THE EPIDEMIOLOGY OF CUCUMBER MOSAIC VIRUS IN NARROW-LEAFED LUPINS (*LUPINUS ANGUSTIFOLIUS*) IN SOUTH AUSTRALIA

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Errata

1. List of scientific names used in the summary, headings, figures and tables.

Plants: *Lupinus angustifolius*.

Aphids: *Aphis craccivora, Brachycaudus rumexicolens, Dysaphis aucupariae, Hyperomyzus lactucae, Lipaphis erysimi, Macrosiphum euphorbiae, Metapolophium dirhodum, Myzus persicae, Rhopalosiphum padi*.

2. Page 28, line 12: Madden, 1990, should read Madden, Knoke and Louie, 1990.

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Summary

(1) Epidemics of CMV in *L. angustifolius* were experimentally initiated in 1987, 1988 and 1989, to study factors affecting the rate of epidemic progress.

(2) Rapid virus spread occurred during spring, and coincided with the plant growth stages of flowering and pod fill.

(3) Field diagnosis of infection by symptoms and by detection of antigen by DAS ELISA was compared. Incidence of infection at crop maturity was underestimated by about 50 % when symptoms were used for diagnosis, due to the occurrence of symptomless infections.

(4) Lupins, which were either infected through seed or inoculated at the seedling stage, were shown to be important primary sources of inoculum. Clumps of infected plants formed following virus spread by aphids. Infection gradients arising from linear sources of inoculum were steep, with incidence of infection decreasing from 100 % to 20 % in a distance of 2.5 m. (5 plant rows). Secondary infection foci also developed from longer distance dispersal of inoculum.

(5) Yellow pan traps were used to monitor aphid flights during the lupin growing season in 1987, 1988 and 1989. *Myzus persicae*, *Lipaphis erysimi*, *Rhopalosiphum padi*, *Aphis craccivora* and *Brachycaudus rumexicolens* were trapped in largest numbers. For all species, most abundant flights were in the period between late August to October. *R. padi* and *M. persicae* were trapped regularly, though in low numbers, through winter.

(6) In 1989, the yellow pans were compared with suction traps, which were mounted at the height of the lupin canopy, and with green tile traps. The green tiles trapped inefficiently and no comparison could be made with the yellow pans and suction traps. Large numbers of *R. padi* and *M. persicae* were collected in the suction traps and these species were

therefore abundant in the boundary layer of the crop where they could alight on the lupins. Abundant flights of *L. erysimi* were detected using the yellow pans, but this species was rarely trapped in the suction traps. It was therefore considered that *L. erysimi* were not flying in the boundary layer of the lupin crop and were therefore not attempting to alight.

(7) The daily flight patterns of aphids on six days in spring, 1989, were monitored, and corresponding weather conditions also measured. The daily flight patterns of *M. persicae*, *R. padi* and *L. erysimi* were variable and affected by temperature and wind speed. Aphid flight was not detected below 10.6 C for *M. persicae*, 9.7 C for *R. padi* and 12.7 C for *L. erysimi*. High wind speeds reduced, but did not inhibit flight, as some aphids were trapped when wind speed was greater than 10 km/hour. The rapid detection of abundant aphid flights following a change in the weather to conditions that favour flight initiation, suggested that the aphid source was close (within 5 km.) to the field site.

(8) From glasshouse transmission tests, *M. persicae*, *R. padi*, *A. craccivora*, *B. rumexicolens*, *D. aucupariae* and *H. lactucae* were shown to be capable of transmitting a lupin isolate of CMV, but not *L. erysimi, Macrosiphum euphorbiae* and *Metapolophium dirhodum*

(9) Field spread of CMV correlated with aphid flights, assuming a 2 week delay between inoculation and detection of systemic infection. *R. padi* was concluded to be an important vector as (a) virus spread in the 1987 field trial correlated with a flight of aphids composed primarily of *R. padi*, (b) *R. padi* was shown to be abundant in the boundary layer of the crop and was found alighting on the lupins and (c) *R. padi* was shown to be capable of transmitting CMV. There was no effect on epidemic progress of either initiating colonies of *A. craccivora* on introduced sources of inoculum, or initiating colonies of *R. padi* on oats, planted next to introduced sources of inoculum.

(10) Epidemic progress in the 1987 field trial was quantified using previously published models proposed to describe the functional relationship between disease increase and vector numbers. The interpretations of the best fitting model were (a) the growth rate of the epidemic increased as the number of alates entering the crop increased, (b) the probabilty of virus acquisition by the aphids increased as incidence of infection increased, as might occur during a polycyclic epidemic, and (c) the probability of transmission decreased as the epidemic progressed.

Infection gradients observed in the 1988 field trial were also quantified using previously published models. The interpretations of the better fitting models were that either most or all of the inoculum originated from the linear source of inoculum, and that inoculum was diluted with increasing distance from the source. Infection gradients with the shape observed, are considered to occur during a monocyclic epidemic, or at the beginning of a polycyclic epidemic. The infection gradients were, in fact, observed soon after the first spring flight of aphids.

(11) Commercially traded lupin seed from South Australia, Victoria and New South Wales, was tested for CMV transmission. Transmission rates ranged between 0 and 11.5 %. CMV transmission was found in seeds from the lupin cultivars 'Danja', 'Illyarrie', 'Warrah', 'Wandoo' and 'Yandee'. CMV transmssion was detected in 23 of the 51 seedlots tested.

(12) Seed transmission rates were dependent on the age of the plant at the time of inoculation. Highest rates of transmission (between 23 and 25 %) occurred when the plant became infected during vegetative growth. The rate of transmission progressively declined with later inoculations after the beginning of flowering. The probability that a seed became infected decreased the more developed the seed at the time of inoculation. Infectious CMV was recovered from the cotyledons and primordial radicle and plumule, suggesting that seed transmission resulted from infection of the embryonic tissues.

(13) Dry matter productivity was only affected when the plant became infected during vegetative growth. Seed productivity was still affected when the plant became infected during flowering. For lupins infected at the seedling stage, the reduction in seed yield was 99.7 % and the reduction in dry matter yield was 98.6 %. Seedlings that were infected through seed showed no greater tolerance to infection than those seedlings that were inoculated at the cotyledon stage.

(12) Largest numbers of infected seed were produced by plants which were inoculated at the beginning of flowering. Virus spread occuring at the beginning of flowering was shown mathematically to be optimal for virus persistence by seed transmission, as for all but the largest of epidemics, maximum seed transmission levels are predicted to occur when the plants are inoculated at this time. It was also shown that CMV could not persist by transmission in lupin seeds if no secondary spread by aphids occurred.

Seed transmission levels were observed to increase in one generation, even when secondary spread by aphids was small.

STATEMENT

This thesis contains no material which has been previously presented for any other degree or diploma in any university and to the best of my knowledge and belief, does not contain material published or written by another person, except where due reference is made in the text. I consent to the thesis being made available for loan and photocopying if accepted for the award of the degree.

Andrew D. W. Geering

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This thesis is dedicated to my parents, Margo and Bill, and to Margaret.

Chapter 1

Introduction

Cucumber mosaic virus (CMV) is an important plant pathogen of world wide importance and causes disease in many vegetable, pasture, grain legume and ornamental species (Nelson and Tuttle, 1969; Lockhart and Fischer, 1976; Conti *et al.*, 1979; Quiot, 1980; Horvath, 1983; Quiot *et al.*, 1983; Alberts *et al.*, 1985; Aly *et al.*, 1986; Jones, 1988; Jones and McKirdy, 1990; Kearney *et al.*, 1990). In this introductory chapter, previous research done to study the biology, epidemiology and control of CMV is discussed.

