated by adding 1.0 ml of 10% trichloroacetic acid (TCA) and 75µl of bovine serum albumin (1 mg per ml). The reaction mixture was then kept on ice for 30 min. and the TCA precipitated Nucleic acids were collected by filtration through Whatman GF/A glass-fibre filters and washing twice with 20 ml of 10% TCA and 10 ml of 80% ethanol. The filters were then dried and counted in 2 ml plastic vials with toluene-based scintillation fluid containing 3.5 g of 2.5- diphenyl oxazole (PPO) and 0.35g of 1,4-bis 2- (5 diphenyloxazolyl) benzene

(POPOP) per litre.

8.2.8 Calculation of estimated percentage of homology

Hybridization was estimated as the percentage of S_1 nuclease resistance (Abu-Samah, 1982) which was:

$$\frac{\text{c.p.m. in aliquot treated with } S_1}{\text{c.p.m. in untreated aliquot}}$$

These values were corrected for self-annealing of the cDNA in the absence of RNA as follows:

$$\text{corrected \% } S_1 \text{ nuclease resistance} = 100 \times \frac{S_1 \text{ nuclease resistance of hybrid (\%)} - S_1 \text{ nuclease resistance of cDNA (\%)}}{100 - S_1 \text{ nuclease resistance of cDNA (\%)}}$$

$$\text{The estimated \% homology in heterologous reactions} = 100 \times \frac{\text{corrected \% } S_1 \text{ nuclease resistance of the heterologous hybrid}}{\text{corrected \% } S_1 \text{ nuclease resistance of the homologous hybrid}}$$

8.2.9 Enzyme - linked imunosorbent assay (ELISA)

The procedures adopted in this thesis for ELISA were essentially as described by Mc Laughlin et al. (1981).

8.2.10 Preparation and purification of immunoglobulin (Ig)

SCRLV antiserum (2.5 ml) was diluted with an equal amount of distilled water and 5 ml of 36% sodium sulphate was added to precipitate immunoglobulin (Ig). After 10 min. at room temperature the mixture was centrifuged at 5000 g for 15 min., the pellet was washed with 10 ml of 18% sodium sulphate and sedimented at 5000 g for 15 min. The pellet was then resuspended in 1.0 ml of phosphate - buffered saline (PBS, 0.02M

sodium phosphate, 0.15M NaCl, 0.003M KCl pH 7.3), then dialyzed against the same buffer at 4°C with two changes. The immunoglobulin fraction was then adjusted to 1mg/ml (E_{280} = 1.5) in PBS and stored as a stock solution with 0.01% (W/v) sodium azide at 4°C for later use.

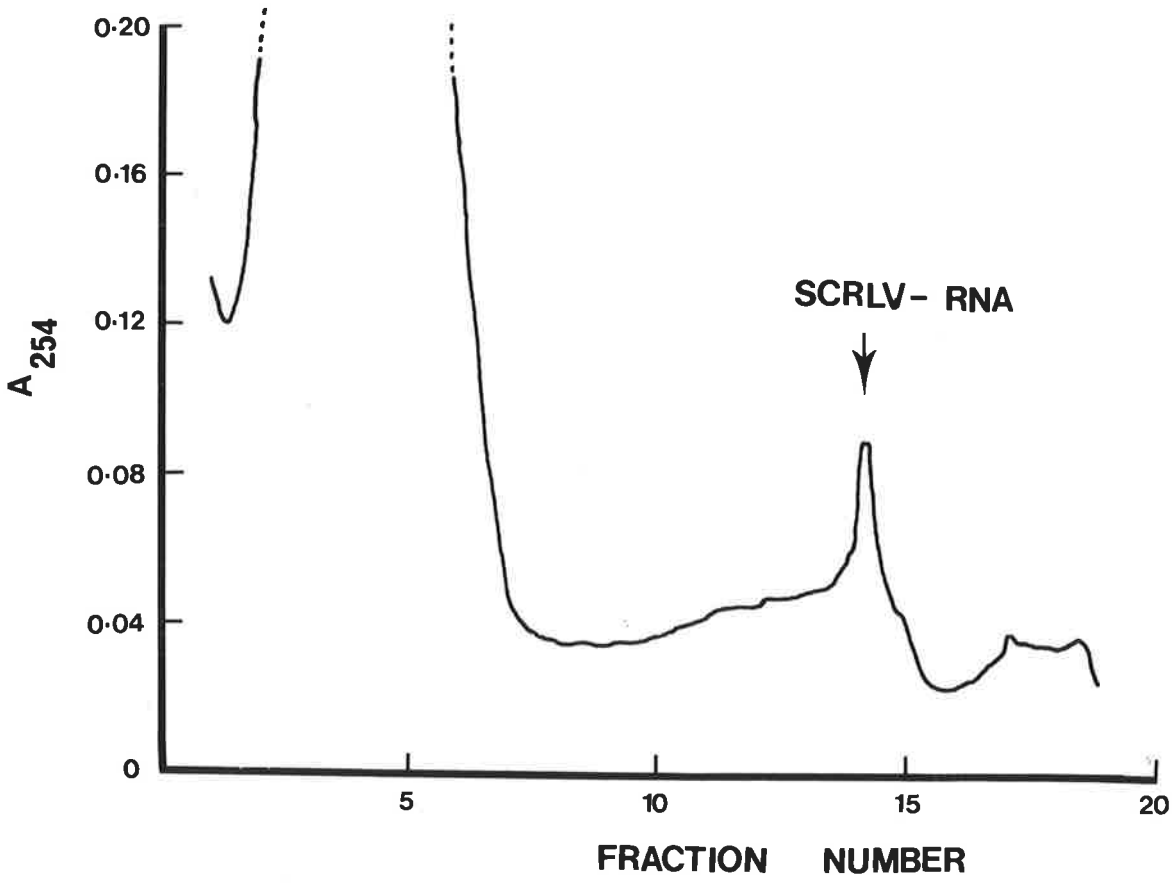
8.2.11 Alkaline phosphatase enzyme conjugation of Ig

Alkaline phosphatase (SIGMA Chemical Co., U.S.A.) (5 mg) was added to 1 ml of Ig stock solution and the mixture was dialyzed against PBS at 4°C overnight with 4 changes. Then 10% glutaraldehyde was added to a final concentration of 0.2%. Preparations were incubated at room temperature for 2 hrs. then dialyzed against PBS buffer overnight at 4°C (with 3 changes) and finally against tris - buffered saline (TBS = 0.05M Tris-HCl, pH8 containing 0.15M NaCl). Conjugates were adjusted to 0.5 mg Ig/ml in TBS. Bovine serum albumin (BSA) and sodium azide were added to give concentrations of 1% (w/v) and 0.01% (w/v) respectively and the conjugates were stored in the dark at 4°C.

8.2.12 ELISA procedure

Polystyrene Micro test plates (Disposable Products Ltd., Adelaide, South Australia) with U shaped wells were used for the assays. The wells, were rinsed with distilled water and sensitized by adding 100µl of coating antibody (2.5µg/ml in 0.5M sodium carbonate coating buffer, pH 9.6 containing 0.01% sodium azide) per well. The plates were incubated for one hour at 5°C, then unabsorbed antibody was rinsed from the wells by three, 3 min. washes in PBS containing 0.05% Tween 20.

Fig. 35 Sucrose density gradient fractionation of nucleic acids isolated from SCRLV which was purified by double PEG precipitation. The high molecular weight peak (arrowed) was used for cDNA synthesis.


0·20
0·16
0·12
0·08
0·04
0
A254
SCRLV- RNA
5
10
15
20
FRACTION NUMBER

Sap extracted from T.subterraneum L. Cv. Mt.Barker in PBS-Tween containing DIECA was added at 200 µl per well, and left overnight at 5°C in the coated wells. After rinsing as before, conjugated Ig diluted in PBS-Tween was added to the wells (100µl/well) and incubated overnight at 4°C. After rinsing antibody - antigen specific reactions were detected by adding 100 µl of substrate (p - nitrophenylphosphate at 1mg/ml in 10% (v/v) diethanolamine, pH 9.8 containing 0.01% (w/v) sodium azide). Plates containing substrate were incubated for 1 hr. or longer at room temperature. Reactions were stopped by adding 50µl of 3M NaOH. The strength of the reaction in each well was determined by comparing colour development at 400nm in wells using a Unicam SP 1800 ultraviolet spectrophotometer.

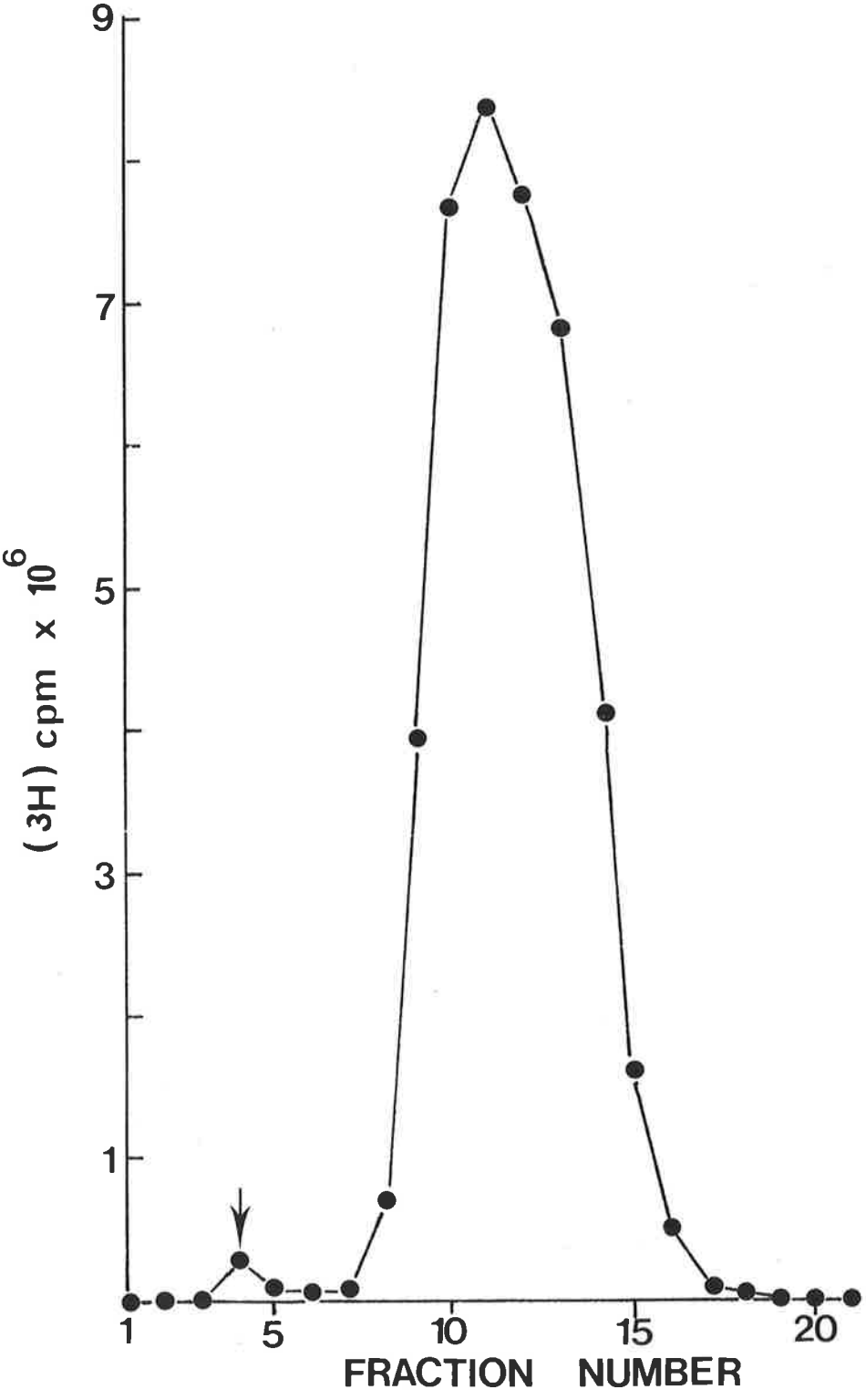
8.3 RESULTS

8.3.1 Characteristics and hybridization specificity of SCRLV-cDNA

From the peak fraction (Fig. 35) containing c. 1µg of nucleic acid about 0.4µg of RNA was recovered by ethanol precipitation. Incorporation of ^{3}H-dCTP into cDNA was about 1% (Fig. 36).

The kinetics of the homologous hybridization between SCRLV ^{3}H-cDNA and SCRLV-RNA, and between the cDNA and total nucleic acids extracted from infected plants and A.solani (K1tb.) are shown in Figs. 37 and 38 respectively. Fourfold serial dilutions of nucleic acid extracts were allowed to hybridize with ^{3}H-cDNA. The kinetics of hybridization (single phase $R_o t$ curves) indicated that the cDNA was not significantly contaminated with

Fig. 36 Separation of 3H-cDNA (arrowed) from unreacted 3H - dCTP. The G-50 sephadex colum was eluted with NH_4HCO_3 pH 9.0. 20µl aliquot was counted of 10µl fraction.


9
7
5
3
1
(3H) cpm x 10^6
1
5
10
15
20
FRACTION NUMBER

sequences not specific for SCRLV-RNA. The level of contamination of the SCRLV-RNA with plant RNA would have been less than 0.002% (Homologous $R_o t\frac{1}{2} \div$ Maximum $R_o t$ value tested = $9 \times 10^{-3} \div 648 \times 100$).

The $R_o t\ \frac{1}{2}$ value for homologous hybridization (^{3}H-cDNA : SCRLV-RNA) (Fig. 37) was 9.0×10^{-3} mol. sec.l^{-1} which is the value expected for an RNA of molecular weight of ca. 2×10^6. This is based on the assumption that $R_o t\ \frac{1}{2}$ and analytical complexity are linearly related (Hell et al.,1976). Gould and Francki (1981) reported that a $R_o t\ \frac{1}{2}$ value 1.12×10^{-2} mol.sec.l^{-1} was obtained for a viral RNA of estimated molecular weight of 2.1×10^6. Abu-Samah (1982) working with BYMV-RNA under conditions of hybridization similar to those described in this Chapter, obtained a $R_o t\ \frac{1}{2}$ of 1.2×10^{-2} mol.sec.l^{-1} for RNA with an estimated molecular weight of 2.6×10^6. For example, the estimated RNA concentration at $R_o t\ \frac{1}{2}$ was 0.037 µg/ml. Therefore $R_o t\ \frac{1}{2}$ =

$$\frac{0.037 \times 78060}{320000} \quad \text{(see Section 8.2.6)}$$

$$= \frac{9.0 \times 10^{-3}}{} \text{ mol.sec.} l^{-1}$$

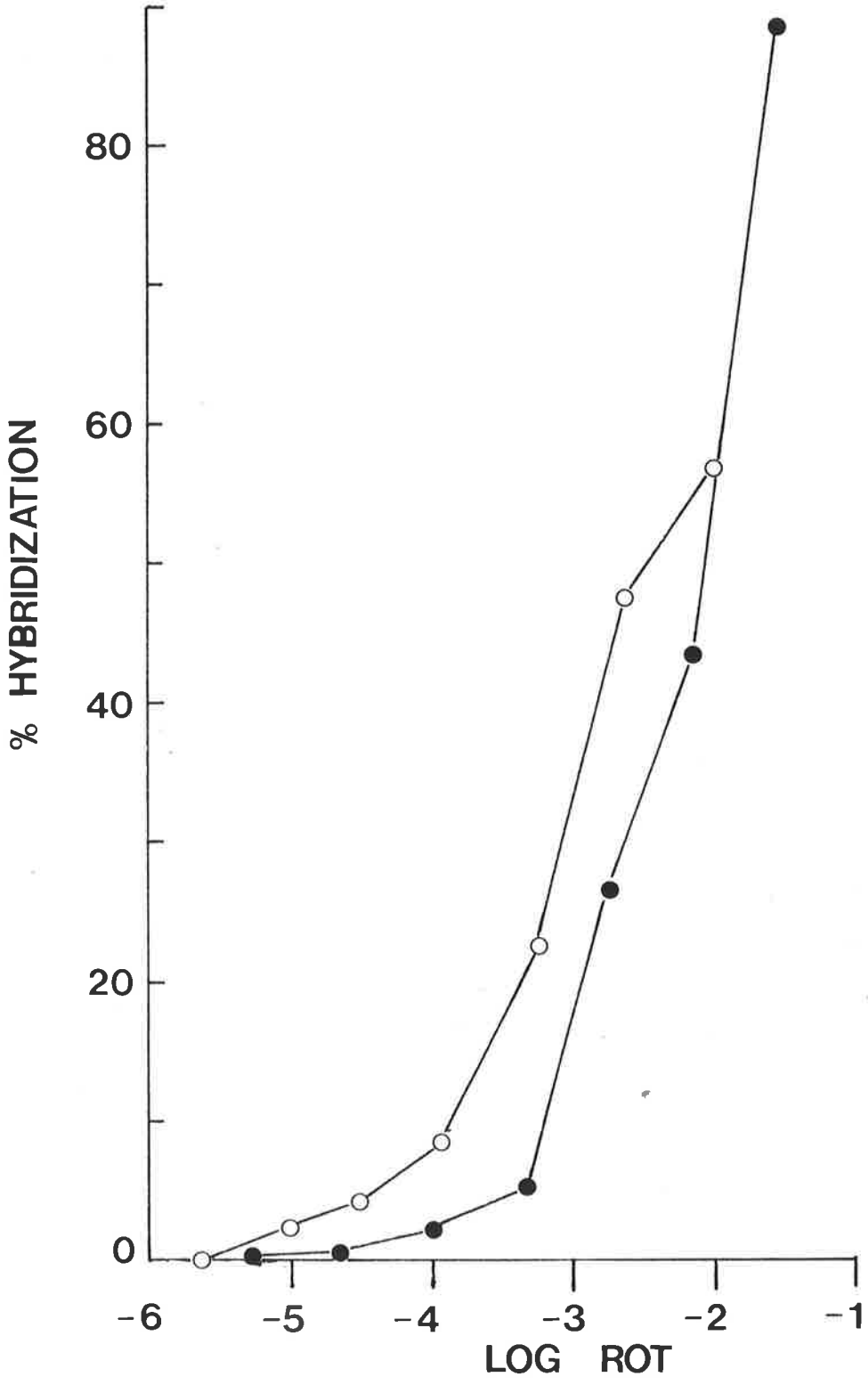
If the $R_o t\ \frac{1}{2}$ of 1.2×10^{-2} mol.sec. l^{-1} is obtained for an RNA of Mo.Wt. 2.6×10^6 (Abu-Samah, 1981) the approximate molecular weight of SCRLV-RNA

$$= \frac{2600000 \times .009}{0.012}$$

$$= \frac{1.95 \times 10^6}{}$$

This estimate of molecular weight is close to that estimated for SCRLV-RNA (see Section 7.3.10) and members of the luteovirus

Fig. 37 Homologous hybridization Kinetics of SCRLV-specific 3H-cDNA with the high molecular weight virus RNA. Four-fold dilutions of the RNA in 0.18M NaCl, 0.01M Tris- H Cl, pH 7.0, 1mM EDTA, 0.05% SDS were incubated with cDNA at 65°C for 21 hrs. and 41 min. (○) or 72 hrs. (●). Percent hybridization was assayed with S_1 nuclease and calculated according to the text (see Section 8.2.8).


% HYBRIDIZATION
80
60
40
20
0
-6
-5
-4
-3
-2
-1
LOG ROT

group (Rochow and Duffus, 1981).

8.3.2 Detection of SCRLV-RNA in plants infected with SCRLV and in aphids fed on SCRLV infected plants

Nucleic acid extracted from SCRLV infected plants or from A.solani (Kltb.) fed on infected T.subterraneum L. showed between 44% and 96% maximum hybridization values whereas nucleic acid extracted from healthy plants and A.solani (Kltb.) not fed on SCRLV source gave values of between 0 and 12% (Table 18). This confirmed that the ^{3}H-cDNA probe was specific for SCRLV and that it did not hybridize with nucleic acids extracted from healthy T.subterraneum L., P.sativum L. and nonviruliferous A.solani (Kltb.). Potato leaf roll virus (PLRV) which shows a distant serological cross reaction with SCRLV (Ashby and Kyriakou, 1982) showed no detectable nucleotide sequence homology when nucleic acids extracted from PLRV-infected Physalis floridana L. were allowed to hybridize with cDNA during the hybridization incubation (Table 18).

It was also observed that the CTAB step in the purification of nucleic acids from plants and aphids was necessary to prevent partial degradation of cDNA during the hybridization incubation (Table 18).

8.3.3 Detection of SCRLV in aphids

Following a 72 hr . acquisition feed by apterae on SCRLV-infected T.subterraneum L. (Table 18), groups of A.solani (Kltb.) but not M.persicae (Sulz.) were found by MHA to have high percentage hybridization values and thus to contain SCRLV-RNA. In a second trial these species were allowed to feed on SCRLV-infected T.subterraneum L.cv. Mt.Barker for 102 hours under continuous light at 25^{o}C. Live fourth and fifth stage

Table 18: Percentage hybridization of SCRLV-RNA; cDNA with RNA in a range of nucleic acid extracts

Source	Weight of the starting material (g)	Preparation	Nucleic acid Amount extracted (µg)	Nucleic acid Concentration in assay (µg/ml)	$R_o t$ (mol.sec.l^{-1})	Hybridization (%)
Purified SCRLV	500	RNA	0.44	0.29	2.3×10^{-1}	88.6[a]
" "	500	RNA	"	"	"	50.0
T.subterraneum L.						
SCRLV-infected	6.5	total nucleic acid	272(304)	800(805)	648(652)	44.5(34.4)[b]
Healthy	6.5	"	316(92)	810(600)	656(486)	12.2(0.6)
P.sativum L.						
SCRLV-infected	10	"	120(500)	800(800)	648(648)	56.5(25.5)
Healthy	10	"	684(424)	800(800)	648(648)	4.2(0.4)
P.floridana L.						
PLRV-infected	5.5	"	88	600	486	0.2
Healthy	1.2	"	174	800	648	0.1

Contd...

Table 18. (Continuation)

Source	Weight of starting material(g)	Preparation	Nucleic Acid		$R_o t$ (mol.sec.l^{-1})	Hybridization (%)
			Amount extracted (µg)	Concentration in assay (µg/ml)		
A.solani (kltb.)						
200 aphids access to SCRLV[e]	0.075	total nucleic acid	112(200)	800(800)	648(648)	95.9(94.1)
20 " " " "	-	"	-	-c	-	46.5(-)[d]
200 nonviruliferous	0.055	"	104(148)	750(800)	608(648)	0 (1.2)
20 "	-	"	-	-c	-	0
M.persicae ((Sulz.)						
200 aphids access to SCRLV[e]	-	-	-	-c	-	0
20 " " " "	-	-	-	-c	-	0

a = Values corrected for self hybridization (0-5%), but not normalized with reference to homologous standard.

b = Values in paranthesis are the samples not subjected to CTAB precipitation during the total nucleic acid extraction.

c = Carrier yeast RNA was used to extract aphid nucleic acid, $R_o t$ was therefore not calculated. Incubation was for 118 hrs. when carrier was added and 72 hrs. otherwise.

d = No data

e = Aphids fed on SCRLV-infected T.subterraneum L. plants for 72 hrs.

Table 19: Detection of SCRLV in *A.solani* (kltb.) and *M.persicae* (Sulz.) by ELISA and MHA, after feeding on infected *T. subterraneum*.

Sample	Access to SCRLV source	ELISA[a] (A_{400})	Hybridization (%)[b]
10 A.solani (kltb.)	102 hours	1.62	-[c]
" "	" "	1.60	-
" "	" "	-	52.9
" "	" "	0.07	-
" "	" "	0.07	-
" "	" "	-	1.1
10 M.persicae (Sulz.)	102 Hours	0.17	-
" "	" "	0.24	-
" "	" "	-	6.6
30 "	" "	-	16.7
10 "	0	0.10	-
10 "	0	0.09	-
30 "	0	-	0
T.subterraneum L.			
SCRLV-infected		0.88	-
Healthy		0.06	-
V.faba L.			
SCRLV-infected		1.74	-
Healthy		0.06	-

a = Coating antibody at 1.25 µg/ml, 1 hr. at 5°C; antigen (10 aphids were ground in 100µl of 20mM phosphate buffered saline, pH 7.3, 0.05% Tween-20, 20mM diethyldithiocarbamate, 20mM 2-mercaptoethanol; leaf was ground in 4 volumes of the same buffer), was allowed to react for 24 hrs. at 5°C, absorbed conjugate was clarified by centrifugation, and used at 1.25µg/ml, incubating 24hrs. at 5°C; substrate was incubated 5 hrs. at 20°C.

b = Hybridization for 119 hrs. values corrected for self-hybridization.

c = No data.

larvae were collected into batches of 10 or 30 and assayed by MHA. Duplicate groups of 10 aphids were also assayed by ELISA. The results in Table 19 show that SCRLV can be detected in A.solani (Kltb.) by both methods. Trace amounts of SCRLV were detected in the M.persicae (Sulz.) groups given access to SCRLV by both ELISA and MHA. Since both viral antigen and nucleic acid were detected it is concluded that whole virus was taken up by M.persicae (Sulz.). This suggests that although SCRLV may be detected in M.persicae (Sulz.) it does not accumulate to the concentration found in A.solani (Kltb.). This is consistent with the results in Chapter 3 that A.solani (Kltb.) but not M.persicae (Sulz.) transmits SCRLV.

An estimate was made of the average SCRLV content in a group of A.solani (Kltb.) given access to an SCRLV-infected T.subterraneum L. plants for 72 hours. The hybridization kinetics are shown in Fig. 38. A $R_o t\ ½$ value (31.5 mol.sec. l^{-1} see below) was obtained for hybridization time of 20 hrs. for the aphid nucleic acid extract at 1/16 dilution. At this dilution the concentration of the total nucleic acid extract was calculated to be 140µg per ml. Therefore the $R_o t\ ½$ =

$$\frac{140 \times 72000}{320000}$$

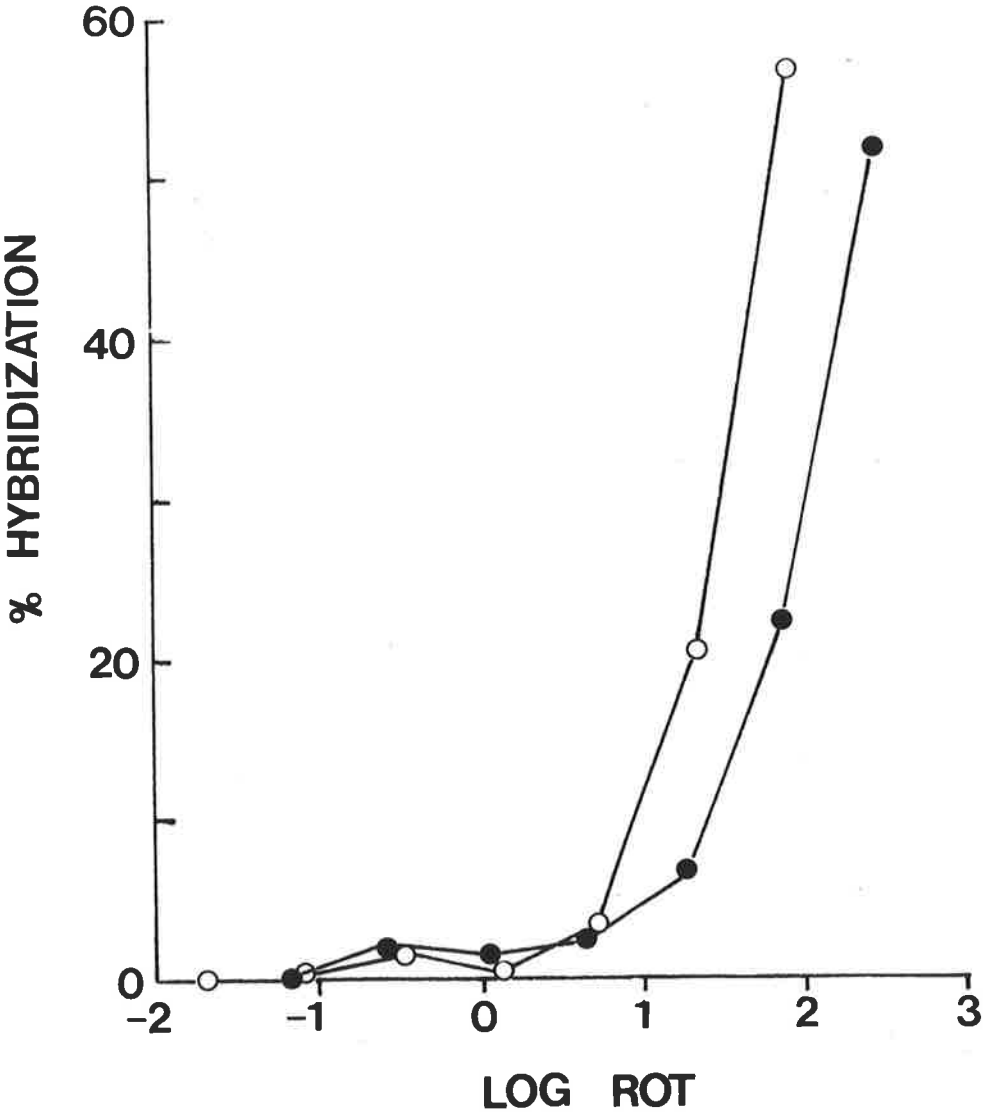
$$= \underline{31.5 \text{ mol. sec.}} l^{-1}$$

By comparing the $R_o t\ ½$ for an aphid extract with the homologous $R_o t\ ½$ (0.009 mol.sec. l^{-1}), SCRLV-RNA was shown to comprise 0.028% (0.009 ÷ 31.5 x 100) of the total nucleic acid extracted from aphids. As the amount of nucleic acid extracted from the 200 aphids was 112 µg, the amount of SCRLV-RNA in 200 aphids

$$= \frac{0.028 \times 112}{100}$$

$$= \underline{31.4 \text{ ng.}}$$

Fig. 38 Hybridization Kinetics of SCRLV-specific cDNA with total nucleic acids extracted from the vector, A.solani (Kltb.) after aphids had been given access to a SCRLV-infected T.subterraneum L. plants for 72 hrs. Aphid nucleic acids were diluted four-fold and incubated as in Fig. 37 for 20 hrs. (○) or 72 hrs. (●).


60
40
20
0
% HYBRIDIZATION
-2
-1
0
1
2
3
LOG ROT

Assuming the RNA content of SCRLV is 20%, the virus content per aphid would be $\frac{100 \times 31.4}{20 \times 200} = 0.785$ng.

Average aphid weight was 375µg, therefore number of aphids per gm. would be 2667 or number of aphids per kg. 2667000. Therefore virus content of the aphids can be estimated to $(7.84 \times 10^{-7}) \times 2667000 = 2.1$mg per kg. of aphids. This value is not corrected for extraction efficiency of the nucleic acid. Although this value is not known for this experiment it seems likely to be approximately 33% from the other experiments using carrier RNA (Randles per.com.).

8.4 DISCUSSION

In this Chapter the ELISA technique was used only as a basis to support MHA data. This is the first evidence to show that MHA can be used to detect SCRLV in virus-infected plants and in aphids given access to the virus-infected plants.

The results shown that SCRLV-RNA was an effective template for reverse transcription into ^{3}H-cDNA, the method described by Taylor et al. (1976). Further, it was shown that less than one microgram of pure RNA is sufficient to synthesize a cDNA probe. This ^{3}H-cDNA probe can probably be used diagnostically for a minimum of 4 years on the basis of previous experience with this type of assay (Randles per. com.).

Although it was observed that DNA (Fig. 32 c) was present in nucleic acids extracted from highly purified SCRLV, it was assumed that this DNA would have sedimented more slowly than the main SCRLV-RNA peak in the sucrose density gradient

(Fig. 35) and would not have interfered with the synthesis of cDNA to SCRLV-RNA in the presence of actinomycin D (Taylor et al., 1976). The specificity of the cDNA for SCRLV-RNA confirms that its synthesis was as expected using the Taylor random primer method (Taylor et al., 1976).

Since SCRLV is largely confined to the vascular system of its plant host (see Chapter 6) successful diagnosis of SCRLV would be expected to depend on using an efficient method to extract nucleic acid from infected plants, as well as its vectors. The results show (Table 18) that the method described in this Chapter to extract nucleic acid is satisfactory. As shown in Table 18, the CTAB step also appears to be necessary in the nucleic acid extraction procedure to prevent partial degradation of cDNA during the hybridization incubation.

The variation in maximum percentage hybridization values obtained in different experiments, may possibly be due to ~~inadvertent~~ variation in S_1 nuclease activity in different assays.

In conclusion, the above experiments show that hybridization analysis using ^{3}H-cDNA to detect SCRLV-RNA in partially purified nucleic acid extracts of plants and aphids is a relatively rapid, sensitive and reliable procedure for the detection of SCRLV. A further use for ^{3}H-cDHA will be in the identification and comparison of SCRLV with other luteoviruses.

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GENERAL DISCUSSION

An understanding of the rate, range, time and pattern of virus spread is necessary to evaluate virus epidemiology, and to formulate appropriate virus control measures. Epidemiology is influenced by properties of the vector, host, virus and environment and quite marked variations in epidemiology can be expected through changes in the environment.

BYMV and SCRLV have become the most important viruses of legume crops in the south east of South Australia since 1975 (Randles pers. com.). The experiments described in Chapter 3 with V.faba L. and T.subterraneum L. firstly, provide a basis for the development of control measures of BYMV and SCRLV in a mediterranean environment typical of the southern part of Australia (winter-spring growing season and dry summer), and secondly, they allow re-evaluation of the principles of the spread of non-persistently and persistently borne aphid transmitted viruses under field conditions. The use of the BYMV-S isolate and the SCRLV-T isolate which induced distinctive symptoms on V.Faba L. minor (Fig. 2) and T.subterraneum L. (Fig. 3) was an advantage because it allowed both viruses to be studied simultaneously. This avoided the variation arising from the use of different sites and times of planting. The indexing of field samples (Section 3.3.1) revealed no other viruses with similar symptomatology in the experimental plots.