1.1 Description of CMV

CMV, together with tomato aspermy virus (TAV) and peanut stunt virus (PSV), are members of the cucumovirus plant virus group (Francki, 1985). The RNA genome of cucumber mosaic virus, which is single-stranded, linear and positive sense, is divided into 3 segments, all of which must be present in inoculum for infection to establish (Peden and Symons, 1973; Lot *et al.*, 1974). The RNA segments differ in molecular weight and are labelled RNA 1, 2 and 3 in order of decreasing size (Lot *et al.*, 1974). RNA's 1 and 2 are thought to contain genes coding for proteins which form the RNA replicase (Nitta *et al.*, 1988). The coat protein gene and another gene thought to code for the transport protein are found on RNA 3 (Davies and Symons, 1988). A smaller sub-genomic RNA, called RNA 4, is transcribed from RNA 3 and is the messenger RNA form of the coat protein gene (Gould and Symons, 1982; Davies and Symons, 1988).

The RNA molecules are encapsidated in a capsid consisting of 180 identical protein subunits, each weighing 26.2 kD (Gould and Symons, 1982). Particle shape is icosahedral and particle diameter 29 nm (Francki *et al.*, 1985). RNA's 1 and 2 are thought to be individually encapsidated, and RNA's 3 and 4 encapsidated together, though particles

containing various combinations of the RNA types may exist (Peden and Symons, 1973; Lot and Kaper, 1976; Kaper and Waterworth, 1981).

1.2 CMV variation and classification of strains

1.2.1 Processes by which variation arises

Many variants of CMV have been described that differ in biological properties such as host range, host reactions to infection, and ability and efficiency of transmission by aphids and through seed (Kaper and Waterworth, 1981; Shintaku and Palukaitis, 1990). Variation in CMV is thought to be generated by:

(a) Mutation.

Viruses with RNA genomes have a high rate of viable mutations due to the absence of RNA proofreading exonucleases associated with the RNA replicase (Holland *et al.*, 1982). For example, variants of CMV quickly arise when the virus is passaged in new hosts, due to different host selection pressures (Lakshman *et al.*, 1985).

(b) Gene assortment through pseudorecombination and recombination.

The term pseudorecombination has been introduced to describe the mixing of RNA segments of viruses with divided genomes. New, viable viruses have been experimentally produced by combining RNA segments from different strains of CMV and even by combining RNA segments from CMV and TAV (Mossop and Francki, 1977; Rao and Francki, 1981; Rao and Francki, 1982; Zitter and Gonsalves, 1990). Evidence for pseudorecombination occurring *in vivo* is lacking.

There is increasing evidence to suggest that genetic recombination may occur in many RNA viruses and this may also be an important source of genetic diversity (Bujarski and Kaesberg, 1986; Jarvis and Kirkegaard, 1991).

(c) Presence of satellite RNA.

In some CMV isolates, an RNA smaller than the genomic components of CMV, called satellite RNA (sat RNA), is found (Kaper *et al.*, 1976). Sat RNA is not part of the CMV genome, but depends on the CMV RNA replicase to replicate itself (Kaper *et al.*, 1976; Kaper and Waterworth, 1977; Gould *et al.*, 1978; Mossop and Francki, 1978). Sat RNA is encapsidated by CMV coat protein and can therefore be aphid transmitted (Chen and Francki, 1990). Sat RNA often modulates the disease symptoms caused by CMV, and in some cases, disease is ameliorated, but in other cases, made more severe. The effect of sat RNA on symptom expression is dependent on characteristics of the sat RNA, the helper CMV strain and the host. For example, typical symptoms in tomato caused by CMV infection, are shoestring and mosaic symptoms of the leaf, however, in association with CARNA 5, a form of sat RNA, symptoms in different tomato accession lines can range from total plant necrosis to mild mosaic (White and Kaper, 1987). A sat RNA investigated by Mossop and Francki (1977) ameliorated symptoms caused by many strains of CMV, however, with some strains, it had no effect on symptoms.

There are few reports of sat RNA being identified as a principal aetiological agent of disease in field crops. Notably, CMV and its sat RNA has been identified as the cause of epidemics of tomato necrosis in France and Italy (Kaper *et al.*, 1990) and also the cause of 'white leaf' disease of tomatoes in New York (Gonsalves *et al.*, 1982). Insufficient surveys have been done to generalise on the abundance of sat RNA in field populations of CMV, but Kearney *et al.* (1990) found it to be rare in their surveys of crops in New York and Bermuda.

1.2.2 Classification and detection of CMV strains

Serological and nucleic acid hybridisation studies have been used to subdivide CMV strains into groups. Comparison of both American and Australian isolates of CMV using cDNA dot blot hybridisation assays has shown that two groups, called subgroups 1 and 2, can be distinguished (Owen and Palukaitis, 1988; Wahyuni *et al.*, 1992). Members of the same subgroup have extensive nucleic acid homology, and with the dot blot assay, will hybridise strongly with each other, but not with members of the other subgroup. Subgroups 1 and 2 are comparable to the DTL and ToRS serogroups, defined by Devergne and Cardin (1975), however significant variation in serological properties was found between members of a subgroup so that monoclonal antibodies that recognised the two serogroups, could not always be used to categorise strains into their respective subgroups (Wahyuni *et al.*, 1992). The two groups, WT and S, defined by Piazzolla *et al.* (1979), who used RNA-RNA hybridisation tests to group strains, also appear equivalent to subgroups 1 and 2. Edwards and Gonsalves (1983) and Kearny *et al.* (1990) could serologically distinguish members from each of the WT and S groups using polyclonal antibodies and double antibody sandwich (DAS) ELISA. Eighty three percent of field isolates collected in New York and Bermuda could be assigned to either the WT or S serotype, however the remaining isolates reacted similarly in ELISA with antibodies prepared against strains from each group. In contrast to the previous mentioned work, Wahyuni *et al.* (1992) found that DAS ELISA, utilising polyclonal antibodies prepared against one strain of CMV, readily detected strains from both subgroups 1 and 2 and was therefore a suitable method to detect a wide range of CMV variants.

At present, no consistent differences in biological properties have been found between members of subgroups 1 and 2 (Wahyuni *et al.*, 1992) and therefore no distinction can be made between the subgroups in terms of their epidemiology. Both subgroups have been isolated from *Lupinus angustifolius* in Western Australia (Wahyuni *et al.*, 1992).

1.3 Types of natural transmission

1.3.1 Aphid transmission

(a) Mechanism of aphid transmission

CMV is transmitted in a non-persistent manner by more than 60 aphid species (Kennedy *et al.*, 1962). CMV is acquired by aphids in as little as 5 seconds, acquisition efficiency decreases after about 2 minutes and aphids remain infectious for short periods of usually less than two hours (Francki *et al.*, 1979).

Evidence on the role of the coat protein in determining aphid transmissibility suggests that retention of CMV by the aphid involves an interaction between a chemical site on the surface of the virus particle and a complementary receptor site on the surface of either the food canal of the maxillae or the foregut (Mossop and Francki, 1977; Gera *et al.*, 1979; Harrison and Murant, 1984; Chen and Francki, 1990; Matthews, 1991). Evidence reviewed by Pirone and Harris (1977), suggests that healthy plant cells could be inoculated by the aphid regurgitating contents from its alimentary canal.

(b) Factors affecting transmission efficiency

The efficiency with which aphids transmit CMV (transmission efficiency) is variable and dependent on the nature of interactions between the virus, host, aphid and the environment. Evidence for variation in transmission efficiency resulting from alteration of the interaction between the virus capsid and the receptor site in the aphid's mouthparts has been provided by experiments in which aphids were membrane-fed purified preparations of CMV, and variation in transmission efficiency was observed when different aphid species or CMV strains were used (Megahed and Pirone, 1966; Gera *et al.*, 1979; Chen and Francki, 1990). This variation may reflect differences in the extent of retention of the virus in the

aphid following acquisition feeds or differences in the extent of particle release during subsequent feeds on healthy plants (Harrison and Murant, 1984).