Aphids have their greatest economic importance in their role as vectors of virus diseases. The timing of flights of the alatae of a particular aphid species gives the most convenient estimate of changes in the size of aphid populations and the times of crop infestation. At the experimental site M.persicae (Sulz.), M.euphorbiae (Thomas), A.solani (Kltb.) and A.craccivora Koch. showed 2 peaks of flight in each year with major flight activity during the spring season and minor flight activity during the autumn (Fig. 16). These four species mentioned above are vectors of BYMV (Kennedy et al., 1962) and A.solani (Kltb.) is the vector of SCRLV (Johnstone, 1978). They showed (Fig. 16) fluctuations in migratory activity similar to those previously reported for the Adelaide hills (Hughes et al., 1964; 1965). The presence (winter-spring season) or absence (summer and autumn) of green plants adjacent to the aphid traps appeared not to influence the timing of peaks of migration (see Chapter 4 and data obtained over 2½ years (Fig. 16)). The earlier published data led to the conclusion that the Moericke trapping technique gives a general and reproducible estimate of vector migration patterns over a wide area. The vectors studied (Fig. 16) showed overall patterns of migration which were consistent and similar for the four species over the period of the study. Therefore, from aphid trapping records (Fig. 16) alone it is not possible to compare the relative importance of the vectors of BYMV as has been done previously, for example, with cauliflower mosaic virus where different species of vectors show different times of flight (Randles and Crowley, 1967). Therefore it will be important to investigate the transmission efficiency

of the aphid vectors of BYMV to gain some idea of their relative importance.

More aphids were trapped in weeks when mean weekly temperature was between 13°C and 17°C (Table 12). Other aphid species also migrate frequently within this temperature range. Therefore temperature is one of the important determinants of aphid population dynamics on source plants (Maelzer, 1981; Martin and Randles, 1981).

It seems probable that the rainfall levels (Fig.17) interrupted flights of alates and hence the sharp but temporary drop in numbers trapped. Furthermore, Table 12 shows that rainfall above 7 mm appeared to effect flights only when mean weekly temperatures were outside the range 13°C to 17°C. However, further investigations are required to check the significance of this observation. Knowledge of factors that determine vector activity should allow virus incidence to be minimized in annual crops such as V.faba L. and such knowledge may help to anticipate the possible course of an epidemic, and it may help to explain variable incidence of these viruses in climatically different areas.

A general relationship between the spread of both viruses and flights of aphids has been confirmed by the exposure of trap plants (Fig. 16). This relationship also implies that factors leading to reduced vector activity (Fig.16) may reduce virus spread, and that monitoring of aphid species should indicate the risk of virus infection in cropping areas for V.faba L. In the cool temperate zone of New Zealand

which favours summer growth Ashby et al. (1979) reported that Trifolium repens L. was a source of BYMV and SCRLV. Johnstone, (1978)reported that T.repens L. and Rumex spp. were sources of SCRLV in Tasmania. If these or other host species that survived the South Australian summer were the source of SCRLV and BYMV, susceptible V.faba L. should have become infected when they (trap plants) were exposed in autumn, when there was aphid activity (Fig. 16). But this was not the case in this study. The trap plants only became infected, when the virus source was artifically introduced to adjacent experimental plots during the winter-spring growing season (Fig. 16) which suggests that virus appears to have come only from the nearby plots.

Simultaneous surveys of the patterns of distribution of the BYMV and SCRLV in the same plots (see Chapter 3) have allowed a more precise comparison to be made of the spread of a non-persistently (BYMV) and a persistently (SCRLV) transmitted aphid borne virus than can be obtained in separate plots. The effect of a primary disease focus on the number of infected plants in a V.faba L. crop at maturity showed that BYMV spread was independent of vector colonization whereas SCRLV spread was dependent on vector colonization. Where the SCRLV source was provided centrally with the vector A.solani (Kltb.) (SCRLV-Vv) the observed spread was from the centre (Fig. 9). The initial spread was probably due to introduced apterae rather than alatae because aphid trapping (Fig. 16) showed at the time of initial spread of SCRLV that no alate

A.solani (Kltb.) were trapped.

The difference in patterns of spread of SCRLV in SCRLV-V treatments during 1979, and 1980 in the V.faba L. trials may have been due to the different types of insecticides used to kill the A.solani (Kltb.) after infecting the central plants with SCRLV in SCRLV-V treatments initially. Even though no quantitative studies have been done, later observations in the glass house showed that the recommended dosage of "Pyrethrum" by the manufacturer, does not control 100 percent of the aphids whereas "Metasystox" does. Therefore it is possible that some A.solani (Kltb.) apterae survived on "Pyrethrum" sprayed plots and ~~were found to~~ spread the virus from the central infected source (Table 1) whereas in "Metasystox" sprayed plots (Fig. 9) the SCRLV spread is only from the outside of the plots, presumably coming from sources outside the plots.

The observed different patterns of spread of both viruses in treatments BYMV-V and SCRLV-V (Fig. 9) are mainly due to the different modes of transmission by their vectors. The relative importance of each can be inferred from a discussion of the modes of transmission of the two viruses. BYMV is a non-persistently transmitted virus (Bos, 1970) and nonviruliferous vectors of non-persistently transmitted viruses can acquire the virus within a few seconds (Matthews, 1981) to become viruliferous. It was observed that vectors of BYMV (Fig. 16) are numerous in the study area and some of them could have acquired the BYMV from the infected central focus and contributed to the further spread of BYMV in the experimental

plots. For example, after completion of the teneral period, M.persicae (Sulz.) alates would be expected to take off from the host under favourable weather conditions (Johnson et al., 1957) for a long distance flight, followed by a brief erratic migratory phase in which the aphids try to find suitable host plants. Virus can be picked up from infected plants either shortly before the beginning of the main flight, which can last for hours (Kennedy and Booth, 1963) or during probing between attack flight and settling. Harrewijn et al. (1981) using radiolabelled M.persicae (Sulz.) found that the first flight can be as short as 1 to 100 meters. Van Hoof (1979) found that the spread of potato virus Y - N isolate (PVY^N) in early flights occurred over relatively short distances from a virus source. Conversely, for persistently transmitted viruses e.g. SCRLV (Kellock, 1971) the aphids have to feed on the infected plants for a considerable period (a day or more) and pass the latent period after the acquisition feed before becoming infective (Broadbent, 1952). Further, Johnson (1953;1957) suggests that migratory aphids would be unimportant as a vector of persistent viruses unless they developed on infected plants, because nonviruliferous migratory aphids that land after an initial flight and remain on infected plants long enough to become viruliferous would be unlikely to leave. The flight behaviour of A.solani (Kltb.) is unknown, but some of the other aphid spp. flight behaviour is known, e.g. M.persicae (Sulz.). If A.solani (Kltb.) behave the same way as M.persicae (Sulz.), migrating A.solani (Kltb.) alighting on the infected plants (SCRLV-Vv, SCRLV-V treatments) for a short period are

unlikely to be effective as vectors because according to Teh (1978) nonviruliferous migratory aphids (A.solani (Klbt.)) take a minimum of 20 hrs. to acquire the SCRLV. The latent period, before aphids become infective is 14 hrs. to 22 hrs. The latent period also depends on the temperature (latent period of 14 hrs. and 22 hrs. at the temperature 20°C and 10°C respectively). On the other hand a colony of alate A.solani (Klbt.) produced on infected plants (SCRLV-Vv treatment) will be viruliferous and the virus can persist in the aphids for 3 weeks or more (Kellock, 1971). Presumably, some of these aphids would have spread SCRLV into the SCRLV-V treatment plots.

From the results shown for the V.faba L. trial it can be inferred that in the trial area, migratory nonviruliferous A.solani (Klbt.) would have been of minor importance in the spread of SCRLV whereas A.solani (Klbt.) moving from colonies on the infected plants would have been the source of spread. Conversely, with BYMV the migrating nonviruliferous vectors of BYMV are equally important in the spread of BYMV. Therefore, when controlling the spread of these two viruses, strategies should be based on the above mentioned characters.

The results in Chapter 3 show that BYMV can spread significantly when virus infected plants were present at that site and control could be achieved by taking measures to prevent the vectors of BYMV entering the crop. The control of SCRLV, where both virus and vector have to be present for significant spread to occur, could presumably be achieved at this site by preventing colonization of the crop with A.solani (Klbt.)

The maximum BYMV infection occurs at the experimental site during October (Fig. 16) when peak numbers of alate BYMV vectors were trapped (Fig. 16). To prevent the crop becoming infected with migratory aphids after feeding on diseased plants in the field, late planting after the aphid activity, could have been helpful, but it is not economical to have a late planted crop because of the high cost of irrigating the crop during summer. The repellent effect of certain colours to aphids (Moericke, 1950; Kennedy et al., 1961) is another possible approach to reduce BYMV incidence in annuals. Aluminium foil also has been used to reduce the incidence of virus diseases introduced into crops by transient aphids (Johnson et al.,1967). This approach is worth testing in the future.

Aphids have a two way interaction between flight and settling (Kennedy, 1965). Aphids tend to adopt rebound flight when they land on non-host plants. Knowledge of this characteristic rebound flight has been utilised for reducing the incidence of virus diseases by cultivating non-host barrier crops within crop plants. Simons (1957); Broadbent (1969); Loebenstein and Raccah (1980) reported that barriers are more effective in protecting plants from non-persistent viruses than persistent ones.

Studies in Chapter 5 describe an attempt to reduce the infection of BYMV and spread of BYMV from nearby infected sources by controlling the aphids using readily available insecticides and barley barriers. Results for BYMV in Table 15 show that the insecticides used were unable to reduce the incidence and spread of BYMV from the nearby infector source presumably because

acquisition and inoculation occur before insecticides are effective on aphids. Barley barrier rows did not reduce the overall accumulated BYMV incidence but influenced the spread of BYMV from the infector row towards the outside of the plots. However, with SCRLV, the systemic insecticides "Disyston" and "Metasystox" (Table 14) reduced the colonization of A.solani (Kltb.) and this was associated with reduced virus incidence relative to the other treatments (Table 15). None of the treatments prevented the spread of SCRLV from the infector row (Fig. 20 T_1....T_5). This is probably because the inoculation threshold was 20 min., (Kellock, 1971) and the insecticides would be able to kill the aphids before they infected the plants (Johnstone and Rapley, 1981). Other possible alternative methods that would be worth investigating are biological control of vectors as suggested by Johnstone and Rapley (1981) because A.solani (Kltb.) has been introduced to Australia without its parasites.

Molecular hybridization assay (MHA) techniques have been used to study the viroids, where only small amounts of RNA are available and concentrations in tissue are very low (Owens, 1978, Palukaitis et al., 1979; Randles and Palukaitis, 1979). The success of this technique depends on obtaining viral RNA of high purity (Abu Samah, 1982). Like other luteoviruses SCRLV is detectable only in phloem transfer cells (Fig. 21, Chapter 6) and Jayasena et al. (1981). Successful purification of SCRLV mainly depends on finding a suitable host to allow SCRLV to multiply faster and isolating SCRLV from transfer cells. Teh (1978) attempted to purify SCRLV unsuccessfully from a number of hosts. As Teh (1978) stated it was easier to

raise and readily transmit SCRLV to T.subterraneum L. Therefore, to purify SCRLV, T.subterraneum L. cv. Mt.Barker was used as described in Chapter 7. A number of methods described for the purification of other luteoviruses (see Chapter 7) were not successful. However, the enzyme assisted method described by Takanami and Kubo (1979a) was modified and as described in Chapter 7 using T.subterraneum L. gave low yields of SCRLV. Changing the virus multiplication host to P.sativum L. cv. Puget ~~with double~~ and using two PEG precipitation steps gave a yield of 1.34 mg/kg of tissue which was sufficient to purify viral RNA. Recently Johnstone et al. (1982) and Ashby and Kyriakou (1982) claimed success in the purification of SCRLV using cellulase. The only difference between the method used by Ashby and Kyriakou (1982) and the method described in Chapter 7 is the clarification procedure. Chloroform-butanol clarification tends to cause loss of virus particles (based on the number of particles observed in the electronmicroscopic field area) when compared to Triton X 100. Waterhouse and Murant (1981) found similar results when they used chloroform-butanol vs Triton X 100 in purification of carrot red leaf virus which is also a luteovirus.

When T.subterraneum L. infected plants were used to purify SCRLV, two types of particles were observed (Fig. 25) (tubular particles and isometric particles). These two types of particles were also observed by Ashby and Kyriakou (1982). The isometric particles were found in infected plants and not in healthy T.subterraneum L. and the isometric particles were indistinguishable from PLRV particles purified (Fig. 22e) in

P.floridana L. using the procedure similar to that used to purify SCRLV from infected T.subterraneum L. The tubular particles were also observed in low concentration in healthy T.subterraneum L. Ashby and Kyriakou (1982) reported that these particles (tubular) were composed of a single polypeptide of molecular weight 54200.

Longer incubation (16 hrs.) of infected plant extract with cellulase (Onozuka R - 10) enzyme (method A - see Chapter 7 Section 7.2.2) gave much more virus than a short 2 hour incubation at 28°C, but purified virus was not aphid transmissible after this treatment. Waterhouse and Murant (1981) suggested that prolonged incubation with enzyme caused damage to the nucleic acid or protein components of the virus and this may be a possible cause for lack of aphid transmission. This warrants further investigation. However, it has been shown, that virus particles purified from method B (see Section 7.2.2) caused red leaf symptoms on healthy T.subterraneum L. seedlings when nonviruliferous A.solani (Kltb.) were allowed to feed on the virus preparation for 48 hrs. at room temperature (see Section 7.2.5). When nonviruliferous M.persicae (Sulz.) were allowed to feed on the same virus preparation as for A.solani (Kltb.) red leaf symptoms on healthy T.subterraneum L. seedlings were not induced. Johnstone (1978) reported that the Tasmanian isolate of SCRLV (SCRLV-T) was transmitted only by A.solani (Kltb.) and not by M.persicae (Sulz.)

The nucleic acids isolated from SCRLV prepared by method A (Fig. 32) indicate that there were two RNAs and some

DNA present. The fast moving RNA in polyacrylamide slab gel (Fig. 32c- No.3) could be a cleavage product of the higher molecular weight RNA (Fig. 32c - No.1). The relationship between RNA 1 and 2 is not known and requires further investigation such as a comparison of nucleotide sequences. The origin of the DNA is unknown and it may be analogous to that which Sarkar (1976)found in PLRV. The DNA source needs to be studied further. However, the work by Rowhani and Stace-Smith, 1979; Mehrad et al., 1979; Takanami and Kubo, 1979b, has proved that PLRV has a single stranded RNA. The slow moving RNA (Fig. 32c No.1) has a molecular weight (Fig. 34) equivalent to BYDV, the type member of the luteovirus group (Rochow and Duffus, 1981). Since the molecular weights of luteovirus RNA's have been so far estimated only on nondenaturing gels e.g. BYDV-RNA (Brakke and Rochow, 1974); PLRV-RNA (Takanami and Kubo, 1979b; Rowhani and Stace-Smith, 1979); PeLRV-RNA (Ashby and Huttinga, 1979) there is uncertainty about the true values of the molecular weight of their RNA's. Therefore a comparison of results, from nondenaturing and denaturing gels with a range of luteovirus RNAs should be undertaken when adequate amounts of RNA become available.

Work in Chapter 8 shows the potential value of MHA for identifying plants infected with viruses which are in low concentration. R_oT analysis (Fig. 37) showed that RNA used to make the cDNA probe was not significantly contaminated with host plant RNA. The virus specific RNA as well as RNA in leaves infected by SCRLV was readily detected with homologous cDNA probes. MHA also showed that aphids feeding on SCRLV infected

plants contain an RNA with a nucleotide sequence similar to that of SCRLV.

MHA is however, more than a diagnostic aid. It can be used as a quantitative assay for the virus in plants or aphids. Furthermore MHA allowed the measurement of the concentration of SCRLV-RNA extracted from leaf tissue as well as from aphids which would be valuable for estimating absolute virus and RNA concentration in virus (see Chapter 8 Section 8.3.3).

The possible application of the direct ELISA method for identification of purified SCRLV particles as well as ~~from~~ in extracts of infected plant material was also investigated (Chapter 8). The results show (Table 19) that infected plants are clearly distinguished from healthy ones as ~~well as~~ are viruliferous from healthy aphids. The direct ELISA method also showed (Table 19) that M.persicae (Sulz.) ~~was~~ although not a vector of SCRLV (Johnstone, 1978), ~~and~~ after feeding on SCRLV infected plants, ~~it~~ showed a positive reaction. This should lead to further investigations on the fate of virus particles in nonvectors.

In this study the efficiency of the direct ELISA method to the indirect ELISA method was not compared. It should be emphasized that the high specificity of direct ELISA raises problems in detecting closely related viruses. For example, conjugated antibodies in at least 5 different antisera would be required to detect the presence of the 5 major strains of BYDV (Rochow and Carmichael, 1979). Hence, the application of direct ELISA may be of restricted use for identification work, although it is very useful for the identification of closely related virus strains (van Regenmortel and Burckard, 1980).

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List of Appendix Tables

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Table 1. BYMV and SCRLV spread from the centre where the virus source was provided centrally with vector on T.subterraneum L. (1979 summer-autumn). 143

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Appendix Table 1: BYMV and SCRLV spread from the centre where the virus source was provided centrally with vector on T.subterraneum L.(1979 summer - autumn)

Date of observation	Treatment	Distance from source (row)	Number of plants infected/~~from~~ healthy plants exposed				Percent Infection		S.E. (mean) (±)	
			SCRLV		BYMV		SCRLV	BYMV	SCRLV	BYMV
			R_1*	R_2	R_1	R_2				
5.3.80	Virus + vector (Vv)	1	3/7	2/8	0/8	0/7	33.92	0	8.95	0
		2	2/13	0/16	0/16	0/16	7.69	0	7.71	0
		3	0/24	1/24	0/23	0/24	2.08	0	2.08	0
		4	0/32	0/32	0/28	0/31	0	0	0	0
		5	0/38	0/39	0/40	0/36	0	0	0	0
		6	0/47	0/48	0/40	0/42	0	0	0	0
19.3.80		1	5/7	5/8	1/8	1/7	66.96	13.39	4.47	0.89
		2	7/13	10/16	0/16	0/16	58.17	0	4.34	0
		3	0/24	2/24	0/23	0/24	4.16	0	4.17	0
		4	0/32	0/32	0/28	0/31	0	0	0	0
		5	0/38	0/39	0/40	0/36	0	0	0	0
		6	0/47	0/48	0/40	0/42	0	0	0	0
2.4.80		1	6/7	7/8	4/8	3/7	86.60	46.42	0.89	3.58
		2	10/13	12/16	0/16	0/16	75.96	0	0.96	0
		3	5/24	3/24	0/23	0/24	16.66	0	4.17	0
		4	3/32	8/32	0/28	0/31	17.18	0	7.83	0
		5	0/38	0/39	0/40	0/36	0	0	0	0
		6	0/47	0/48	0/40	0/42	0	0	0	0

Contd.