Normand and Pirone (1968) and Zitter and Gonsalves (1991) have shown that the primary cause of differences between some CMV strains in transmission efficiency is due to differences in the level of replication in the host, rather than differences in the properties of the coat protein. Early reports of variation in transmission efficiency resulting from a change in the virus host species, the environmental conditions during host growth and the position of the source leaf (Simons, 1955; Stimmann and Swenson, 1967), most probably reflect differences in the rate of replication and the concentration of CMV in the leaf.

In a field situation, the efficiency with which an aphid transmits a non-persistently transmitted virus is also influenced by the aphid's behaviour. The term 'vector propensity' has been introduced to describe the probability of an aphid transmitting the virus following an opportunity to acquire it and provided the aphid lands on a healthy virus host plant (Irwin and Ruesink, 1986). Vector propensity is determined by both the innate ability of the aphid to transmit, as well as the type of feeding behaviour exhibited by the aphid on the source plant (Irwin and Ruesink, 1986). Transmission of non-persistently transmitted viruses is more likely to occur if the aphid moves rapidly between plants, and is not favoured by settling behaviour of the aphid. Glasshouse transmission tests in which the time periods of virus acquisition and transmission are controlled are likely to give poor estimates of the field vector propensity due to the manipulation of the aphid's normal feeding behaviour.

(c) Role of alate aphids as field vectors of CMV

Field spread of CMV and other non-persistently transmitted viruses often correlates with migratory flights of aphids (Van Hoof, 1977; Halbert *et al.*, 1981; Raccah *et al.*, 1985; Raccah *et al.*, 1988). Aphid alates are attracted to foliage of a young physiological age, rather than that associated with any particular plant species (Kennedy *et al.*, 1961). Host

selection is made following brief probes into the leaf, during which CMV acquisition and transmission can occur, and therefore both colonising and non-colonising may be important vectors. For example, *Aphis gossypii* and other *Aphis* species are the most important vectors of CMV in Israeli pepper crops (Raccah *et al.*, 1985). These species do not colonise peppers and are found in relatively small numbers on the peppers compared with other colonising aphids such as *Myzus persicae* and *Macrosiphum euphorbiae* (Raccah *et al.*, 1985). They do, however, land in large numbers on the peppers and transmission tests of aphids trapped alive in suction traps show that they are more frequently found to be viruliferous than the colonising species (Raccah *et al.*, 1985).

(d) Distance of dispersal by aphids

Little research has been conducted to determine the distance of dispersal of CMV by aphids and to describe the infection gradients that may form. Aly *et al.* (1986) found that at the end of the growing season, the incidence of infection of gladiolus with CMV decreased from a maximum of between 36-62 % for plots adjacent to the inoculum source, to 2.7 % for a plot located 100 metres from the inoculum source. Generally, aphid spread of non-persistently transmitted viruses is only thought to occur over short distances due to the short retention time of the virus by the aphid.

Zeyen and Berger (1990) have challenged the notion that long distance transport of non-persistently transported viruses does not occur. They argue that the short retention times reported for non-persistently transmitted viruses are artifacts of the experimental methods used and that if the aphids are prevented from probing whilst they are contained before the inoculation feed, as would occur during flight, then a greater percentage of aphids will transmit after a given time. Also, if large sample sizes are used in order to detect low levels of transmission, then estimates of the maximum retention time are increased by many hours. They argue that in the mid-west of the USA, low level jet winds may transport large numbers of aphids over three hundred kilometres in as little as four hours and provide a

means of long distance dispersal of non-persistently transmitted viruses. Although jet winds can transport aphids over large distances, Taylor (1986) concludes that high density drift of aphids mostly occurs over smaller distances of between 5-50 kilometres.

In many cases, the primary inoculum source of CMV and other viruses is located in or close to the crop and the aphid acquires the virus following a period of migratory flight. At the end of migratory flight, alate aphids have a period of 'trivial flight', whereby they hop from plant to plant, and probe the leaf to assess its palatability (Robert, 1987). Short distance flight of this nature would be a contributing factor towards the formation of steep infection gradients, as the aphid quickly loses infectivity following multiple probes.

1.3.2 Seed transmission

(a) Mechanism of seed transmission

Mandahar (1981) lists 19 plant species in which CMV is seed transmitted. Not all CMV strains are seed transmitted in a particular plant species (Davis and Hampton, 1986). Rates of transmission ranging between 0 and 40 % have been reported for different plant species (Mandahar, 1981). Seed transmission of plant viruses can result from infection of the embryonic tissue, and in some cases, by contamination of the seed coat (Bennett, 1969; Mandahar, 1981). CMV is labile outside host tissue and would therefore be expected to be transmitted by infection of the embryonic tissue, as those viruses that are transmitted by contamination of the seed coat, such as tobamoviruses, are typically very stable and infectious for long periods (Bennett, 1969).

Embryo infection may occur by infection of the megagametophyte (embryo sac) prior to fertilisation, by pollen transmission during fertilisation or by direct infection of the developing embryo (Bennett, 1969). Davis and Hampton (1986) found that seed transmission of CMV in *Phaseolus vulgaris* only occurred when the plant was inoculated

before flowering commenced. From this result, they proposed that seed transmission resulted from infection of the megagametophyte.

The mechanism of seed transmission of barley stripe mosaic virus is better understood than that of CMV and known to result from infection of the embryo by either infection of the megagametophyte or by pollen transmission (Carroll, 1981). For the megagametophyte to become infected, the sporogenous cells must become infected (Carroll, 1981). The sporogenous cells enlarge to form the megaspore mother cells, which divide meiotically, then mitotically, to form the megagametophyte. The megaspore mother cells and the megagametophyte are surrounded by a callose layer, and only the sporogenous cells have plasmodesmatal connections to the surrounding tissue, through which the virus can infect the cells (Carroll, 1981). The developing embryo also lacks plasmodesmatal connections to the surrounding maternal tissue and it has been proposed that this may be the reason why the virus cannot directly infect the immature embryo (Carroll, 1981).

(b) Factors affecting rate of seed transmission

The rate of seed transmission is variable and depends on the interaction between the host plant, virus and environment. Davis and Hampton (1986) investigated variation in rates of seed transmission of CMV in *Phaseolus vulgaris* that arose from differences in the combination of host cultivar and virus isolate. For the cultivar 'Topcrop', the rate varied between 0 and 49 percent for different virus isolates. CMV isolate B was shown to be seed transmitted in only 2 of the 14 cultivars tested.

Rate of seed transmission is also dependent on the age of the plant at the time of inoculation. As previously mentioned, Davis and Hampton (1986) showed that CMV was seed transmitted in plants of *P. vulgaris* inoculated at the seedling stage, but not in plants inoculated immediately before or during flowering. This effect of plant age is thought to be

primarily due to the existence of a cut-off point in the development of the embryo, after which time the embryo escapes infection if the plant becomes infected.

Rate of seed transmission of soybean mosaic virus in soybeans is also affected by plant age at the time of inoculation (Irwin and Goodman, 1981). With highly determinant cultivars that flower over short periods, seed transmission does not occur when the plant is inoculated after the commencement of flowering (Irwin and Goodman, 1981). In comparison, for indeterminant cultivars in which flowers continue to emerge over long periods, seed transmission will occur, though at reduced rates, in plants inoculated after the commencement of flowering (Irwin and Goodman, 1981). Seeds produced by the later maturing flowers become infected and providing plant senescence does not restrict seed maturation, these seeds will transmit the virus.