Continuation of Appendix Table: 1

Date of observation	Treatment	Distance from source (row)	Number of plants infected from healthy plants				Percent infection		S.E. (mean) (±)	
			SCRLV		BYMV		SCRLV	BYMV	SCRLV	BYMV
			R_1	R_2	R_1	R_2				
16.4.80		1	7/7	7/8	5/8	6/7	93.75	74.10	6.26	11.63
		2	11/13	12/16	4/16	4/16	79.80	25.0	4.81	0
		3	13/24	16/24	3/23	2/24	60.41	10.68	6.26	2.36
		4	4/32	8/32	4/28	3/31	18.75	11.97	6.26	2.31
		5	2/38	3/39	2/40	2/36	6.47	5.27	1.21	0.27
		6	2/47	1/48	2/40	4/42	3.16	7.26	1.08	2.26
30.4.80		1	7/7	8/8	8/8	7/7	100	100	0	0
		2	13/13	15/16	8/16	9/16	96.81	53.12	3.13	3.13
		3	21/24	20/24	5/23	8/24	85.41	27.53	2.04	5.81
		4	19/32	16/32	5/28	7/31	54.68	20.21	4.69	2.37
		5	20/38	17/39	3/40	4/36	48.10	9.30	4.53	1.81
		6	16/47	16/48	6/40	4/42	33.68	12.26	0.35	2.74
14.5.80		1	7/7	8/8	8/8	7/7	100	100	0	0
		2	13/13	16/16	11/16	10/16	100	65.62	0	3.13
		3	21/24	22/24	6/23	9/24	89.58	31.79	2.08	5.72
		4	22/32	20/32	5/28	7/31	65.62	20.21	3.13	2.37
		5	23/38	20/39	4/40	4/36	55.9	10.55	4.63	0.55
		6	17/47	16/48	6/40	5/42	34.75	13.45	1.42	1.55

Continuation of Appendix Table: 1

Date of observation	Treatment	Distance from source (row)	Number of plants infected from healthy plants				Percent infection		S.E. (mean) (±)	
			SCRLV		BYMV		SCRLV	BYMV	SCRLV	BYMV
			R_1	R_2	R_1	R_2				
5.3.80	Control (c)	1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	0/24	0/24	0/24	0	0	0	0
		4	0/28	0/31	0/32	0/32	0	0	0	0
		5	0/40	0/36	0/38	0/39	0	0	0	0
		6	0/40	0/42	0/47	0/48	0	0	0	0
19.3.80		1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	0/24	0/24	0/24	0	0	0	0
		4	0/28	0/31	0/32	0/32	0	0	0	0
		5	0/40	0/36	0/38	0/39	0	0	0	0
		6	0/40	0/42	0/47	0/48	0	0	0	0
2.4.80		1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	0/24	0/24	0/24	0	0	0	0
		4	0/28	0/31	0/32	0/32	0	0	0	0
		5	0/40	0/36	0/38	0/39	0	0	0	0
		6	0/40	0/42	0/47	0/48	0	0	0	0

contd....

Continuation of Appendix Table: 1.

Date of observation	Treatment	Distance from source (row)	Number of plants infected from healthy plants				Percent infection		S.E. (±) (mean)	
			SCRLV		BYMV		SCRLV	BYMV	SCRLV	BYMV
			R_1	R_2	R_1	R_2				
16.4.80		1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	0/24	0/24	0/24	0	0	0	0
		4	0/28	0/31	1/32	0/32	0	1.56	0	1.56
		5	1/40	1/36	2/38	2/39	2.6	5.19	0.1	0.07
		6	3/40	5/42	3/47	4/48	9.7	7.35	2.20	0.97
30.4.80		1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	0/24	0/24	0/24	0	0	0	0
		4	4/28	6/31	3/32	1/32	16.81	6.24	2.54	3.13
		5	5/40	9/36	5/38	6/39	18.75	14.26	6.26	1.11
		6	8/40	10/42	8/47	10/48	21.9	18.92	1.9	1.91
14.5.80		1	0/8	0/7	0/7	0/8	0	0	0	0
		2	0/16	0/16	0/13	0/16	0	0	0	0
		3	0/23	2/24	0/24	0/24	4.16	0	4.17	0
		4	5/28	6/31	3/32	1/32	18.6	6.24	0.75	3.13
		5	8/40	10/36	7/38	6/39	23.88	16.9	3.89	1.52
		6	16/40	11/42	10/47	10/48	33.09	21.05	6.92	0.22

* R = Replicate

Appendix Table 2 : Mean fortnightly percentage rate of increase in incidence of BYMV and SCRLV in T.subterraneum L. trial

Treatment	Date of observation	Number of diseased plants out of number of healthy plants available		Average percent infection
		R^{**}_1	R_2	
SCRLV-Vv*	5/3/80	5/161	3/167	2.44
	19/3	7/156	14/164	6.50
	2/4	12/149	13/150	8.35
	16/4	15/137	17/137	11.67
	30/4	57/122	45/120	42.11
	14/5	7/65	10/75	12.04
C	5/3	0/155	0/156	0
	19/3	0/155	0/156	0
	2/4	0/155	0/156	0
	16/4	4/155	6/156	3.21
	30/4	13/151	19/150	10.63
	14/5	12/138	4/131	5.87
BYMV-Vv	5/3	0/155	0/156	0
	19/3	1/155	1/156	0.64
	2/4	3/154	2/155	1.61
	16/4	16/151	18/153	11.17
	30/4	15/135	18/135	12.22
	14/5	5/120	3/117	3.36
C	5/3	0/161	0/167	0
	19/3	0/161	0/167	0
	2/4	0/161	0/167	0
	16/4	6/161	6/167	3.65
	30/4	10/155	11/161	6.64
	14/5	4/145	0/150	1.37

Vv* = Virus + vector

C = No virus, no vector

R** = Replicates

Appendix Table 3: The amount of BYMV and SCRLV spread from epidemiological plots after virus source was provided (1979 winter-spring trial).

Date of observation	Treatment	Distance from source (m)	Number of diseased plants out of healthy plants						Percent infection (mean)	
			SCRLV			BYMV			SCRLV	BYMV
			R_1*	R_2	R_3	R_1	R_2	R_3		
5.10.79	Virus + vector (Vv)	1	0/40	0/40	0/40	2/40	3/40	0/40	0	4.16
		2	0/40	0/40	1/40	1/40	0/40	0/40	0.83	0.83
		3	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		4	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		5	0/40	0/40	0/40	0/40	0/40	0/40	0	0
18.10.79		1	4/40	3/40	0/40	3/40	3/40	2/40	8.33	6.66
		2	1/40	2/40	2/40	1/40	1/40	0/40	4.16	1.66
		3	0/40	2/40	1/40	1/40	0/40	0/40	2.5	0.83
		4	1/40	0/40	1/40	0/40	0/40	0/40	1.66	0
		5	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
2.11.79		1	4/40	3/40	3/40	3/40	3/40	2/40	8.33	6.66
		2	3/40	2/40	2/40	1/40	1/40	0/40	5.83	1.66
		3	1/40	2/40	1/40	1/40	0/40	0/40	3.33	0.83
		4	1/40	0/40	1/40	0/40	0/40	0/40	1.66	0
		5	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0

Contd.

Continuation of Appendix Table: 3

Date of observation	Treatment	Distance from source (m)	Number of diseased plants out of healthy plants						Percent infection (mean)	
			SCRLV			BYMV			SCRLV	BYMV
			R_1	R_2	R_3	R_1	R_2	R_3		
16.11.79		1	4/40	3/40	3/40	3/40	3/40	2/40	8.33	6.66
		2	3/40	2/40	2/40	1/40	1/40	0/40	5.83	1.66
		3	1/40	2/40	2/40	1/40	0/40	0/40	4.16	0.83
		4	1/40	1/40	1/40	0/40	0/40	0/40	2.5	0
		5	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
5.10.79	Virus only (V)	1	0/40	0/40	0/40	1/40	0/40	0/40	0	0.83
		2	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		3	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		4	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		5	0/40	0/40	0/40	0/40	0/40	0/40	0	0
18.10.79		1	2/40	2/40	1/40	2/40	0/40	0/40	4.16	1.66
		2	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
		3	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		4	0/40	1/40	0/40	0/40	0/40	0/40	0.83	0
		5	0/40	0/40	1/40	0/40	0/40	0/40	0.83	0

Contd..

Continuation of Appendix Table: 3

Date of observation	Treatment	Distance from source (m)	Number of diseased plants out of healthy plants						Percent infection (mean)	
			SCRLV			BYMV			SCRLV	BYMV
			R_1	R_2	R_3	R_1	R_2	R_3		
2.11.79		1	2/40	2/40	2/40	2/40	0/40	0/40	5.0	1.66
		2	1/40	1/40	1/40	0/40	0/40	0/40	2.5	0
		3	2/40	0/40	1/40	0/40	0/40	0/40	2.5	0
		4	1/40	1/40	0/40	0/40	0/40	0/40	1.66	0
		5	1/40	1/40	1/40	0/40	0/40	0/40	2.5	0
16.11.79		1	2/40	2/40	2/40	2/40	0/40	0/40	5.0	1.66
		2	1/40	1/40	1/40	0/40	0/40	0/40	2.5	0
		3	2/40	0/40	1/40	0/40	0/40	0/40	2.5	0
		4	1/40	1/40	0/40	0/40	0/40	0/40	1.66	0
		5	1/40	1/40	1/40	0/40	0/40	0/40	2.5	0
5.10.79	Control	1	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		2	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		3	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		4	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		5	0/40	0/40	0/40	0/40	0/40	0/40	0	0

Contd..

Continuation of Appendix Table: 3

Date of observation	Treatment	Distance from source (m)	Number of diseased plants out of healthy plants						Percent infection (mean)	
			SCRLV			BYMV			SCRLV	BYMV
			R_1*	R_2	R_3	R_1	R_2	R_3		
18.10.79		1	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		2	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		3	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		4	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		5	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
2.11.79		1	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		2	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		3	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
		4	0/40	1/40	1/40	0/40	0/40	0/40	1.66	0
		5	3/40	2/40	1/40	0/40	0/40	0/40	5.0	0
16.11.79		1	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		2	0/40	0/40	0/40	0/40	0/40	0/40	0	0
		3	1/40	0/40	0/40	0/40	0/40	0/40	0.83	0
		4	0/40	1/40	2/40	0/40	0/40	0/40	2.5	0
		5	3/40	2/40	1/40	0/40	0/40	0/40	5.0	0

* Replicates.

Appendix Table: 4 : Movement of BYMV and SCRLV on V.faba L. after virus source was provided with or wihout vector - 1980 winter-spring.

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1*	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
28.8.80	Virus + vector (Vv)	1	0/8	1/8	0/8	0/8	0/8	0/8	4.16	0	4.18	0
		2	1/13	0/16	0/14	0/13	0/16	0/14	2.56	0	2.57	0
		3	1/23	0/23	0/24	0/23	0/23	0/24	1.44	0	1.45	0
		4	0/31	0/31	0/32	0/31	0/31	0/32	0	0	0	0
		5	0/35	0/39	0/39	0/35	0/39	0/39	0	0	0	0
		6	0/42	0/47	0/48	0/42	0/47	0/48	0	0	0	0
		7	0/54	0/54	0/56	0/54	0/54	0/56	0	0	0	0
		8	0/60	0/59	0/63	0/60	0/59	0/63	0	0	0	0
		9	0/68	0/68	0/70	0/68	0/68	0/70	0	0	0	0
		10	0/72	0/76	0/76	0/72	0/76	0/76	0	0	0	0
10.9.80		1	1/8	2/8	1/8	2/8	2/8	1/8	16.66	20.83	4.18	4.18
		2	1/13	0/16	0/14	1/13	0/16	0/14	2.56	2.56	2.57	2.57
		3	1/23	0/23	0/24	1/23	3/23	0/24	1.44	5.79	1.47	3.85
		4	0/31	0/31	0/32	1/31	1/31	0/32	0	2.14	0	1.07
		5	0/35	0/39	0/39	1/35	0/39	0/39	0	0.95	0	0.95
		6	0/42	0/47	0/48	0/42	0/47	0/48	0	0	0	0
		7	0/54	0/54	0/56	0/54	0/54	0/56	0	0	0	0
		8	0/60	0/59	0/63	0/60	0/59	0/63	0	0	0	0
		9	0/68	0/68	0/70	0/68	0/68	0/70	0	0	0	0
		10	0/72	0/76	0/76	0/72	0/76	0/76	0	0	0	0

Contd..

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
23.9.80		1	2/8	2/8	3/8	3/8	4/8	3/8	29.16	41.66	4.18	4.18
		2	2/13	4/16	1/14	2/13	6/16	1/14	15.84	20.00	5.18	9.11
		3	4/23	2/23	3/24	4/23	4/23	0/24	12.86	11.59	2.53	5.82
		4	6/31	3/31	0/32	3/31	3/31	1/32	9.67	7.48	5.61	2.19
		5	3/35	4/39	1/39	1/35	1/39	0/39	7.12	1.80	2.34	0.91
		6	0/42	0/47	1/48	5/42	3/47	0/48	0.69	6.09	0.69	3.45
		7	1/54	1/54	0/56	1/54	1/54	1/56	1.23	1.82	0.61	0.02
		8	0/60	0/59	0/63	2/60	2/59	0/63	0	2.23	0	1.12
		9	0/68	1/68	0/70	6/68	3/68	0/70	0.49	4.41	0.49	2.55
		10	0/72	0/76	0/76	4/72	3/76	0/76	0	3.14	0	1.64
9.10.80		1	2/8	2/8	4/8	5/8	5/8	3/8	33.33	54.16	8.37	8.37
		2	3/13	5/16	5/14	4/13	10/16	1/14	30.0	33.46	3.72	16.12
		3	6/23	4/23	5/24	4/23	8/23	1/24	21.43	18.77	2.53	8.91
		4	9/31	10/31	1/32	9/31	8/31	3/32	21.48	21.40	9.27	6.11
		5	7/35	6/39	3/39	4/45	2/39	0/39	14.35	5.51	3.60	3.31
		6	7/42	7/47	2/48	10/42	3/47	0/48	11.90	10.06	3.92	7.14
		7	4/54	5/54	2/56	3/54	7/54	2/56	6.74	7.36	1.68	2.87
		8	6/60	4/59	1/63	4/60	3/59	1/63	6.11	4.44	2.46	1.50
		9	2/68	1/68	1/70	12/68	9/68	0/70	1.94	10.29	0.50	5.32
		10	2/72	1/76	3/76	5/72	8/76	5/76	2.67	8.01	0.76	1.26

Contd..

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
22.10.80		1	2/8	2/8	4/8	5/8	6/8	4/8	33.33	62.5	8.37	7.25
		2	3/13	5/16	5/14	5/13	10/16	2/14	30.0	38.41	3.72	13.99
		3	6/23	4/23	9/24	5/23	8/23	2/24	26.99	21.58	5.85	7.65
		4	9/31	11/31	2/32	11/31	8/31	4/32	23.58	24.59	8.91	6.69
		5	7/35	6/39	5/39	7/35	4/39	0/39	16.06	10.08	2.11	5.80
		6	7/42	7/47	2/48	10/42	3/47	0/48	11.90	10.06	3.92	7.14
		7	6/54	5/54	3/56	4/54	8/54	3/56	8.57	9.18	1.70	2.88
		8	6/60	4/59	1/63	4/60	4/59	1/63	6.11	5.0	2.46	1.72
		9	3/68	1/68	2/70	12/68	12/68	0/70	2.91	11.76	0.85	5.91
		10	3/72	2/76	3/76	5/72	14/76	5/76	3.57	10.64	0.48	3.89
28.8.80	Virus only (V)	1	0/7	0/5	0/8	0/7	0/5	0/8	0	0	0	0
		2	0/16	0/8	0/16	0/16	0/8	0/16	0	0	0	0
		3	0/22	0/18	0/24	0/22	0/18	0/24	0	0	0	0
		4	0/30	0/26	0/32	0/30	0/26	0/32	0	0	0	0
		5	0/39	0/30	0/39	0/39	0/30	0/39	0	0	0	0
		6	0/48	0/37	0/48	0/48	0/37	0/48	0	0	0	0
		7	0/53	0/48	0/56	0/53	0/48	0/56	0	0	0	0
		8	0/59	0/55	0/63	0/59	0/55	0/63	0	0	0	0
		9	0/69	0/62	0/71	0/69	0/62	0/71	0	0	0	0
		10	0/74	0/59	0/78	0/74	0/59	0/78	0	0	0	0

Contd..