(c) Epidemiological significance of seed transmission

Seed transmission is important in the ecology of plant viruses, as it allows survival of the virus in periods when host growth is prevented and it also provides a means of long distance dispersal of the virus through either natural movement or the commercial trade of infected seed. Generally, those viruses that are embryo borne can remain viable for as long as the seed remains viable (Stace-Smith and Hamilton, 1987). Seed transmission of CMV has been shown to occur in seed of *Stellaria media* that has been buried for as long as 2 years (Tomlinson and Walker, 1973). Seed transmission in crop seed ensures that primary sources of inoculum are randomly dispersed throughout the following crop generation (Stace-Smith and Hamilton, 1987).

Seed transmission of CMV is a serious problem to plant breeders in the maintenance of germplasm collections. Jones (1988) found widespread infection of the *L. angustifolius* germplasm collection in Western Australia, including the cultivars 'Illyarrie', 'Chittick' and 'Yandee', and newly released cultivars such as Wandoo.

1.4 Role of weed and ornamental plants in the ecology of CMV

Weed plants play an important role in the ecology of some CMV strains and are often important primary sources of inoculum from which annual crop plants become infected. To illustrate the importance of CMV in the ecology of CMV, some case studies are presented.

In the higher latitudes of Europe and North America, cold winters restrict plant growth and annual crops are grown during Spring and Summer. CMV is an important pathogen of lettuce crops and weeds are important as overwintering hosts of the CMV strains infecting lettuce. In the state of New York, CMV survives over winter in the dormant roots, rhizomes and rosettes of the perennials *Asclepias syriaca*, *Barbarea vulgaris*, *Rorippa islandica* and *Linaria vulgaris* (Rist and Lorbeer, 1989). These weeds are commonly found in the irrigation ditches. New spring growth of these perennials is infectious and from these plants, CMV spreads into lettuce crops (Rist and Lorbeer, 1991). Similarly, in England, many common annual and perennial weeds of lettuce crops have also been found to be infected. The annual chickweed (*Stellaria media*) is both abundant and frequently infected and seed transmission of CMV in this plant is considered to be an important means of overwintering of the virus (Tomlinson and Carter, 1970; Tomlinson and Walker, 1973). Chickweed and some other annual weeds, such as *Senecio vulgaris* and *Tripleurospermum maritimum*, and perennial weeds, such as *Lamium album* and *Malva sylvestris*, can also survive through the winter, and when infected, also act as primary sources of inoculum in spring (Tomlinson *et al.*, 1970).

In the Rhone Valley of France, two strains of CMV, designated B and C, are found infecting weeds and vegetable crops. The comparative ecology of these two strains is reviewed by Quiot (1980), from which the following discussion is derived. The two CMV strains, which can be distinguished by serological properties and symptoms of infection in *Nicotiana tabacum* 'Xanthi', also differ in their response to high temperature. Replication of strain B is inhibited by high temperatures and when the temperature of plant growth is 32°C,

strain C will quickly replace strain B in a mixed infection. This difference in temperature sensitivity is reflected in the natural distribution of the two viruses. CMV strain B is found in winter growing perennial weeds such as *Rubia perigrina* and strain B predominates over strain C in spring crops of tomatoes. Conversely, strain C is found in summer growing weeds such as *Portulacca oleracea* and in summer crops of muskmelon and tomato. Although there is a continuum of susceptible weed and crop plants with overlapping growth periods, CMV B can only survive through summer by infecting perennial or annual hosts that survive from one winter to the next or by seed transmission in weeds such as *Stellaria media*. During summer, the concentration of strain B in infected *Rubia perigrina* is very low, and infectious CMV cannot be recovered and these plants and others only become efficient sources of inoculum when conditions become cooler.

In Arizona, cantaloupe (*Cucumis melo* var. *reticulatus*) crops are grown in irrigation areas in spring and summer. The non-cropping period is during winter, a period when other annual weeds that are susceptible to CMV infection also do not grow. Crops with severe epidemics are located near towns and country homes (Nelson and Tuttle, 1969). The perennial garden ornamental, periwrinkle (~~*Vinca rosea*~~ *Catharanthus rosea*), was found to have a high incidence of infection, and together with some other garden plants, was identified as an important overwintering host from which cantaloupe crops became infected (Nelson and Tuttle, 1969).

A limitation to some epidemiological studies where weeds have been identified as alternative virus hosts is that no information is provided as to when these weeds became infected in relation to spread of the virus within the crop. In the lettuce growing areas of New York, CMV has a natural host range of 18 weed species (Rist and Lorbeer, 1989). Incidence of infection in commercial lettuce crops at maturity strongly correlates with incidence of infection in weeds early in the season (Rist and Lorbeer, 1991). Early in the growing season, only four weed species, *A. syriaca, B. vulgaris, L. vulgaris* and *R. islandica* are important as primary sources of inoculum (Rist and Lorbeer, 1989, 1991).

CMV is seed transmitted in *L. angustifolius* and the role of alternative hosts in the epidemiology is unclear. Both Alberts *et al.* (1985) and Jones (1988) have identified a number of annual weeds of diseased lupin crops that are frequently found to be infected with CMV. These weeds may, however, have become infected from inoculum arising from the lupin crop and not *vice versa*. If the strain is not seed transmitted in these weed hosts, then these plants would be unimportant in overseasoning of the virus over the summer drought of Southern Australia, and unimportant as infection reservoirs for the lupin epidemic.

1.5 Control of CMV

1.5.1 Eradication or geographical isolation from sources of inoculum

(a) Reduction of seed transmission levels

Epidemics of viruses that are introduced into the crop via seed transmission and which are subsequently spread by aphids, can be controlled by reducing the level of seed transmission. Desirably, seed should be free of virus infection, though this is often not practically possible and impossible to ensure when only samples of a seedlot are tested (Russell, 1988). Large samples of seed can be tested for virus infection, using sensitive detection methods such as ELISA, and rates of seed transmission accurately estimated (Jones, 1988; Russell, 1988).

The term inoculum threshold has been introduced to describe the "maximum amount of inoculum that can be tolerated without an appreciable constraint to yield and its concomitant limitation of economic yield" (Stace-Smith and Hamilton, 1987). The inoculum threshold in any given year is not a constant, but affected by many biological variables such as the timing and magnitude of vector activity, as well as economic variables such as the cost of seed and herbicides, that also determine the overall profit margin of the crop (Stace-Smith and Hamilton, 1987). Generally, from the limited amount of information available, the

inoculum threshold for seed-borne viruses that are also efficiently transmitted by aphids is very low (Stace-Smith and Hamilton, 1987). Zink *et al.* (1956) concluded that for lettuce mosaic virus, seed transmission levels greater than 0.1 % were likely to cause unacceptable disease epidemics.

(b) Eradication of alternative hosts

Where CMV persists between crops in alternative hosts such as weeds, and these are the most important sources of inoculum, then eradication of these plants will prevent epidemics in the crop. Eradication of the infection reservoirs may not always be practically possible. Such circumstances may arise when infected plants are components of pasture, are garden ornamentals, are found in neighbouring farms or are closely related to the crop plant and selective herbicides are not available.

(c) Separation from the inoculum reservoir

Control can be achieved by separating the crop, in space or time, from the inoculum reservoir. For example, farmers in Israel do not plant their bell pepper crops before mid-April because of the risk of CMV infection. After early May, the risk of infection is much decreased as the weed hosts of CMV have dried (Loebenstein and Raccah, 1980). In Arizona, highest disease incidence occurs in crops close to settlement where the infection reservoirs are found (Nelson and Tuttle, 1969). The risk of crop disease decreases with increasing distance from these sources of inoculum. Control by physical separation from the infection reservoir can be enhanced when a barrier crop of a non-susceptible species is planted around the crop to be protected. The barrier crop is effective by intercepting viruliferous aphids, which lose infectivity when they probe on the barrier crop plants (Jayasena and Randles, 1985).