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
10.9.80		1	0/7	0/5	0/8	1/7	0/5	1/8	0	8.92	0	4.50
		2	0/16	0/8	0/16	0/16	0/8	1/16	0	2.08	0	2.09
		3	0/22	0/18	0/24	0/22	0/18	0/24	0	0	0	0
		4	0/30	0/26	0/32	0/30	0/26	0/32	0	0	0	0
		5	0/39	0/30	0/39	0/39	0/30	0/39	0	0	0	0
		6	0/48	0/47	0/48	0/48	0/37	0/48	0	0	0	0
		7	0/53	0/48	0/56	0/53	0/48	0/56	0	0	0	0
		8	0/59	0/55	0/63	0/59	0/55	0/63	0	0	0	0
		9	0/69	0/62	0/71	0/69	0/62	0/71	0	0	0	0
		10	0/74	0/59	0/78	0/74	0/59	0/78	0	0	0	0
23.9.80		1	0/7	0/5	0/8	3/7	3/5	6/8	0	59.28	0	9.33
		2	0/16	0/8	0/16	3/16	1/8	4/16	0	18.75	0	3.62
		3	0/22	0/18	0/24	4/22	0/18	3/24	0	10.22	0	5.39
		4	0/30	0/26	0/32	3/30	0/26	2/32	0	5.41	0	2.93
		5	0/39	0/30	0/39	0/39	0/30	0/39	0	0	0	0
		6	0/48	0/37	0/48	0/48	0/37	1/48	0	0.69	0	0.69
		7	0/53	1/48	0/56	0/53	0/48	2/56	0.69	1.19	0.69	1.19
		8	0/59	0/55	0/63	0/59	1/55	3/63	0	2.19	0	1.39
		9	1/69	0/62	0/71	1/69	2/62	3/71	0.48	2.96	0.48	0.81
		10	0/74	1/59	0/78	1/74	1/59	6/78	0.56	3.57	0.56	2.06

Contd.

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
9.10.80		1	0/7	0/5	0/8	5/7	4/5	6/8	0	75.47	0	2.50
		2	0/16	0/8	0/16	11/16	3/8	7/16	0	50.0	0	9.59
		3	0/22	0/18	0/24	10/22	3/18	8/24	0	31.81	0	8.38
		4	0/30	0/26	0/32	11/30	2/26	6/32	0	21.03	0	8.48
		5	0/39	0/30	1/39	12/39	0/30	4/39	0.85	13.67	0.85	9.09
		6	1/48	0/37	0/48	6/48	0/37	6/48	0.69	8.33	0.69	4.18
		7	0/53	2/48	1/56	4/53	1/48	9/56	1.98	8.56	1.21	4.09
		8	0/59	1/55	0/63	0/59	1/55	7/63	0.60	4.30	0.60	3.45
		9	1/69	1/62	1/71	7/69	3/62	7/71	1.48	8.27	0.06	1.73
		10	2/74	3/59	4/78	9/74	2/59	9/78	4.3	9.02	0.80	2.84
22.10.80		1	0/7	0/5	0/8	5/7	4/5	7/8	0	79.64	0	4.66
		2	0/16	0/8	0/16	11/16	3/8	7/16	0	50.0	0	9.59
		3	0/22	0/18	0/24	10/22	3/18	10/24	0	34.59	0	9.07
		4	0/30	0/26	0/32	11/30	4/26	6/32	0	23.59	0	6.63
		5	0/39	0/30	1/39	12/39	0/30	6/39	0.85	15.38	0.85	8.92
		6	2/48	0/37	1/48	6/48	0/37	7/48	2.08	9.02	1.20	4.57
		7	3/53	2/48	1/56	4/53	1/48	9/56	3.86	8.56	1.13	4.09
		8	2/59	2/55	1/63	0/59	2/55	8/63	2.86	5.44	0.64	3.79
		9	2/69	3/62	2/71	7/69	4/62	10/71	3.51	10.22	0.66	2.21
		10	3/74	3/59	4/78	11/74	3/59	11/78	4.75	11.34	0.35	3.15

Contd.

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
28.8.80	Control (C)	1	0/9	0/8	0/8	0/9	0/8	0/8	0	0	0	0
		2	0/15	0/15	0/11	0/15	0/15	0/11	0	0	0	0
		3	0/23	0/22	0/20	0/23	0/22	0/20	0	0	0	0
		4	0/31	0/30	0/26	0/31	0/30	0/26	0	0	0	0
		5	0/40	0/38	0/36	0/40	0/38	0/36	0	0	0	0
		6	0/47	0/47	0/40	0/47	0/47	0/40	0	0	0	0
		7	0/55	0/54	0/46	0/55	0/54	0/46	0	0	0	0
		8	0/62	0/61	0/56	0/62	0/61	0/56	0	0	0	0
		9	0/69	0/71	0/58	0/69	0/71	0/58	0	0	0	0
		10	0/78	0/77	0/68	0/78	0/77	0/68	0	0	0	0
10.9.80		1	0/9	0/8	0/8	0/9	0/8	0/8	0	0	0	0
		2	0/15	0/15	0/11	0/15	0/15	0/11	0	0	0	0
		3	0/23	0/22	0/20	0/23	0/22	0/20	0	0	0	0
		4	0/31	0/30	0/26	0/31	0/30	0/26	0	0	0	0
		5	0/40	0/38	0/36	0/40	0/38	0/36	0	0	0	0
		6	0/47	0/47	0/40	0/47	0/47	0/40	0	0	0	0
		7	0/55	0/54	0/46	0/55	0/54	0/46	0	0	0	0
		8	0/62	0/61	0/56	0/62	0/61	0/56	0	0	0	0
		9	0/69	0/71	0/58	0/69	0/71	0/58	0	0	0	0
		10	0/78	0/77	0/68	0/78	0/77	0/68	0	0	0	0

Contd..

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
23.9.80		1	0/9	0/8	0/8	0/9	0/8	0/8	0	0	0	0
		2	0/15	0/15	0/11	0/15	0/15	0/11	0	0	0	0
		3	0/23	0/22	0/20	0/23	0/22	0/20	0	0	0	0
		4	0/31	0/30	0/26	1/31	0/30	0/26	0	1.07	0	1.07
		5	0/40	0/38	0/36	1/40	0/38	0/36	0	0.83	0	0.83
		6	0/47	0/47	0/40	2/47	0/47	0/40	0	1.41	0	1.42
		7	0/55	1/59	0/46	2/55	1/54	0/46	1.34	1.82	0.67	1.05
		8	0/62	0/61	0/56	2/62	1/61	0/56	0	1.61	0	0.93
		9	0/69	1/71	0/58	2/69	3/71	0/58	0.46	2.37	0.46	1.25
		10	0/78	0/77	1/68	4/78	1/77	0/68	0.49	2.13	0.49	1.54
9.10.80		1	0/9	0/8	0/8	0/9	0/8	0/8	0	0	0	0
		2	0/15	0/15	0/11	0/15	0/15	0/11	0	0	0	0
		3	0/23	0/22	0/20	0/23	1/22	0/20	0	1.51	0	1.52
		4	0/31	0/30	0/26	2/31	0/30	0/26	0	2.15	0	2.16
		5	0/40	0/38	0/36	2/40	0/38	1/36	0	2.59	0	1.45
		6	0/47	1/47	0/40	2/47	1/47	0/40	0.70	2.12	0.71	1.23
		7	1/55	1/54	1/46	2/55	1/54	2/46	1.94	3.89	0.11	0.22
		8	1/62	1/61	1/56	3/62	2/61	1/56	1.67	3.29	0.05	0.88
		9	2/69	2/71	0/58	4/69	3/71	3/58	1.90	5.06	0.95	0.45
		10	1/78	3/77	3/68	5/78	8/77	6/68	3.30	8.53	0.01	1.16

Contd.

Continuation of Appendix Table: 4

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants						Percent infection mean		S.E. of mean (±)	
			SCRLV			BYMV						
			R_1*	R_2	R_3	R_1	R_2	R_3	SCRLV	BYMV	SCRLV	BYMV
22.10.80		1	0/9	0/8	0/8	0/9	0/8	0/8	0	0	0	0
		2	0/15	0/15	0/11	0/15	0/15	0/11	0	0	0	0
		3	0/23	0/22	0/20	0/23	1/22	0/20	0	1.51	0	1.52
		4	1/31	0/30	0/26	2/31	0/30	0/26	1.07	2.15	1.07	2.16
		5	0/40	0/38	0/36	3/40	0/38	1/36	0	3.42	0.86	2.20
		6	1/47	2/47	0/40	2/47	1/47	2/40	2.12	3.77	1.23	0.86
		7	4/55	1/54	1/46	3/55	2/54	2/46	3.76	4.49	1.76	0.51
		8	4/62	2/61	2/56	4/62	2/61	3/56	4.43	5.02	1.01	0.93
		9	5/69	4/71	1/58	4/69	4/71	3/58	4.86	8.11	1.64	0.54
		10	2/78	4/77	3/68	9/78	10/77	6/68	4.05	11.11	0.78	1.22

* Replicates.

Appendix Table: 5 Mean fortnightly percentage rate of increase in incidence of BYMV and SCRLV in V.faba L. trial

Treatment	Date of observation	Number of diseased plants out of number of healthy plants R_1**	R_2	R_3	Average percent infection
SCRLV-Vv*	28/8/80	2/406	1/422	0/430	0.24
	10/9	1/404	1/421	1/430	0.23
	23/9	15/403	15/420	9/429	3.12
	9/10	29/388	27/405	27/420	6.85
	22/10	6/359	3/378	10/393	1.66
-V	28/8	0/416	0/349	0/436	0
	10/9	0/416	0/349	0/436	0
	23/9	1/416	2/349	0/436	0.27
	9/10	4/415	4/347	7/436	1.23
	22/10	4/411	4/343	3/429	0.94
-C	28/8	0/428	0/425	0/370	0
	10/9	0/428	1/425	0/370	0.07
	23/9	0/428	1/424	2/370	0.25
	9/10	6/428	6/423	5/368	1.38
	22/10	12/422	8/417	7/363	2.22
BYMV-Vv	28/8	0/406	0/422	0/430	0
	10/9	10/406	8/422	0/430	1.45
	23/9	23/396	24/414	3/430	4.09
	9/10	27/373	30/390	7/427	5.51
	22/10	8/345	15/360	4/420	2.47
-V	28/8	0/416	0/349	0/436	0
	10/9	5/416	1/349	10/436	1.25
	23/9	10/411	7/348	19/426	2.96
	9/10	60/401	11/341	37/407	9.09
	22/10	2/341	5/330	14/370	1.95
-C	28/8	0/428	0/425	0/370	0
	10/9	3/428	1/425	0/370	0.31
	23/9	11/425	5/424	5/370	1.70
	9/10	6/414	7/419	6/365	1.58
	22/10	3/411	2/412	4/359	0.77

Vv* = Virus + vector

V = Virus only

C = No virus, no vector

R** = Replicates

Appendix Table 6 : Key to aphid identification

Alatae viviparae

1. Myzus persicae (Sulzer)	Abdomen almost same width from thorax to bases of cornicles, then sides gently rounded to meet the cauda abruptly. Head with prominent inward-pointing antennal tubercles. Cornicles slightly swollen on apical half, cauda short, Abdomen green or pink with a more or less solid dark patch.
2. Macrosiphum euphorbiae (Thomas)	Body elongate, wedge-shaped. Frontal tubercles high, diverging; antennal hairs long, longer than half diameter of antennal segment III; abdomen pale. All veins of front wings of same thickness. Cornicles extremely long, the tips without tacklike flanges. Antennal segment III (except extreme base) and rest of the antenna usually blackish.
3. Aulacorthum solani (Kaltenbach)	Abdomen with dark, irregularly shaped, transverse, segmental bars of broken patches; head spinulose lateroventrally; frontal tubercles parallel-sided. Basal veins of front wings slightly darker and thicker than other veins. Cornicles straight with prominent flanges.
4. Aphis craccivora Koch	Frontal tubercles slightly curved flat; body egg shaped, shining black dorsum; cornicles short black; cauda pointed apically black.

Contd.

Continuation of Appendix Table 6

Apterae viviparae	
1. Myzus persicae (Sulzer)	Abdomen almost same width from thorax to bases of cornicles, then sides gently rounded to meet the cauda abruptly. Head with prominent inward-pointing antennal tubercles. Cornicles slightly swollen on apical half; cauda short.
2. Macrosiphum euphorbiae (Thomas)	Frontal tubercles high, diverging; antennal hairs long, more than half as long as diameter of base of antennal segment III. Body elongate; legs and antennae long. Cornicles cylindrical, flared outward, about one-third the length of the body; cauda about one-third the length of the cornicles, both extending about the same distance past end of body.
3. Aulacorthum solani (Kaltenbach)	Head spinulose; frontal tubercles parallel-sided; abdomen globular, widest just ahead of cornicles, tapering to insignificant upturned cauda. Cornicles not swollen, slightly tapered with prominent flanges on the dark tips; legs and antennae with dark joints.
4. Aphis craccivora Koch.	Frontal tubercles slightly curved flat; body egg shaped, shining black dorsum with pronounced reticulation; cornicles short black; cauda pointed apically black.

Appendix Table:7 : Weekly catches of alate aphids in traps in the experimental plot (E.P.) area and adjacent to the trap plants (T.P.)

Date	T.P.						E.P.					
	M.p*	M.e	A.s	A.c	O	T	M.p	M.e	A.s	A.c	O	T
27.7.79	0	0	0	0	1	1	0	0	0	0	0	0
3.8.79	0	0	0	0	2	2	7	0	0	0	2	9
10.8.79	0	0	0	0	2	2	7	0	0	0	3	10
17.8.79	0	0	1	0	1	2	2	0	0	0	3	5
24.8.79	1	0	0	0	1	2	8	1	1	1	2	13
31.8.79	1	0	0	0	0	1	6	1	1	0	15	23
7.9.79	1	0	0	0	0	1	1	0	0	0	2	3
14.9.79	0	0	1	0	1	2	7	0	0	0	0	1
21.9.79	0	0	1	1	0	2	3	0	1	0	4	8
28.9.79	0	2	1	21	16	40	41	11	14	133	75	274
5.10.79	0	0	0	0	2	2	17	41	7	7	1	73
12.10.79	1	1	0	0	3	5	24	16	1	1	13	55
19.10.79	0	2	4	3	13	22	18	11	2	13	48	92
26.10.79	4	1	0	2	11	18	31	50	0	11	174	266
2.11.79	0	2	1	1	7	11	0	9	0	0	15	24
9.11.79	0	0	0	0	0	0	3	6	0	0	9	18
16.11.79	0	0	0	0	4	4	0	0	0	0	5	5
21.11.79	0	0	0	0	0	0	0	0	0	2	1	3
30.11.79	0	0	0	0	1	1	0	0	0	2	2	4
7.12.79	0	0	0	2	1	3	3	0	0	2	3	8
14.12.79	0	0	0	0	0	0	1	0	0	0	0	1
21.12.79	0	0	0	2	1	3	0	0	0	0	1	1
28.12.79	0	0	0	0	0	0	0	0	0	0	0	0
4. 1.80	0	0	0	0	1	1	0	0	0	0	1	1
11. 1.80	0	0	0	0	2	2	0	0	0	0	0	0
18. 1.80	0	0	0	0	4	4	0	0	0	0	1	1

Continuation of Appendix Table: 7

Date	T.P						E.P					
	M.p	M.e	A.s	A.c	O	T	M.p	M.e	A.s	A.c	O	T
25.1.80	0	0	0	0	1	1	0	0	0	0	1	1
1.2.80	0	0	0	0	4	4	0	0	0	0	2	2
8.2.80	3	0	0	0	6	9	8	0	0	0	8	16
15.2.80	0	0	0	0	3	3	1	0	0	0	5	6
22.2.80	0	0	0	0	3	3	0	0	0	0	0	0
29.2.80	1	0	0	1	5	7	0	0	0	1	9	10
7.3.80	1	0	0	2	22	25	0	0	0	0	24	24
14.3.80	0	0	0	0	6	6	0	0	0	0	3	3
21.3.80	2	0	0	0	1	3	0	0	1	0	0	1
28.3.80	0	0	0	0	0	0	0	0	0	0	0	0
4.4.80	1	0	0	0	2	3	0	0	0	0	2	2
11.4.80	2	0	0	3	5	10	0	0	0	1	3	4
18.4.80	1	0	0	0	2	3	3	0	0	2	4	9
24.4.80	0	0	0	0	0	0	0	0	0	0	1	1
2.5.80	4	0	0	0	1	5	5	1	0	0	4	10
9.5.80	5	0	0	0	8	13	25	0	0	1	36	62
16.5.80	0	0	0	0	0	0	0	0	0	1	5	6
23.5.80	0	0	0	0	0	0	0	0	0	0	0	0
30.5.80	0	0	0	0	1	1	7	0	0	2	4	13
6.6.80	4	0	0	0	1	5	1	0	0	1	0	2
13.6.80	1	0	0	0	2	3	8	1	0	0	5	14
20.6.80	0	0	0	0	0	0	17	0	0	0	2	19
27.6.80	2	0	0	0	2	4	11	0	0	0	4	15
4.7.80	1	0	0	0	1	2	7	0	0	0	2	9
11.7.80	1	0	0	0	5	6	7	0	0	0	7	14
18.7.80	2	0	0	0	4	6	18	0	0	0	1	19
25.7.80	0	0	1	0	7	8	15	0	0	0	3	18

Contd.

Continuation of Appendix Table: 7

Date	T.P						E.P					
	M.p	M.e	A.s	A.c	O	T	M.p	M.e	A.s	A.c	O	T
1.8.80	0	0	0	0	5	5	11	0	0	0	5	16
8.8.80	4	0	0	0	0	4	29	0	0	0	3	32
15.8.80	4	0	0	0	5	9	27	1	0	0	6	35
22.8.80	9	0	0	0	13	23	55	0	0	0	8	63
29.8.80	5	1	3	1	16	26	104	2	4	1	45	156
5.9.80	10	1	0	2	19	32	207	5	2	4	97	315
12.9.80	55	3	6	3	114	181	2442	88	2	16	3050	5598
19.9.80	10	3	0	13	46	72	135	17	0	20	122	294
26.9.80	24	3	0	4	70	101	1770	82	10	24	194	2080
3.10.80	11	1	0	9	69	90	693	56	2	8	776	1535
10.10.80	2	5	1	1	20	29	978	88	40	16	72	1194
17.10.80	0	5	0	0	4	9	49	162	4	5	34	254
24.10.80	0	4	0	1	3	8	10	40	1	8	28	87
31.10.80	0	0	0	0	5	5	2	7	0	1	28	38
7.11.80	0	0	0	0	4	4	0	1	0	0	10	11
14.11.80	0	3	0	0	4	7	0	6	0	0	8	14
21.11.80	0	0	0	0	3	3	0	0	0	0	4	4
28.11.80	0	0	0	0	0	0	0	1	0	0	3	4
5.12.80	0	0	0	0	1	1	0	0	0	0	0	0
12.12.80	0	0	0	0	2	2	0	0	0	0	0	0
19.12.80	0	0	0	0	0	0	0	0	0	0	0	0
26.12.80	0	0	0	0	0	0	0	0	0	0	0	0
2. 1.81	1	0	0	0	1	2	0	0	0	0	3	3
9. 1.81	2	0	0	0	1	3	0	0	0	0	3	3
16. 1.81	0	0	0	0	3	3	0	0	0	0	6	6
23. 1.81	0	0	0	0	0	0	0	0	0	0	6	6
30. 1.81	0	0	0	0	3	3	0	0	0	0	3	3

Contd..

Continuation of Appendix Table: 7

Date	T.P						E.P					
	M.p	M.e	A.s	A.c	O	T	M.p	M.e	A.s	A.c	O	T
6.2.81	0	0	0	0	4	4	0	0	0	0	3	3
13.2.81	0	0	0	0	0	0	0	0	0	0	9	9
20.2.81	0	0	0	0	0	0	0	0	0	0	1	1
27.2.81	0	0	0	0	4	4	0	0	0	0	10	10
6.3.81	0	0	0	0	0	0	0	0	0	0	3	3
13.3.81	0	0	0	0	4	4	0	0	0	0	3	3
20.3.81	2	0	0	1	9	12	1	1	0	2	21	25
27.3.81	4	0	1	1	24	30	0	0	0	0	30	30
3.4.81	20	0	0	0	53	73	12	0	0	0	63	75
10.4.81	21	1	0	3	39	64	6	0	0	0	20	26
17.4.81	23	0	0	0	12	35	7	0	1	0	8	16
24.4.81	26	2	0	0	15	43	6	3	0	0	14	23
1.5.81	68	4	0	1	191	264	15	3	0	1	71	90
8.5.81	296	6	0	3	95	400	34	2	0	1	20	57
15.5.81	91	0	0	3	111	205	110	2	0	2	141	255
22.5.81	18	0	0	2	14	34	24	1	0	0	11	36
29.5.81	3	0	0	0	3	6	9	1	0	0	2	12
5.6.81	2	0	0	0	5	7	4	0	0	0	4	8
12.6.81	1	0	0	0	2	3	3	0	0	0	3	6
19.6.81	0	0	0	1	5	6	4	0	0	0	17	21
26.6.81	0	0	0	0	0	0	3	0	0	0	1	4
3.7.81	0	0	0	0	0	0	1	0	0	0	1	2
10.7.81	0	0	0	0	0	0	0	0	0	0	1	1
17.7.81	0	0	0	0	0	0	0	0	0	0	3	3
24.7.81	0	0	0	0	0	0	1	0	0	0	1	2
31.7.81	2	0	0	0	0	2	1	0	0	0	0	1

Continuateion of Appendix Table: 7

Date	T.P						E.P					
	M.p	M.e	A.s	A.c	O	T	M.p	M.e	A.s	A.c	O	T
7. 8.81	0	0	0	0	0	0	0	0	0	0	0	0
14. 8.81	0	0	0	0	0	0	0	0	0	0	0	0
28. 8.81	0	0	0	0	0	0	0	0	0	0	0	0
4. 9.81	0	0	0	0	1	1	1	0	0	0	0	1
11. 9.81	2	0	0	0	12	14	16	1	0	0	21	38
18. 9.81	8	1	2	2	14	27	120	9	1	8	138	276
25. 9.81	16	0	0	7	26	49	194	15	0	24	217	450
2.10.81	35	16	2	16	53	122	660	47	9	61	268	1045
9.10.81	96	36	4	6	54	196	1192	134	8	40	306	1680
16.10.81	42	70	12	18	94	236	378	166	0	34	194	772
23.10.81	22	52	12	6	48	140	172	214	0	38	84	508
30.10.81	42	94	0	12	42	190	62	278	16	102	70	528
6.11.81	0	1	0	0	5	6	2	11	0	1	8	22
13.11.81	0	0	0	0	2	2	1	9	0	2	4	16
20.11.81	0	0	0	1	0	1	0	0	0	0	0	0
27.11.81	0	0	0	1	0	1	0	0	0	0	0	0
4.12.81	0	0	0	0	0	0	0	0	0	0	0	0

* Mp = Myzus persicae
Me = Macrosiphum euphorbiae
As = Aulacorthum solani
Ac = Aphis Craccivora
O = Other aphid species
T = Total number of aphids
Total number of aphid species trapped for 29 months 22081
Number of 4 aphid species trapped for the same period 13730

Appendix Table: 8 : Meterological data for Strathalbyn from August 1979 to December 1981.

Date	Mean weekly temperature (C^{o})	Weekly rainfall (mm)	Mean weekly Windspeed (knots)
4.8.79 - 10.8	11.42	15.60	13.92
11 - 17	10.30	11.80	11.5
18 - 24	10.75	5.8	10.75
25 - 31	11.17	18.6	10.85
1.9.79 - 7	10.77	22.6	17.07
8 - 14	14.18	17.8	16.21
15 - 21	12.91	0	11.71
22 - 28	15.29	11.8	9.5
29 -5.10.79	13.53	21.6	12.64
6 - 12	12.86	41.8	19.35
13 - 19	13.35	3.6	11.71
20 - 26	16.77	0.8	13.57
27 -2.11.79	15.05	0	12.64
3 - 9	18.05	0	12.57
10 - 16	18.46	32.2	15.85
17 - 23	17.96	5.6	13.71
24 - 30	16.59	2.4	9.78
1.12.79- 7	20.77	0	13.42
8 - 14	18.12	6.0	13.28
15 - 21	18.78	0	11.57
22 - 28	20.18	0	13.28
29 -4.1.80	20.50	9.6	10.5
5 - 11	18.50	0.8	13.21
12 - 18	19.68	2.6	13.64
19 - 25	18.95	1.2	11.16
26 -1.2.80	18.32	0	13.21
2 - 8	15.93	0	12.92
9 - 15	21.5	0	13.5
16 - 22	23.08	0.4	12.21
23 - 29	18.69	2.6	13.07
1.3.80 - 7	17.72	3.6	12.07
8 - 14	16.66	0	13.28
15 - 21	20.79	0	11.5
22 - 28	17.37	0	7.35
29 -4.4.80	16.99	0.2	11.85
5 - 11	17.67	0	12.71
12 - 18	21.43	29.4	10.07
19 - 25	18.45	34.8	13.14
26 -2.5.80	12.3	0	9.28
3 - 9	17.33	3.4	10.4
10 - 16	14.98	4.4	9.8
17 - 23	12.31	6.0	10.71
24 - 30	14.36	2.8	8

Contd.

Continuation of Appendix Table: 8

Date	Mean weekly temperature ($C^{o}C$)	Weekly rainfall (mm)	Mean weekly Windspeed (knots)
31 - 6.6.80	11.92	21.8	8.85
7 - 13	11.57	12.0	5.21
14 - 20	11.53	18.8	12.14
21 - 27	12.67	11	12.05
28 - 4.7.80	11.31	27.2	14
5 - 11	11.18	3.8	9.78
12 - 18	12.04	6.4	11.33
19 - 25	9.2	3.6	7.85
26 - 1.8.80	11.15	6.6	11.85
2 - 8	10.45	11.2	12.64
9 - 15	11.85	7.4	15.35
16 - 22	11.59	6.2	14.42
23 - 29	11.65	1.6	16.92
30 - 5.9.80	12.95	3.8	17.78
6 - 12	14.32	4.2	17.78
13 - 19	12.95	36.6	13.92
20 - 26	14.79	0.2	13.92
27 - 3.10.80	14.78	0	13.92
4 - 10	14.62	40.2	10.85
11 - 17	14.55	32.4	11.57
18 - 24	15.9	7.4	14.64
25 - 31	14.70	7.4	14.35
1 .11.80 - 7	14.79	28.6	13.5
8 - 14	19.77	0	14.35
15 - 21	20.27	1.4	12.35
22 - 28	19.77	0	14
29 - 5.12.80	17.10	3.6	14.92
6 - 12	23.8	0	11.71
13 - 19	15.66	11.2	13.71
20 - 26	21.83	0	14.42
27 - 2.1.81	-	-	-
3 - 9	-	-	9.83
10 - 16	23.21	0	9.21
17 - 23	21.15	0.6	10.85
24 - 30	22.6	23.0	13.07
31 - 6. 2.81	21.5	2.8	16.67
7 - 13	21.96	0	13.64
14 - 20	21.12	4.0	15
21 - 27	19.28	0	13.07
28 - 6. 3.81	17.1	29.2	15.35
7 - 13	17.28	7.6	13
14 - 20	17.67	0.8	10.57
21 - 27	14.94	1.4	9.21
28 - 3. 4.81	19.84	4	7.64
4 - 10	17.15	0.6	10.14
11 - 17	17.58	0.4	12.07

Contd.

Continuation of Appendix Table: 8

Date			Mean weekly temperature (C^{o})	Weekly rainfall (mm)	Mean weekly Windspeed (knots)
18	-	24	17.72	0.4	12.07
25	-	1.5.81	15.26	0	12.28
2	-	8	15.99	6	13.07
9	-	15	14.24	5.6	9.57
16	-	22	10.35	4.6	9.5
23	-	29	13.3	10.15	13.92
30	-	5.6.81	12.66	41.8	12.78
6	-	12	11.84	15.6	14.85
13	-	19	10.92	8.2	10.57
20	-	26	9.76	49.4	14.14
26	-	3.7.81	-	-	-
4	-	10	10.01	17.4	13.85
11	-	17	10.44	15.2	13.64
18	-	24	12.27	28.6	10.21
25	-	31	10.71	12.2	12.21
1.8.81	-	7	11.56	8.4	12.07
8	-	14	10.35	70.2	15.71
15	-	21	12.65	27.8	21.42
22	-	28	9.76	9.4	10.57
29	-	4.9.81	10.15	17.4	13.28
5	-	11	15.42	0	14.85
12	-	18	15.32	0	8.78
19	-	25	13.81	8.6	12.21
26	-	2.10.81	-	4	-
3	-	9	15.07	12.8	16.85
10	-	16	12.88	.8	14.0
17	-	23	14.04	3.6	15.57
24	-	30	17.13	3.8	9.64
31	-	6.11.81	16.47	1.4	10.85
7	-	13	16.83	20.4	12.28
14	-	20	14.24	13.2	7.57
21	-	27	22.21	1.0	15.07
28	-	4.12.81	-	-	-

Appendix Table: 9 The number of trap plants (V.faba L. cv. Acquadulce) infected with BYMV and SCRLV after exposing them at Strathalbyn for 28 day period.

Period		Number of plants exposed	Number of plants showing		Percent infection	
			SCRLV	BYMV	SCRLV	BYMV
20.7.79	- 17.8	100	2	0	1	0
17.8	- 14.9	100	2	0	2	0
14.9	- 12.10	100	4	0	4	0
12.10	- 9.11	100	0	4	0	4
9.11	- 7.12	100	0	9	0	9
7.12	- 4.1.80	100	0	0	0	0
4.1	- 1.2	100	0	0	0	0
1.2	-29.2	100	0	1	0	1
29.2	-28.3	100	0	0	0	0
28.3	-24.4	100	0	0	0	0
24.4.	-23.5	100	0	0	0	0
23.5	-20.6	100	0	0	0	0
20.6	-18.7	100	0	0	0	0
18.7	-15.8	100	0	0	0	0
15.8	-12.9	100	0	1	0	1
12.9	-10.10	100	2	7	2	7
10.10	- 7.11	100	1	1	1	1
7.11	- 5.12	100	0	0	0	0
5.12	- 2.1.81	100	0	0	0	0
2.1	-30.1	100	0	0	0	0
30.1	-27.2	100	0	0	0	0
27.2	-27.3	100	0	0	0	0
27.3	-24.4	100	0	0	0	0
24.4	-22.5	100	0	0	0	0
22.5	-19.6	100	0	0	0	0
19.6	-17.7	100	0	0	0	0
17.7	-14.8	100	0	0	0	0
14.8	-11.9	100	0	0	0	0
11.9	- 9.10	100	2	16	2	16
9.10	- 6.11	100	2	11	2	11
6.11	- 4.12	100	0	0	0	0

Appendix Table: 10 Number of aphids present in 10 shoots of V.faba L. in each replicate on 5 occasions.

Date	Aphid species	Replicate	Treatments				
			T_1*	T_2	T_3	T_4	T_5
21/8/81	M.persicae	R_1	0(0)	0(0)	0(0)	0(0)	0(0)
		R_2	0(0)	0(0)	0(0)	0(0)	0(0)
		R_3	0(0)	0(0)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(0)	0(0)	0(0)	0(0)	0(0)
	M.euphorbiae	R_1	0(0)	0(0)	0(0)	0(0)	0(0)
		R_2	0(0)	0(0)	0(0)	0(0)	0(0)
		R_3	0(0)	0(0)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(0)	0(0)	0(0)	0(0)	0(0)
	A.solani	R_1	2(0)	0(0)	0(0)	3(0)	1(0)
		R_2	1(0)	1(0)	1(0)	1(0)	0(0)
		R_3	1(0)	0(0)	0(0)	0(0)	0(0)
		R_4	2(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(0)	0(0)	0(0)	0(0)	2(0)
	A.craccivora	R_1	1(0)	0(0)	0(0)	0(0)	0(0)
		R_2	2(0)	0(0)	0(0)	0(0)	2(0)
		R_3	0(0)	0(0)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	1(0)	0(0)
		R_5	0(0)	0(0)	0(0)	0(0)	0(0)

Contd.

Continuation of Appendix Table: 10

Date	Aphid species	Replicate	Treatments T_1*	T_2	T_3	T_4	T_5
21/8/81	+ Other species including young of ++ Mp; Me; As; Ac.	R_1	0(0)	0(0)	0(0)	0(0)	0(0)
		R_2	0(0)	0(0)	0(0)	0(0)	0(0)
		R_3	0(0)	0(0)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(0)	0(0)	0(0)	0(0)	0(0)
3/9/81	M.persicae	R_1	0(0)	0(0)	0(0)	0(0)	0(0)
		R_2	0(3)	0(0)	0(0)	0(0)	0(1)
		R_3	0(0)	0(0)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(2)	0(0)	0(0)	0(2)	0(1)
	M.euphorbiae	R_1	0(0)	0(0)	0(0)	0(0)	0(0)
		R_2	0(0)	0(0)	0(0)	0(0)	0(1)
		R_3	0(0)	0(1)	0(0)	0(0)	0(0)
		R_4	0(0)	0(0)	0(0)	0(0)	0(0)
		R_5	0(1)	0(0)	0(1)	0(0)	0(2)
	A.solani	R_1	1(0)	0(0)	0(0)	1(0)	5(0)
		R_2	1(0)	0(0)	0(0)	0(0)	1(0)
		R_3	0(0)	0(0)	0(0)	2(0)	1(0)
		R_4	2(0)	1(0)	0(0)	0(0)	0(0)
		R_5	0(0)	0(0)	0(0)	1(0)	4(0)

Contd.

Continuation of Appendix Table: 10

Date	Aphid species	Replicate	Treatments				
			T_1*	T_2	T_3	T_4	T_5
3/9/81	A.craccivora	R_1	0(0)	0(0)	0(1)	0(0)	0(1)
		R_2	0(2)	0(1)	0(0)	0(2)	2(4)
		R_3	0(1)	0(1)	0(1)	0(1)	0(2)
		R_4	0(0)	0(3)	0(2)	0(1)	0(1)
		R_5	0(1)	0(2)	0(0)	0(1)	0(3)
	+ Other species	R_1	0	0	1	0	12
	including young	R_2	0	0	0	0	1
	of	R_3	0	0	0	4	0
	++ Mp; Me; As; Ac.	R_4	0	0	0	1	4
		R_5	0	0	0	0	6
17/9/81	M.persicae	R_1	0(10)	0(4)	0(6)	0(2)	0(3)
		R_2	0(15)	0(9)	0(24)	0(6)	0(17)
		R_3	0(7)	0(7)	0(11)	0(9)	0(11)
		R_4	0(13)	0(23)	0(10)	0(10)	0(14)
		R_5	0(15)	0(1)	0(26)	0(15)	0(16)
	M.euphorbiae	R_1	2(0)	3(0)	2(0)	7(0)	0(5)
		R_2	2(0)	0(7)	2(0)	0(4)	2(5)
		R_3	8(0)	0(0)	0(0)	6(4)	3(0)
		R_4	2(3)	1(7)	1(2)	2(7)	1(4)
		R_5	0(5)	0(1)	0(6)	0(0)	0(12)

Contd.