1.5.2 Control by preventing aphid spread

(a) Insecticides

Generally, insecticides are ineffective in preventing spread of non-persistently transmitted viruses. Insecticides do not act quickly enough to prevent virus spread, as they take many hours to kill and virus transmission can occur in as little as a few minutes (Loebenstein and Raccah, 1980; Tomlinson, 1987; Matthews, 1991).

Application of insecticides may help control of virus epidemics when the crop plant is both the virus and aphid host and prevention of aphid colonisation may reduce the total number of vectors.

(b) Mineral Oils

Mineral oils, when sprayed onto plant foliage, have been demonstrated to reduce spread of CMV in peppers and cucumbers (Loebenstein *et al.*, 1966; Loebenstein *et al.*, 1970) and when used in combination with coarse nets, control spread of CMV in gladiolus (Aly *et al.*, 1986). The mode of action of mineral oils in interfering with aphid transmission is not clear, however, it is possible that they may act by preventing virus attachment to the aphid's stylet, perhaps by changing the surface charge of the stylet, and they also may act by altering the feeding behaviour of the aphid (Loebenstein and Raccah, 1980; Matthews, 1991).

Mineral oils are particularly applicable to control of viruses in horticultural crops and are most effective when vector activity occurs during relatively short periods when the plants are small (Loebenstein and Raccah, 1980). Mineral oils are of low toxicity to humans and other animals, but are volatile and sometimes phytotoxic (Matthews, 1991). There are also problems with application of the oil and its effectiveness may be reduced by poor coverage of the foliage and the oil being washed off by rain (Matthews, 1991).

(c) Reflective mulches and coarse nets

Reflective mulches reduce the number of alate aphids alighting on the crop plants and by so doing, are effective in reducing spread of CMV and other non-persistently transmitted aphid-borne viruses (Loebenstein *et al.*, 1975; McLean *et al.*, 1982; Lecoq and Pitrat, 1983). Migratory alate aphids, after an extended period of flight, become attracted to light of wavelength greater than 500 nm and are repelled by shorter wavelength light in the ultraviolet and blue range (Robert, 1987). This phototactic behaviour guides aphids towards plants. Mulches such as straw, aluminium foil and white or grey polyethylene, reflect a wide spectrum of light, including short wavelength light and so deter aphids from settling on the plants.

Coarse white nets suspended over crops have also been demonstrated to reduce the number of alate aphids alighting and by so doing, control spread of CMV. These nets are not physical barriers to aphid movement, but appear to act by repelling aphids (Cohen, 1981).

Both reflective mulches and coarse nets are expensive to use and only suited to high value crops covering small areas. Problems also exist with the disposal of non-biodegradable mulches after their use (Nameth *et al.*, 1986).

(c) Avoiding vector flights

Spread of CMV by aphids can be controlled by altering the sowing date so as to avoid vector flights, or to allow crop maturation before the vector flights occur. With increased maturity, plant susceptibility may decrease, and also, the effects of virus infection on growth are usually less severe.

1.5.3 Plant resistance to virus infection

(a) Naturally occurring resistance

Naturally occurring resistance to CMV infection has been found in members of the cucurbit family, including cucumber (*Cucumis sativus*), muskmelon (*Cucumis melo*) and marrow (*Cucumis pepo*) and also in lettuce (*Lactuca sativa*), spinach (*Spinacea oleracea*) and cowpea (*Vigna unguiculata*) (Shifriss *et al.*, 1942; Pound and Cheo, 1952; Sinclair and Walker, 1955; Webb and Bohn, 1962; Walkey and Pink, 1984; Provvidenti *et al.*, 1980).

The genetics of resistance to CMV and the mechanisms by which the genes confer resistance, are not well understood. In many resistant cucurbits, systemic infection of the plant occurs, but virus concentration in the leaf is reduced and consequently symptoms of infection are less severe (Karchi *et al.*, 1975; Barbara and Wood, 1974; Coutts *et al.*, 1978). There are conflicting reports on the mode of inheritance of this type of resistance in the cucumber (*C. sativus*) cultivar 'Chinese Long'. Earlier reports suggested that the resistance was controlled by three complementary dominant genes and modifiers (Shifriss *et al.*, 1942). However, a later report suggested that the resistance was due to a single dominant gene (Wasuwat and Walker, 1961)). Fraser (1986) concluded that the first report of polygenic resistance resulted from a failure to control environmental conditions during tests to assess the inheritance of resistance, and an attempt to explain genetic and environmental interactions in genetic terms only. Treatment of the leaves of the resistant cucumber 'China' (resistance derived from 'China Long') soon after inoculation, with compounds that block DNA transcription, reduced the effectiveness of the resistance (Barbara and Wood, 1974). This suggested that resistance is an active response by the plant to infection and that an inhibitor of virus multiplication is produced. Similar treatment of cucumber protoplasts did not affect the resistance response, which led Boulton *et al.* (1985) to suggest that plant resistance, in the form of reduced CMV multiplication, was due to both an inherent property of the cell and also a response triggered after the initiation of infection and involving a cell-to-cell interaction.

A problem of sole reliance on plant resistance to control CMV is the great variability of CMV and the potential for rapid evolution of the virus when new host selection pressures arise. Plant resistance involving a single mechanism may be overcome, as is the case for the form of resistance found in lettuce by Provvidenti *et al.*, (1980), which was effective against some but not all of the naturally occurring CMV isolates.

The muskmelon cultivar 'Songwhan Charmi' has been identified by French breeders as an important source of durable resistance and has several mechanisms of resistance. This cultivar is both immune to 65 % of the CMV isolates collected in a survey of naturally infected plants in France and resistant to transmission of CMV by the important vector *A. gossypii* (Lecoq *et al.*, 1979; Leroux *et al.*, 1979). The resistance to transmission by *A. gossypii*, which is controlled by a single dominant gene, appears to be related to the non-preference for this host by *A. gossypii* (Pitrat and Lecoq, 1979). This resistance also prevents transmission of other melon viruses by *A. gossypii*, but is ineffective against transmission of CMV by other aphid species (Lecoq, Labonne and Pitrat, 1980). 'Song' strains of CMV infect 'Songwhan Charmi', however, virus replication is reduced and the plants are less efficient as sources of inoculum compared with the susceptible cultivar 'Cantaloup Charentais' (Lecoq *et al.*, 1979). The rate of progress of epidemics with the 'Song' strains of CMV is therefore slowed (Lecoq and Pitrat, 1983).

(b) Resistance from cross protection.

Plants systemically infected with a mild strain of CMV have an induced resistance, called cross-protection, to a second infection by another strain of CMV (Dodds *et al.*, 1985). Transgenic plants producing the coat protein of CMV have been shown to be resistant to the virus, a phenomenon that supports the role of coat protein in cross protection (Cuozzo *et al.*, 1988). There are potential problems with this form of genetically engineered resistance. *In vitro*, CMV coat protein can non-specifically encapsidate virus RNA, including that from non-aphid transmitted viruses (Chen and Francki, 1990). If this occurs *in vivo*, it may

allow such viruses to become aphid transmissible. In addition, to ensure the success of this form of resistance, expression of coat protein would probably need to be continual and at a high level, and the creation of this nutrient sink may have unfavourable consequences such as reducing the nutritional quality of edible crops (Courtice, 1987).