Continuation of Appendix Table: 10

Date	Aphid species	Replicate	Treatments				
			T_1*	T_2	T_3	T_4	T_5
17/9/81	A.solani	R_1	2(0)	1(0)	0(0)	8(0)	4(0)
		R_2	8(0)	0(0)	1(0)	4(0)	11(0)
		R_3	5(0)	3(0)	0(0)	3(0)	6(0)
		R_4	4(0)	1(0)	0(0)	10(0)	12(0)
		R_5	5(0)	2(0)	3(1)	30(0)	8(1)
	A.craccivora	R_1	5(14)	4(21)	1(33)	0(6)	12(12)
		R_2	2(13)	3(15)	6(9)	2(7)	9(45)
		R_3	1(20)	3(8)	15(12)	0(4)	12(5)
		R_4	3(24)	5(46)	0(25)	1(13)	8(29)
		R_5	1(33)	1(19)	0(43)	0(26)	10(18)
	+ Other species including young of ++ Mp; Me; As; Ac.	R_1	47	32	13	6	27
		R_2	91	38	25	18	38
		R_3	49	23	41	7	20
		R_4	58	39	33	24	40
		R_5	63	20	26	15	86
1/10/81	M.persicae	R_1	4(34)	16(20)	2(2)	14(36)	16(14)
		R_2	48(50)	18(20)	8(20)	24(12)	6(16)
		R_3	10(8)	10(8)	0(6)	30(18)	56(16)
		R_4	42(8)	4(10)	10(14)	14(16)	22(14)
		R_5	2(14)	28(10)	2(6)	14(10)	8(4)

Contd.

Continuation of Appendix Table: 10.

Date	Aphid species	Replicate	Treatments				
			T_1*	T_2	T_3	T_4	T_5
1/10/81	M.euphorbiae	R_1	24(10)	0(12)	0(2)	6(6)	22(4)
		R_1^2	30(20)	0(22)	0(20)	2(19)	4(6)
		R_2^2	4(2)	4(14)	0(4)	10(6)	22(4)
		R_4^4	0(12)	0(2)	0(0)	0(4)	16(10)
		R_5	6(6)	0(14)	0(1)	0(4)	12(8)
	A.solani	R_1	26(6)	10(0)	1(0)	42(0)	4(2)
		R_1^2	78(0)	22(4)	0(2)	8(0)	24(0)
		R_2^3	22(0)	0(0)	0(2)	4(0)	10(0)
		R_3^4	18(2)	10(0)	4(0)	36(0)	34(0)
		R_4^5	18(0)	8(0)	3(0)	32(0)	32(2)
	A.craccivora	R_1	38(12)	12(17)	1(5)	12(28)	26(26)
		R_1^2	56(18)	24(20)	2(24)	2(6)	30(54)
		R_2^3	14(8)	6(6)	0(2)	14(12)	34(20)
		R_3^4	26(22)	4(0)	0(2)	6(14)	48(42)
		R_4^5	25(2)	20(18)	0(10)	18(12)	40(36)
	+Other species including young of ++Mp; Me; As; Ac.	R_1	334	119	14	108	172
		R_1^2	718	522	38	96	576
		R_2^3	152	156	21	201	390
		R_3^4	366	97	23	143	257
		R_4^5	193	103	52	172	309

Contd.

Continuation of Appendix Table: 10

Date	Aphid species	Replicate	Treatments T_1*	T_2	T_3	T_4	T_5
15/10/81	M. persicae	R_1	52(39)	14(16)	0(1)	54(32)	2(2)
		R_2	94(46)	4(5)	4(0)	32(16)	12(6)
		R_3	0(5)	8(2)	0(1)	60(22)	26(8)
		R_4	10(16)	56(4)	0(2)	26(17)	14(6)
		R_5	16(14)	22(12)	0(3)	18(11)	18(2)
	M.euphorbiae	R_1	20(6)	4(13)	0(3)	4(6)	4(10)
		R_2	37(16)	0(10)	2(3)	2(9)	8(14)
		R_3	2(4)	2(8)	0(4)	2(5)	6(13)
		R_4	10(16)	2(6)	0(7)	4(8)	0(21)
		R_5	2(6)	6(8)	0(8)	0(8)	6(24)
	A.solani	R_1	16(0)	16(0)	0(0)	14(0)	2(0)
		R_2	14(0)	3(0)	2(1)	10(2)	26(3)
		R_3	2(0)	4(2)	0(0)	23(0)	8(0)
		R_4	10(0)	6(0)	0(0)	24(0)	12(0)
		R_5	8(2)	12(0)	0(0)	18(0)	24(1)
	A. craccivora	R_1	38(0)	11(0)	0(0)	10(5)	3(1)
		R_2	71(0)	1(0)	1(0)	13(2)	14(4)
		R_3	5(0)	8(2)	0(1)	15(9)	11(0)
		R_4	30(3)	23(1)	0(0)	6(6)	20(3)
		R_5	22(7)	9(1)	0(2)	36(6)	32(2)

Contd.

Continuation of Appendix Table: 10

Date	Aphid species	Replicate	Treatments T_1*	T_2	T_3	T_4	T_5
15/10/81	+ Other species	R_1	748	159	22	416	71
	including	R_2	1108	54	26	230	298
	young of	R_3	92	196	23	363	251
	++ Mp; Me; As; Ac.	R_4	462	82	31	332	341
		R_5	299	212	56	292	218

T_1* = Control; T_2 = Disyston ; T_3 = Metasystox ; T_4 = Malathion : T_5 = Barley barrier

( ) = Data in parenthesis represent wing form of aphid species.

\+ = Wing and wingless species all included.

++ = Mp = M.persicae; Me = M.euphorbiae ; As = A.solani ; Ac = A.craccivora.

Appendix Table: 11 Movement of SCRLV on V.faba L. from centre row after virus source was artificially introduced with vector - 1981 winter-spring.

Date of observation	Treatment	Distance from source row	Number of plants infected out of healthy plants					% SCRLV infection (mean of 5 replicates)
			R_1[a]	R_2	R_3	R_4	R_5	
3/9/81	Control	1	2/20, 0/20	2/19, 7/19	2/20, 2/20	1/20, 1/20	1/20, 0/20	9.2(± 3.31)[b]
		2	1/20, 0/19	1/19, 2/18	3/19, 1/19	0/20, 2/20	0/20, 0/20	5.2(1.75)
		3	0/19, 2/19	1/19, 1/19	1/20, 0/20	0/20, 1/20	0/20, 0/20	3.1(1.15)
		4	1/20, 1/19	0/19, 1/19	1/20, 0/20	0/20, 2/20	2/20, 0/18	4.0(1.25)
		5	0/20, 0/20	0/19, 0/15	1/20, 0/20	0/20, 1/20	0/20, 1/20	1.5(0.76)
17/9/81		1	8/20, 7/20	8/19, 9/19	9/20,11/20	10/20,18/20	14/20,16/20	54.4(5.8)
		2	6/20, 3/19	5/19, 7/18	8/19, 5/19	13/20,13/20	16/20,16/20	46.9(7.4)
		3	2/19, 3/19	2/19, 1/19	7/20, 9/20	10/20,13/20	14/20,13/20	37.2(8.0)
		4	2/20, 1/19	0/19, 1/19	1/20, 6/20	9/20, 8/20	5/20, 7/18	20.4(5.4)
		5	2/20, 1/20	3/19, 1/15	4/20, 5/20	7/20, 4/20	3/20, 2/20	16.2(2.8)
1/10/81		1	16/20,17/20	13/19,12/19	14/20,17/20	13/20,18/20	18/20,19/20	79.1(3.6)
		2	11/20, 8/19	9/19, 8/18	13/19,11/19	16/20,16/20	19/20,17/20	65.5(5.9)
		3	4/19,10/19	2/19, 7/19	9/20,12/20	14/20,13/20	16/20,16/20	52.1(7.5)
		4	4/20, 7/19	5/19, 6/19	3/20,10/20	13/20,12/20	12/20,13/18	43.6(6.4)
		5	6/20, 2/20	6/19, 3/15	8/20, 8/20	13/20, 6/20	8/20, 8/20	34.6(4.6)

Contd.

Continuation of Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants					% SCRLV infection (mean of 5 replicates)
			R_1[a]	R_2	R_3	R_4	R_5	
15/10/81	Control	1	18/20,17/20	17/19,16/19	18/20,17/20	17/20,19/20	18/20,20/20	89.3 (1.5)[b]
		2	12/20,13/19	14/19, 8/19	15/19,14/19	19/20,19/20	19/20,18/20	77.4 (5.3)
		3	10/19,13/19	11/19,11/19	13/20,15/20	18/20,15/20	20/20,18/20	73.1 (5.0)
		4	10/20,11/19	8/19,10/19	7/20,14/20	15/20,15/20	14/20,13/18	59.9 (4.6)
		5	12/20, 3/20	6/19, 3/15	8/20,14/20	15/20,11/20	14/20, 9/20	48.1 (6.7)
3/9/81	Disyston	1	1/20, 1/20	0/20, 0/20	1/20, 2/20	2/20, 2/20	4/19, 4/19	8.7 (±2.36)
		2	0/20, 0/20	0/20, 0/20	0/20, 1/20	1/20, 0/20	0/19, 1/18	1.5(.79)
		3	0/20, 0/20	0/20, 0/20	1/20, 0/20	0/20, 0/20	0/19, 1/19	1.0 (.68)
		4	0/20, 0/20	0/20, 0/20	1/20, 0/19	0/20, 0/19	0/19, 0/19	0 (0)
		5	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 0/19	0 (0)
17/9/81		1	4/20, 1/20	6/20,13/20	10/20,13/20	5/20, 8/20	4/19, 9/19	36.8 (6.3)
		2	1/20, 0/20	9/20, 6/20	5/20, 3/20	3/20, 4/20	6/19, 4/18	20.8 (4.1)
		3	0/20, 0/20	3/20, 4/20	7/20, 3/20	2/20, 1/20	0/19, 1/19	10.5 (3.5)
		4	0/20, 0/20	2/20, 3/20	3/20, 6/19	1/20, 3/19	2/19, 0/19	10.2 (3.1)
		5	0/20, 0/20	1/20, 1/20	5/20, 0/20	0/20, 1/20	2/19, 0/19	5.0 (2.4)

Contd.

Continuation of Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants SCRLV R_1[a]	R_2	R_3	R_4	R_5	% SCRLV infection (mean of 5 replicates).
1/10/81	Disyston	1	15/20,14/20	15/20,17/20	14/20,15/20	14/20,15/20	13/19,15/19	74.2 (1.5)[b]
		2	4/20,11/20	11/20,17/20	12/20, 9/20	4/20, 8/20	8/19, 7/18	46.0 (6.0)
		3	5/20, 5/20	11/20,12/20	7/20,12/20	5/20, 8/20	6/19, 7/19	39.3 (4.4)
		4	2/20, 5/20	4/20, 9/20	3/20, 9/19	3/20, 8/19	2/19, 4/19	25.1 (4.5)
		5	3/20, 3/20	5/20, 1/20	5/20, 0/20	0/20, 3/20	2/19, 2/19	12.1 (2.8)
15/10/81		1	16/20,16/20	18/20,19/20	17/20,17/20	14/20,15/20	13/19,16/19	81.2 (2.6)
		2	4/20,14/20	11/20,17/20	16/20,12/20	6/20,12/20	10/19, 7/18	55.1 (6.6)
		3	5/20,10/20	11/20,12/20	7/20,12/20	5/20,8/20	6/19,10/19	43.4 (4.3)
		4	5/20, 5/20	4/20,11/20	3/20, 9/19	3/20, 8/19	8/19, 6/19	31.8 (4.4)
		5	3/20, 3/20	6/20, 4/20	6/20, 3/20	3/20, 3/20	5/19, 4/19	20.2 (2.0)
3/9/81	Metasystox	1	0/20, 0/20	0/20, 0/20	0/20, 0/20	3/20, 3/20	0/19, 0/20	4.0 (2.0)
		2	0/19, 1/19	0/20, 0/19	0/20, 2/20	1/20, 0/20	1/20, 0/20	2.5 (1.1)
		3	1/20, 0/19	0/20, 1/20	0/19, 1/20	0/20, 0/20	1/20, 0/20	2.0 (0.8)
		4	0/19, 0/20	0/20, 0/20	0/19, 0/20	0/20, 0/19	0/20, 0/20	0 0
		5	0/18, 0/20	0/18, 0/18	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 0

Contd.

Continuation of Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants (SCRLV)					% SCRLV infection (mean of 5 replicates)
			R_1[a]	R_2	R_3	R_4	R_5	
17/9/81	Metasystox	1	2/20, 2/20	2/20, 4/20	8/20,10/20	11/20,17/20	6/19,11/20	36.6 (7.9)[b]
		2	0/19, 1/19	1/20, 0/19	8/20, 3/20	7/20, 6/20	8/20, 6/20	20.0 (5.2)
		3	0/20, 0/19	0/20, 1/20	1/19, 3/20	6/20, 6/20	3/20, 4/20	13.0 (3.5)
		4	0/19, 0/20	0/20, 1/20	0/19, 3/20	0/20, 2/19	2/20, 2/20	5.0 (1.8)
		5	0/18, 0/20	0/18, 0/18	0/20, 1/20	1/20, 1/20	2/20, 0/20	2.5 (1.1)
1/10/81		1	7/20,11/20	5/20, 4/20	9/20,10/20	16/20,18/20	10/20,15/20	52.7 (7.3)
		2	10/19, 6/19	3/20, 2/19	8/20, 4/20	11/20, 8/20	9/20,11/20	36.4 (5.2)
		3	8/20, 2/19	3/20, 3/20	1/19, 3/20	6/20, 6/20	3,20, 5/20	20.0 (3.3)
		4	2/19, 3/20	3/20, 4/20	0/19, 3/20	3/20, 8/19	3/20, 5/20	17.2 (3.4)
		5	3/18, 2/20	0/18, 0/18	0/20, 1/20	3/20, 2/20	2/20, 0/20	6.6 (2.0)
15/10/81		1	15/20,11/20	9/20,11/20	10/20,14/20	17/20,18/20	13/19,15/20	66.8 (4.7)
		2	11/19,10/19	7/20, 7/19	8/20, 8/20	11/20, 8/20	11/20,12/20	47.2 (3.0)
		3	8/20, 2/19	6/20, 4/20	3/19, 9/20	6/20, 6/20	4/20, 8/20	28.1 (3.6)
		4	3/19, 4/20	3/20, 4/20	2/19, 7/20	3/20, 8/19	5/20, 5/20	22.3 (3.1)
		5	3/18, 2/20	4/18, 2/18	1/20, 3/20	3/20, 2/20	2/20, 0/20	11.4 (1.9)

Contd.

Continuation of Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants SCRLV R_1[a]	R_2	R_3	R_4	R_5	% SCRLV infection (mean of 5 replicates)
3/9/81	Malathion	1	3/19, 1/19	2/18, 2/20	0/20, 1/20	7/20, 8/20	0/19, 0/20	12.2 (4.5)[b]
		2	2/19, 1/19	2/20, 1/20	1/20, 1/20	2/20, 2/20	0/20, 0/20	6.0 (1.2)
		3	1/19, 2/19	1/20, 0/20	0/20, 1/20	1/19, 1/20	0/20, 1/20	4.1 (1.0)
		4	0/19, 2/19	0/20, 0/20	0/20, 0/20	1/17, 0/20	0/20, 0/20	1.6 (1.1)
		5	0/19, 0/18	0/20, 0/20	0/20, 0/17	0/20, 0/20	0/19, 0/19	0 (0)
17/9/81		1	8/19, 5/19	6/18, 9/20	5/20, 3/20	13/20,16/20	16/19,18/20	50.5 (8.6)
		2	9/19, 4/19	5/20, 2/20	1/20, 1/20	13/20,12/20	17/20,13/20	37.8 (8.7)
		3	2/19, 2/19	4/20, 1/20	0/20, 1/20	5/19,10/20	9/20,15/20	24.7 (7.7)
		4	2/19, 2/19	0/20, 0/20	0/20, 0/20	1/17, 7/20	5/20,10/20	13.6 (5.5)
		5	0/19, 0/18	3/20, 1/20	0/20, 0/17	7/20, 4/20	3/19, 3/19	10.6 (3.7)
1/10/81		1	13/19,13/19	10/18,10/20	9/20,10/20	19/20,20/20	18/19,18/20	71.7 (6.7)
		2	14/19,12/19	10/20, 4/20	9/20, 4/20	18/20,15/20	15/20,17/20	59.6 (7.9)
		3	6/19, 8/19	11/20, 6/20	1/20, 3/20	13/19,15/20	17/20,15/20	48.2 (8.7)
		4	7/19, 7/19	4/20, 4/20	0/20, 3/20	5/17,15/20	12/20,19/20	38.8 (9.3)
		5	1/19, 6/18	6/20, 2/20	0/20, 1/17	9/20,11/20	9/19,13/19	30.0 (7.5)

Contd.

Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants					% SCRLV infection (mean of 5 replicates).
			R_1[a]	R_2	R_3	R_4	R_5	
15/10/81	Malathion	1	13/19,14/19	12/18,12/20	19/20,18/20	20/20,20/20	18/19,18/20	83.8 (4.7)[b]
		2	14/19,15/19	15/20, 6/20	17/20,10/20	18/20,18/20	15/20,17/20	73.2 (6.0)
		3	6/19,11/19	11/20, 7/20	6/20,10/20	14/19,15/20	17/20,15/20	56.8 (6.3)
		4	9/19, 7/19	4/20, 5/20	5/20, 6/20	5/17,16/20	13/20,19/20	45.3 (8.2)
		5	1/19, 7/18	7/20, 2/20	5/20, 4/17	9/20,11/20	13/19,13/19	37.4 (7.0)
3/9/81	Barley barrier	1	0/20, 0/19	0/19, 1/19	0/19, 3/19	1/19, 4/20	1/17, 2/19	6.2 (2.2)
		2	0/19, 0/20	0/20, 0/20	2/19, 3/18	1/20, 2/20	1/19, 2/19	5.7 (1.8)
		3	0/19, 0/20	0/20, 0/20	1/19, 1/19	1/20, 1/20	0/18, 0/18	2.0 (0.8)
		4	0/19, 1/20	0/20, 0/20	0/19, 1/19	1/20, 2/20	1/19, 0/19	2.5 (1.1)
		5	0/20, 0/20	0/20, 0/20	0/19, 0/19	0/19, 0/20	1/18, 0/17	0.5(0.5)
17/9/81		1	1/20, 5/19	14/19,10/19	3/19, 4/19	1/19, 5/20	8/17,13/19	34.0 (7.8)
		2	2/19, 1/20	13/20,13/20	2/19, 3/18	1/20, 2/20	13/19,12/19	31.9 (9.1)
		3	0/19, 1/20	7/20,10/20	3/19, 1/19	3/20, 5/20	7/18,10/18	24.5 (6.1)
		4	0/19, 1/20	7/20,10/20	0/19, 4/19	1/20, 2/20	7/19, 8/19	20.4 (6.4)
		5	0/20, 0/20	2/20, 3/20	1/19, 0/19	3/19, 2/20	5/18, 4/17	10.7 (3.1)

Contd..

Continuation of Appendix Table: 11

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants SCRLV R_1[a]	R_2	R_3	R_4	R_5	% SCRLV infection (mean of 5 replicates).
1/10/81	Barley barrier	1	15/20, 8/19	16/19,14/19	8/19,11/19	5/19, 6/20	12/17,14/19	57.5 (6.6)[b]
		2	19/19,13/20	17/20,14/20	10/19, 3/18	9/20, 7/20	13/19,16/19	62.1 (7.9)
		3	12/19,12/20	12/20,13/20	7/19, 6/19	5/20, 7/20	13/18,15/18	53.2 (6.2)
		4	7/19,12/20	11/20,11/20	3/19, 5/19	5/20, 5/20	13/19,12/19	43.0 (6.0)
		5	10/20, 9/20	4/20, 4/20	2/19, 3/19	3/19, 4/20	6/18, 8/17	27.7 (4.6)
15/10/81		1	18/20, 9/19	16/19,15/19	8/19,14/19	5/19, 6/20	13/17,16/19	63.3 (7.6)
		2	19/19,17/20	17/20,16/20	14/19, 7/18	11/20,9/20	13/19,16/19	71.5 (6.2)
		3	15/19,18/20	14/20,14/20	10/19,10/19	12/20,10/20	15/18,16/18	70.1 (4.6)
		4	13/19,16/20	11/20,15/20	9/19, 9/19	8/20, 6/20	14/19,12/19	57.9 (5.2)
		5	13/20,17/20	8/20,11/20	4/19, 6/19	4/19, 4/20	8/18, 8/17	42.9 (6.7)

R^a = Replicate. (in each replicate, virus spread from both sides of infector row included)

( )[b] = Standard error ±

Appendix Table: 12 Movement of BYMV on V.faba L. from centre row after virus source was artificially introduced with vector - 1981 winter-spring

Date of observation	Treatment	Distance from source row	Number of plants infected out of healthy plants BYMV R_1[a]	R_2	R_3	R_4	R_5	% BYMV infection (mean of 5 replicates)
3/9/81	Control	1	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (± 0)
		2	0/20, 0/19	0/19, 0/18	0/19, 0/19	0/20, 0/20	0/20, 0/20	0.5 (0.5)
		3	0/19, 0/19	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
		4	0/20, 0/19	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/18	0 (0)
		5	0/20, 0/20	0/19, 0/15	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
17/9/81		1	0/20, 0/20	0/19, 2/19	0/20, 0/20	3/20, 3/20	5/20, 2/20	7.5 (2.82)
		2	0/20, 0/19	0/19, 0/18	0/19, 0/19	3/20, 2/20	1/20, 0/20	3.5 (1.67)
		3	0/19, 0/19	1/19, 0/19	0/20, 0/20	2/20, 1/20	0/20, 1/20	2.5 (1.12)
		4	0/20, 0/19	0/19, 0/19	0/20, 0/20	0/20, 1/20	0/20, 0/18	0.5 (0.5)
		5	0/20, 0/20	0/19, 0/15	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
1/10/81		1	0/20, 0/20	5/19, 3/19	3/20, 2/20	8/20,10/20	9/20, 9/20	24.7 (6.0)
		2	0/20, 0/19	4/19, 6/18	0/19, 2/19	7/20, 5/20	4/20, 6/20	18.0 (4.2)
		3	0/19, 0/19	5/19, 1/19	1/20, 2/20	6/20, 4/20	3/20, 7/20	14.6 (4.0)
		4	0/20, 0/19	1/19, 2/19	1/20, 0/20	0/20, 2/20	1/20, 3/18	5.2 (1.7)
		5	0/20, 0/20	0/19, 0/15	4/20, 1/20	2/20, 8/20	2/20, 4/20	10.5 (4.1)

Contd.

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV R_1[a]	R_2	R_3	R_4	R_5	% BYMV infection (mean of 5 replicates).
15/10/81	Control	1	0/20, 3/20	14/19,10/19	11/20,11/20	19/20,17/20	17/20,14/20	58.6 (9.7)[b]
		2	6/20, 4/19	9/19, 7/18	5/19, 7/19	16/20,15/20	13/20,10/20	47.0 (6.4)
		3	3/19, 5/19	11/19, 8/19	5/20, 4/20	14/20,14/20	10/20,14/20	44.7 (6.9)
		4	2/20, 1/19	8/19, 6/19	9/20, 3/20	13/20,11/20	8/20,10/18	36.4 (6.5)
		5	4/20, 0/20	5/19, 6/15	13/20, 6/20	17/20,14/20	13/20,16/20	48.1 (9.0)
3/9/81	Disyston	1	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 0/19	0 (±0)
		2	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 0/18	0 (0)
		3	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 0/19	0 (0)
		4	0/20, 0/20	0/20, 0/20	0/20, 0/19	0/20, 0/19	0/19, 0/19	0 (0)
		5	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 0/19	0 (0)
17/9/81		1	0/20, 0/20	1/20, 0/20	0/20, 0/20	0/20, 1/20	0/19, 0/19	1.0 (0.6)
		2	0/20, 0/20	0/20, 0/20	0/20, 0/20	1/20, 1/20	1/19, 0/18	1.5 (0.7)
		3	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	1/19, 2/19	1.5 (1.1)
		4	0/20, 0/20	0/20, 0/20	0/20, 0/19	0/20, 0/19	1/19, 0/19	0.5 (0.5)
		5	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/19, 1/19	0.5 (0.5)

Contd.

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV R_1[a]	R_2	R_3	R_4	R_5	% BYMV infection (mean of 5 replicates)
1/10/81	Disyston	1	0/20, 0/20	6/20, 6/20	1/20, 3/20	0/20, 2/20	12/19, 9/19	20.5 (6.9)[b]
		2	0/20, 0/20	2/20, 2/20	0/20, 2/20	4/20, 2/20	8/19, 7/18	14.0 (4.8)
		3	0/20, 1/20	1/20, 0/20	0/20, 0/20	2/20, 1/20	14/19, 6/19	13.0 (7.3)
		4	0/20, 0/20	2/20, 0/20	1/20, 1/19	2/20, 1/19	8/19, 6/19	10.9 (4.5)
		5	0/20, 0/20	3/20, 0/20	2/20, 1/20	1/20, 2/20	6/19, 8/19	11.8 (4.5)
15/10/81		1	6/20, 5/20	10/20, 9/20	10/20,13/20	5/20, 9/20	17/19,18/19	51.9 (7.7)
		2	4/20, 5/20	5/20, 8/20	7/20, 9/20	8/20, 6/20	16/19,15/18	42.7 (7.2)
		3	5/20, 4/20	3/20, 8/20	2/20, 5/20	6/20, 4/20	18/19,15/19	35.8 (8.9)
		4	2/20, 2/20	6/20, 7/20	6/20, 5/19	3/20, 8/19	14/19, 9/19	31.9 (6.1)
		5	5/20, 5/20	9/20, 7/20	9/20,10/20	7/20,11/20	17/19,17/19	49.3 (7.3)
3/9/81	Metasystox	1	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	1/19, 0/20	0.5 (0.5)
		2	0/19, 0/19	0/20, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
		3	0/20, 0/19	0/20, 0/20	0/19, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
		4	0/19, 0/20	0/20, 0/20	0/19, 0/20	0/20, 0/19	0/20, 0/20	0 (0)
		5	0/18, 0/20	0/18, 0/18	0/20, 0/20	0/20, 0/20	0/20, 0/20	0 (0)

Contd..

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV R_1a	R_2	R_3	R_3	R_5	% BYMV infection (mean of 5 replicates)
17/9/81	Metasystox	1	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 0/20	2/19, 0/20	1.0 (1.0)
		2	0/19, 0/19	0/20, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/20	1.5 (1.0)
		3	0/20, 0/19	0/20, 0/20	0/19, 0/20	0/20, 0/20	0/20, 0/20	0 (0)
		4	0/19, 0/20	0/20, 0/20	0/19, 0/20	0/20, 0/19	0/20, 0/20	0 (0)
		5	0/18, 0/20	0/18, 0/18	0/20, 0/20	0/20, 1/20	0/20, 0/20	0 (0)
1/10/81		1	0/20, 0/20	2/20, 2/20	3/20, 3/20	10/20,13/20	7/19, 6/20	23.1 (6.8)
		2	1/19, 1/19	2/20, 1/19	0/20, 0/20	9/20, 4/20	5/20,10/20	16.5 (5.7)
		3	0/20, 0/19	1/20, 0/20	2/19, 1/20	12/20,10/20	6/20, 3/20	17.5 (6.9)
		4	1/19, 0/20	2/20, 0/20	3/19, 3/20	10/20, 5/19	3/20, 3/20	15.2 (4.6)
		5	1/18, 0/20	0/18, 0/18	2/20, 1/20	11/20,13/20	0/20, 8/20	18.0 (7.9)
15/10/81		1	7/20,11/20	14/20,16/20	11/20, 6/20	19/20,18/20	15/19,17/20	67.3 (7.1)
		2	7/19,14/19	14/20,14/19	7/20,10/20	14/20,11/20	12/20,19/20	61.9 (5.8)
		3	7/20, 8/19	13/20, 9/20	7/19, 5/20	15/20,10/20	13/20,13/20	50.3 (5.1)
		4	8/19, 9/20	11/20, 9/20	7/19, 3/20	15/20, 7/19	9/20,13/20	46.0 (5.1)
		5	8/18, 9/20	7/18, 9/18	14/20,10/20	15/20,16/20	5/20,16/20	55.8 (6.0)

Contd.

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV					% BYMV infection (mean of 5 replicates)
			R_1[a]	R_2	R_3	R_4	R_5	
3/9/81	Malathion	1	0/19, 0/19	0/18, 0/20	0/20, 0/20	0/20, 0/20	0/19, 1/20	0.5 (0.5)[b]
		2	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/20, 0/20	0/20, 1/20	0.5 (0.5)
		3	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/19, 0/20	1/20, 0/20	0.5 (0.5)
		4	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/17, 0/20	0/20, 0/20	0 (0)
		5	0/19, 0/18	0/20, 0/20	0/20, 0/17	0/20, 0/20	0/19, 0/19	0 (0)
17/9/81		1	0/19, 0/19	1/18, 0/20	0/20, 0/20	3/20, 1/20	1/19, 3/20	4.5 (1.8)
		2	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/20, 2/20	4/20, 1/20	3.5 (2.1)
		3	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/19, 1/20	1/20, 1/20	1.5 (0.7)
		4	0/19, 0/19	0/20, 0/20	0/20, 0/20	0/17, 0/20	0/20, 1/20	0.5 (0.5)
		5	0/19, 0/18	0/20, 0/20	0/20, 6/17	0/20, 1/20	0/19, 0/19	0.5 (0.5)
1/10/81		1	0/19, 0/19	2/18, 4/20	0/20, 0/20	3/20, 5/20	9/19, 8/20	15.8 (5.4)
		2	0/19, 0/19	0/20, 2/20	0/20, 0/20	2/20, 5/20	12/20, 7/20	14.0 (6.4)
		3	0/19, 0/19	0/20, 3/20	1/20, 0/20	1/19, 5/20	9/20, 9/20	14.0 (5.7)
		4	0/19, 0/19	2/20, 0/20	0/20, 0/20	2/17, 2/20	5/20, 9/20	10.1 (4.6)
		5	0/19, 0/18	0/20, 0/20	1/20, 1/17	0/20, 3/20	3/19,10/19	9.9 (5.0)

Contd.

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV R_1[a]	R_2	R_3	R_4	R_5	% BYMV infection (mean of 5 replicates).
15/10/81	Malathion	1	10/19,10/19	10/18, 8/20	11/20,10/20	17/20,15/20	18/19,18/20	65.0 (6.1)[b]
		2	5/19,10/19	7/20, 6/20	6/20, 6/20	10/10,14/20	17/20,14/20	47.8 (6.6)
		3	3/19, 6/19	8/20, 5/20	5/20,11/20	12/19,11/20	16/20,13/20	45.5 (6.6)
		4	7/19, 8/19	5/20, 5/20	8/20,11/20	8/17,11/20	11/20,16/20	46.0 (5.1)
		5	5/19, 7/18	6/20, 7/20	8/20, 5/17	10/20,12/20	12/19,13/19	44.1 (4.8)
3/9/81	Barley barrier	1	0/20, 0/19	0/19, 0/19	0/19, 0/19	0/19, 0/20	0/17, 0/19	0 (0)
		2	0/19, 0/20	0/20, 0/20	0/19, 0/18	0/20, 0/20	0/19, 0/19	0 (0)
		3	0/19, 0/20	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/18, 0/18	0 (0)
		4	0/19, 0/20	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/19, 0/19	0 (0)
		5	0/20, 0/20	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/18, 0/17	0 (0)
17/9/81		1	0/20, 0/19	0/19, 0/19	0/19, 0/19	0/19, 0/20	0/17, 0/19	0 (0)
		2	0/19, 0/20	0/20, 1/20	0/19, 0/18	0/20, 0/20	0/19, 0/19	1.0 (0.6)
		3	0/19, 0/20	0/20, 1/20	0/19, 0/19	0/20, 0/20	0/18, 0/18	0.5 (0.5)
		4	0/19, 0/20	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/19, 2/19	1.0 (1.0)
		5	0/20, 0/20	0/20, 0/20	0/19, 0/19	0/20, 0/20	0/18, 0/17	0 (0)

Contd.

Continuation of Appendix Table: 12

Date of observation	Treatment	Distance from source (row)	Number of plants infected out of healthy plants BYMV R_1[a]	R_2	R_3	R_4	R_5	% BYMV infection (mean of 5 replicates)
1/10/81	Barley barrier	1	0/20, 0/19	0/19, 1/19	0/19, 0/19	0/19, 0/20	1/17, 2/19	2.1 (1.1)[b]
		2	0/19, 0/20	5/20, 6/20	2/19, 0/18	1/20, 2/20	3/19, 3/19	11.2 (3.3)
		3	2/19, 0/20	4/20, 4/20	1/19, 0/19	1/20, 2/20	3/18, 7/18	12.6 (3.7)
		4	1/19, 0/20	5/20, 3/20	1/19, 2/19	3/20, 1/20	2/19, 6/19	12.3 (3.0)
		5	1/20, 1/20	1/20, 4/20	3/19, 0/19	3/20, 1/20	4/18, 9/17	14.6 (4.8)
15/10/81		1	0/20, 0/19	1/19, 6/19	0/19, 1/19	0/19, 0/20	2/17, 3/19	6.9 (3.2)
		2	2/19, 3/20	7/20,11/20	3/19, 2/18	3/20, 4/20	12/19, 5/19	26.6 (5.9)
		3	2/19, 0/20	13/20,12/20	8/19, 5/19	7/20, 7/20	13/18,10/18	40.1 (7.4)
		4	5/19, 2/20	11/20,10/20	14/19, 6/19	9/20, 9/20	13/19, 8/19	44.7 (6.0)
		5	6/20, 3/20	8/20, 13/20	14/19, 6/19	11/20, 9/20	10/18,15/17	50.1 (7.0)

R^a = Replicate. (in each replicate, virus spread from both sides of infector row included)

( )[b] = Standard error ±

Appendix Table: 13 SCRLV particle diameter measured from electron micrographs.

Number of particles measured	Particle diameter (mm)
1	5.8
2	5.8
3	5.8
4	5.9
5	5.8
6	5.8
7	5.9
8	5.8
9	6.0
10	5.8
Mean diameter	5.84
S.E.	$\pm$ 0.022
Therefore diameter of particle	$= \frac{5.84 \times 100}{19.2}$
	= 30.4 nm

(19.2 mm = 100 nm on the electron micrographs)

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