(c) Resistance from satellite RNA

Tomatoes and capsicums have been protected from severe disease caused by infection with aggressive strains of CMV by pre-inoculating them with a mild strain of CMV containing a satellite RNA that ameliorates disease (Yoshida *et al.*, 1985; Wu *et al.*, 1989; Gallitelli *et al.*, 1991; Montasser *et al.*, 1991). These plants show mild or no symptoms of infection and resistance to more severe disease caused by other CMV strains results mostly from the effects of the satellite RNA and partly from cross protection by the mild strain of CMV. Transgenic plants expressing biologically active satellite RNA also protect the plant from damage by aggressive strains of CMV and unlike transgenic plants with coat protein genes, transcription of satellite RNA is only induced when the plant becomes infected (Harrison *et al.*, 1987). A drawback to the use of this form of protection is that a point mutation to the satellite RNA may transform host response from mild symptoms to lethal necrosis (Sleat and Palukaitis, 1990). Also, the effects of sat RNA on disease caused by CMV are host specific and symptomless infection in one host may be more severe if the CMV and sat RNA is transmitted to another host.

1.6 Scope of the thesis

Past investigations have shown that for organisms with fast reproductive rates, a strong selection pressure will cause the genetic composition of the population to rapidly change so as to overcome the selection pressure. For example, the reliance on chemicals to control insect populations has led to the emergence of insect populations with resistance to the insecticide (Devonshire and Moores, 1982). The philosophy currently used for pest

control is integrated pest management (IPM), in which the objective is to achieve both short and long term control of the pest by exploiting weak links in the pest's life cycle (Maelzer, 1986). A similar strategy where all control options are considered is needed to ensure sustainable control of a plant virus.

To allow an integrated approach to be developed for control, it is necessary to understand the epidemiology of the virus. From the previous discussion on the biology of CMV, it is evident that the severity of a disease epidemic is dependent on the nature of interactions that occur between the virus, plant host, aphid vectors and the environment. Control may be achieved by altering any one of these components of the epidemic.

In making the decision of whether to control the pathogen, and what control method to use, the farmer must balance the cost of control against the value of yield increase obtained by controlling the pathogen (Garrett, 1986). For crops with low yield potential or low market values, it would be expected that only inexpensive control methods could be considered, unless damage from the pathogen was severe. There is no effective means of curing a plant of a virus infection, and control measures therefore need to be prophylactic (Matthews, 1991). A prophylactic control measure, when routinely applied, may be uneconomical in the long term if severe disease epidemics occur only sporadically. An understanding of the epidemiology of the pathogen is therefore important in determining if, when and what types of control measures need to be applied. It may also allow prediction of the magnitude of yield loss caused by the pathogen in any one year.

This thesis describes investigations of the epidemiology of CMV in *L. angustifolius* in South Australia. The following contributions have been made to the knowledge of this topic.

(1) Epidemics of CMV in *L. angustifolius* were experimentally initiated in 1987, 1988 and 1989. Spatial and temporal progression of the epidemics were described.

(2) The role of infected lupins, which were inoculated at the seedling stage or infected through seed, as primary sources of inoculum, was investigated.

(3) The pattern of aphid migration during the lupin growth season was monitored and the common aphid species identified.

(4) Development of the epidemic in relation to aphid flights and colonisation was investigated.

(5) The role of common aphid migrants as field vectors was investigated.

(6) The daily patterns of aphid flights in Spring, 1989, ~~was~~ were monitored, and climatic factors affecting the pattern of flight investigated.

(7) Previously published gradient and vector models were fitted to data obtained from the epidemics in 1987 and 1988. Biological interpretations of the best fitting models were made.

(8) The effect of CMV infection on seed and dry matter productivity was investigated.

(9) Factors affecting rate of seed transmission were investigated.

(10) The importance of aphid and seed transmission for the survival of CMV in *L. angustifolius* is discussed.

Chapter 2

General Materials and Methods

2.1 CMV isolate

A single lesion isolate of B_{SA}, a subgroup 2 strain of CMV (Wahyuni *et al.*, 1991), obtained from the Waite Institute collection, was used in the experiments. This lupin isolate was collected in 1983 from a *Vicia faba* plant growing next to a severely diseased *L. angustifolius* crop in the Coomandook district, South Australia. The single lesion isolate (CMV-B_{SA}) was obtained after 3 successive passages through beet *(Beta vulgaris).*

2.2 Storage of CMV

Systemically infected leaves of *Nicotiana glutinosa* were shredded into fine strips, rapidly dried over silica gel under reduced pressure in a vacuum desiccator at 4°C, and stored over $CaCl_2$ in a kimble tube at 4°C.

2.3 Maintenance of the CMV isolate in the glasshouse

The isolate was maintained in *N. glutinosa.* When used for field trials, or aphid transmission experiments, CMV was freshly recovered from stored dried leaf and mechanically passaged not more than twice before use.

2.4 Inoculation and biological indexing

Virus inoculum was prepared by grinding the plant tissue in a chilled mortar and pestle with tap water (*ca.* 1:5 w/v). To biologically index for CMV infection, inoculum was rubbed onto the leaves of small *Nicotiana clevelandii*, *N. glutinosa* and *Chenopodium*

quinoa plants and symptom development recorded two weeks later. Systemic infection with CMV was confirmed by ELISA.

Patch graft inoculations were done by grafting 0.5-1.0 cm scions from infected stem to the basal part of the stem of the recipient plant.

2.5 Serological testing

A double antibody sandwich (DAS) ELISA (Clark *et al.*, 1986), was used for the detection of CMV in seed and leaf sap extracts. Antiserum, prepared in a rabbit against a seedborne isolate of CMV from South Australia, was obtained from the Waite Institute collection. ELISA buffers, methods for purification of γ globulin, preparation of the alkaline phosphatase-antibody (E-Ab) conjugate and the procedure for the DAS ELISA were as described by Clark and Adams (1977), with the following modifications. Bovine alkaline phosphatase (Sigma) was used; coating antibody (2.5μg/ml) was incubated at 25 C in wells of a microtitre plate (Nunc) for 3 hours; E-Ab conjugate (*ca.* 1 mg/ml) was diluted 1:1000 in PBS-tween 20 with 2% polyvinyl pyrrolidone 40T (Sigma) and 0.2 % bovine serum albumin (Sigma) and was incubated in the wells at 25 C for 4 hours; the enzyme substrate, *p* nitrophenyl phosphate (Sigma), was used at a rate of 1 mg/ml in diethanolamine buffer pH 9.8 and incubated for 20-40 minutes at room temperature. Coating antibody, E-Ab conjugate and enzyme substrate solution was added at 100 μl per well; 200 μl of sample was added to each well. Extent of colour change (absorbance at 405 nm) of the enzyme substrate solution was measured with a Bio-Rad Model 2550 EIA reader.

Leaf and seed tissue were extracted in 10-20 volumes (w/v) of sample buffer, which consisted of 0.4 M trisodium citrate, 2.0 % (w/v) polyvinyl pyrrolidone-40T (Sigma), 0.5 % thioglycollic acid, 5 mM EDTA and 1 % tween 20, adjusted to a pH of 6.5. Samples were crushed in a small plastic bag, with a pestle pressed against the bench. Samples were

stored at -20 C (Ward *et al.*, 1987). Samples were clarified in a micro centrifuge at 12,000 r.p.m. for 1 minute.

The sample buffer used was a modification of the citrate buffer used by Francki and Hatta (1980) for serological detection of CMV. The concentration of trisodium citrate was reduced from 0.5 to 0.4 M to improve solubility of the salt and addition of tween 20 and PVP 40T was shown to reduce non-specific reactions in ELISA. The citrate buffer improved the sensitivity of ELISA to detect CMV in lupin sap when compared with the phosphate based sample buffer used by Clark and Adams (1976), as is shown in Table 2.1. Francki (1964) showed that extraction of CMV from leaves using a phosphate based buffer led to a reduction in titre and this resulted from precipitation of the virus. When the capsid was stabilised by glutaraldehyde fixation, the effect of lupin sap on virus detection was only small, as is shown in Fig. 2.1. This suggests that lupin sap causes disruption of the virus particle and this is followed by sedimentation of the coat protein. The small reduction in titre of fixed virus in leaf sap may be because there had been some particle degradation prior to fixation.

ELISA could detect purified CMV-B_{SA} at concentrations $\geq$ 10 ng/ml. For batch testing, 1 infected seed in 100 or 1 infected leaf in 1000 could be detected.

2.6 Management of field trials

Field trials were conducted at the Charlick Experimental Station of the Waite Institute, Strathalbyn, South Australia. This site is representative of and close to important lupin growing areas of south-east S.A.

Standard management procedures for the cultivation of *L. angustifolius* were used in the field trials and are summarised in Table 2.2.

Table 2.1: Effect of sample buffer on sensitivity of DAS ELISA. (-) is sample buffer containing healthy *L. angustifolius* 'Illyarrie' sap diluted 1:10 and (+) differed only in the inclusion of purified CMV at 0.5 µg/ml. CMV was purified using the method described by Francki *et al.* (1979).

[1] Sample buffer	PBS		0.4 M trisodium citrate		0.4 M trisodium citrate + 5 mM EDTA + 0.5 % thioglycollic acid	
	(-)	(+)	(-)	(+)	(-)	(+)
ELISA reading Abs. @ 405 nm.	0.001 ± 0.006	0.130 ± 0.009	- 0.006 ± 0.003	0.703 ± 0.055	0.005 ± 0.004	1.124 ± 0.024

[1] In addition to the components listed, all sample buffers contained 1.0 % tween 20 and 2.0 % polyvinyl pyrrolidone 40 T.

2.7 Seed source

The seed of *L. angustifolius* 'Illyarrie', used in the 1987 field trial, was obtained from Mr. D. Klitscher, Coonalpyn, South Australia.

The seed of *L. angustifolius* 'Illyarrie', used in the 1988 field trial and the 1989 seed transmission experiment, was obtained from Dr. R.A.C. Jones, West Australian Department of Agriculture.

The seed of *L. angustifolius* 'Warrah', used in the 1989 field trial, was obtained from Mr. D. Schinkler.

Fig. 2.1: The effect of lupin sap on detection of native (▩) and fixed (■) CMV by DAS ELISA.

CMV was purified as described by Francki *et al.* (1979) and the capsid structure stabilised by fixation with 0.2 % glutaraldehyde (Rao *et al.* 1982). The ELISA sample buffer used consisted of PBS, with 1% tween-20 and 2.0 % polyvinyl pyrrolidone 40T. All treatments had a final concentration of 0.5 µg/ml CMV-BSA. *L. angustifolius* 'Illyarrie' sap was added to a final dilution of 1:10 for treatment 2, 1:100 for treatment 3 and 1:1000 for treatment 4.

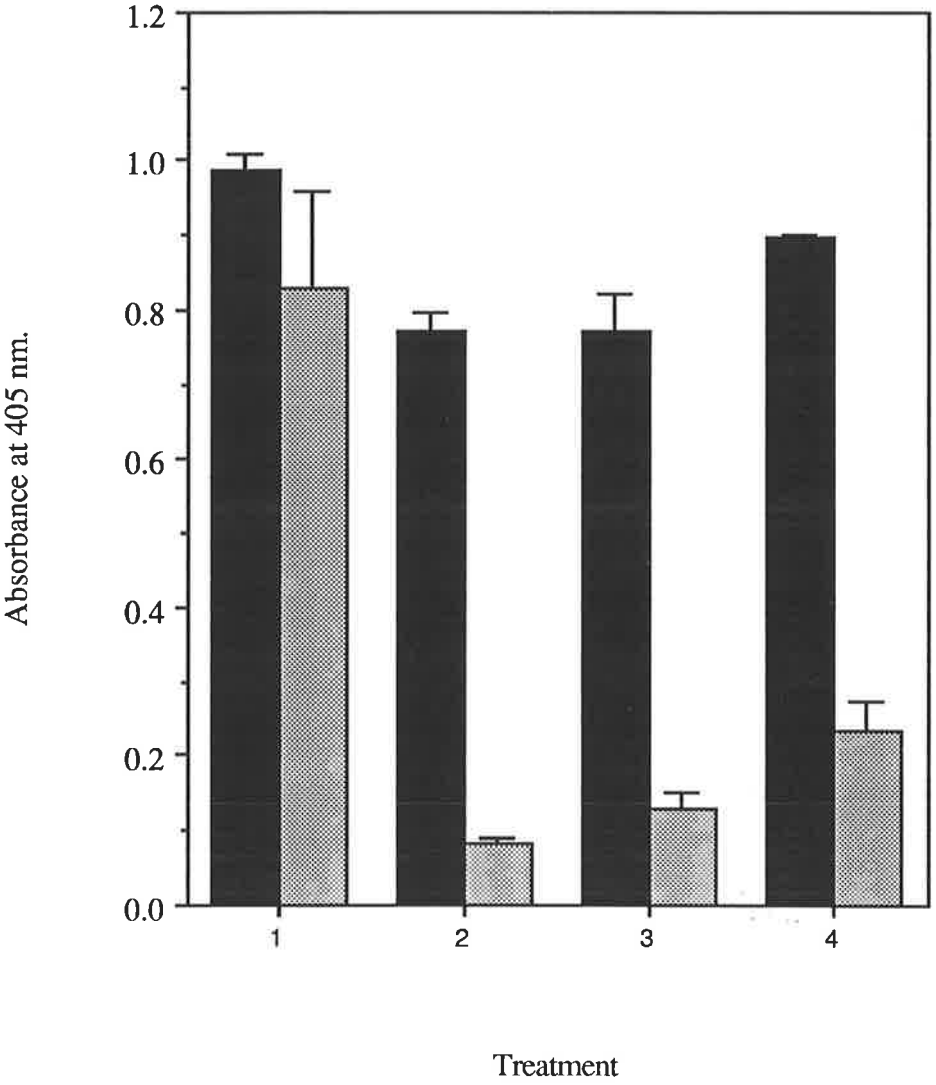

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance at 405 nm.
1
2
3
4
Treatment

Table 2.2: Description of the management of the field trials and comparison of this with the general recommendations for lupins in South Australia.

General recommendations were extracted from Hawthorne and Mowatt (1986), except [1] herbicide information which was extracted from Swarbrick (1984).

	General recommendations	Field trials
Soil type	Well drained soil, pH 4.5-7.5. Soil types include sands, deep sandy loams, sands over clays and well structured loams.	Transitional solonised brown soil-red brown earth. pH 6.5-6.9.
Sowing depth	No greater than 3 cm.	As recommended.
Nodulation	Inoculate seed with Rhizobium Group G using 1.5 % methyl cellulose as an adhesive.	Field sown seeds were coated with Rhizobium Group G (Nitrogerm), as recommended. Plants that were transplanted into the field were inoculated by pouring a water slurry of the peat culture of *Rhizobium* into the pot, prior to transplanting.
Time of sowing	Mid-May in the Mid and Lower South East. Mid-May on deep sands in the Upper South East. By the end of May on shallow sand over clay soils in the Upper South East. Before the end of May in all other districts.	The 1987 field trial was sown on May 7. The 1988 field trial was sown on May 30. The 1989 field trial was sown on May 17. The 1989 seed transmission experiment was sown on May 19.
Sowing rate	75-100 kg/ha (75% germinable seed).	In 1987 and 1988, seeds were sown in a grid pattern with 50 cm spacing. In 1989, seeds were sown 5 cm. apart in rows with 25 cm. spacing.
Plant protection	Dependent on the weed or pest problem. Weed control can be achieved by use of cultivation and/or chemicals. [1] Recommended herbicides include: Seedbed/knockdown herbicide - paraquat/diquat mixture. Pre-emergence residual herbicides - simazine, metribuzin.	In every field experiment, Lupazine was used. In 1988, Sprayseed was used in combination with the Lupazine. Le-mat was used as needed to control lucerne flea and red-legged earth mite. The interplot areas were regularly cultivated.

how does this relate to recommended rate?

2.8 Plant protection

The source, active constituents and application details for the chemicals used for plant protection in the field trials are provided in Table 2.3.

Table 2.3: List of herbicides and pesticides used in field and glasshouse experiments

Brand name	Manufacturer	Active constituent(s)	Application rate of product
Disyston 5	Bayer	50 g/kg disulfoton	70 g/10 m^2
Lemat	Bayer	580 g/l omethoate	50 ml/ha
Lupazine	Incitec	500 g/l simazine	2 l/ha
Perfekthion EC400	BASF	400 g/l dimethoate	1:1000 dilution applied with a hand sprayer
Pirimor	ICI	500 g/kg pirimicarb	8 g dissolved in 15 l applied with a hand sprayer
Rovral	May & Baker	500 g/kg Iprodione	250 g/100kg of seed
Sprayseed	ICI	125 g/l paraquat dichloride & 75 g/l diquat dibromide monohydrate	2 l/ha

2.9 Introduction of infected lupin seedlings into field plots

Lupin seed, precoated with the fungicide Rovral, was germinated in the glasshouse and newly emerged seedlings transplanted into 5 inch diameter polythene pots. The seedlings were mechanically inoculated 7-8 days after sowing, then transferred outside to allow acclimatisation for at least 2 weeks. Seedlings which were systemically infected with CMV (as confirmed by ELISA 2-3 weeks after inoculation), were transplanted into the field plots to provide sources of inoculum.

Bird damage to transplanted seedlings was severe in 1987. Thus, in 1988 and 1989, the source seedlings were protected by covering with bird netting (Sarlon Antibird Net; 4 x 4 m. diamond mesh). Black netting was chosen to minimise possible effects of colour change on detection of source plants by migrant aphids.

Chapter 3

Description of the CMV epidemic in *L. angustifolius*

3.1 Introduction

Two alternative approaches to epidemiological studies involve either extensive surveying of a number of commercial crops in a farming district (Haack, 1986; Alberts *et al.*, 1985; Rist and Lorbeer, 1991), or more limited studies of specifically planted experimental plots or single farming blocks (Jayasena and Randles, 1984, 1985; Madden *et al.*, 1987). Apart from the favourable logistics of small scale field trials and the ability to undertake intensive monitoring of the components of the epidemic, there are other advantages to this approach. The specific placement of disease foci allows spatial progression of the pathogen to be accurately ascertained (Jayasena and Randles, 1984; Madden, 1990). At sites where the virus naturally occurs, the use of distinct and easily recognisable virus strains, such as the vein banding strain of BYMV used by Jayasena and Randles (1984), may provide the only means of accurately monitoring the dispersal of the virus from a disease focus. Specifically designed and replicated experiments also allow comparisons of treatments in which components of the epidemic are altered and potential control measures evaluated (Jayasena and Randles, 1985; Gray *et al.*, 1986). Thresh (1985) does warn that when compared with the commercial situation, artifacts may arise from specifically designed experiments owing to "atypical size, disposition or management" of these trials. Studying selected transects or quadrats of commercial plantings would avoid these problems. The approach of using specifically designed experimental plots was chosen to investigate the epidemiology of CMV in lupins.

The overall objectives of the field trials described in this chapter were to describe the spatial and temporal progress of the CMV epidemic in *L. angustifolius*, to determine the

importance of infected lupin seedlings as primary sources of inoculum and to identify the most important aphid vector species.

3.2 Materials and Methods

3.2.1 The 1987 field trial

A field trial was conducted in 1987 with the objective of initiating a CMV epidemic in *L. angustifolius* to follow development of the epidemic. The trial compared the effects of introducing virus alone, and virus together with colonies of the aphid vector, *Aphis craccivora.*

3.2.1.1 Design

A latin square design was used to test 3 treatments which were replicated 3 times. For each treatment, a plot of 20 x 20 rows of *L. angustifolius* 'Illyarrie' was sown on May 7. For treatments VV (virus source + vector) and V (virus source only), 9 infected lupin seedlings were transplanted into the centre of the plot in an arrangement of 3 x 3 plants (forming a square of 0.5 m^2). No infected seedlings were introduced to treatment C (control). For treatment VV, *A. craccivora* were introduced onto the transplanted infected seedlings. Plots were separated from each other by 15 metres of bare fallow.

3.2.1.2 Establishment of colonies of *A. craccivora* in treatment VV.

Colonies of *A. craccivora* were raised on *L. angustifolius* 'Illyarrie' in aphid cages located outside at the Waite Institute. On May 29, five apterae were transferred onto each of the introduced infected lupin seedlings in treatment VV (a total of 45 per plot). Mesh cages (1 x 1 x 0.6m) were placed over these aphids for 5 days to provide protection from natural predators during establishment of colonies.

3.2.1.3 Surveying for virus infection

To determine the initial levels of plant infection, all plants in the trial were sampled on June 10 for serological testing. To monitor increase in incidence of plant infection, all plants were surveyed for disease symptoms every 2 weeks. Initially, plants with symptoms were also sampled for serological testing. Plants with symptoms of stunting, leaf epinasty and distortion were always found to be infected with CMV. After September 16, symptoms alone were used for diagnosis of plant infection. Symptoms, when doubtful, were confirmed by observation 2 weeks later.

To determine the time delay between initial detection of systemic infection by ELISA and the first appearance of symptoms, plants in 5 rows in each of the control plots were routinely sampled for serological testing in conjunction with the surveys. Incidence of virus infection was obtained from this survey.

3.2.2 The 1988 field trial

A field trial was conducted in 1988 with the following objectives:

(a) To investigate the role of seedling-infected lupins as primary sources of inoculum and to describe patterns of infected plants derived from these infection foci.

(b) To test the effect of introducing a reservoir of the cereal aphid *Rhopalosiphum padi* next to the inoculum source on the progress of the epidemic.

3.2.2.1 Design

A randomised complete block design was used to test four treatments, which were arranged randomly in each of three blocks. The experimental design is illustrated in Fig.

3.1. For each treatment, a plot of 10 x 12 rows of *L. angustifolius* 'Illyarrie' was sown on May 30. For treatments V (virus source only), VO (virus source and oats) and VOA (virus source and oats with aphid colonisation), lines of 10 infected lupin seedlings were transplanted along two opposite sides of the plot on June 30. For treatment C (control), there was no introduced source of inoculum. With treatments VOA and VO, a strip of oats was grown outside the line of infected lupins. For treatment VOA, *R. padi* were introduced onto the oats. For treatment VO, aphid colonisation of the oats was prevented by regular insecticide treatment. Neighbouring treatments were separated by 4 border rows and there were 2 border rows at each end of each of the blocks.

3.2.2.2 Establishment of the oats.

Oats (*Avena sativa* 'N.Z. Cape') were sown on May 4 in three rows, spaced 25 cm. apart. The oats were sown 26 days prior to the lupins, to allow development of a sward on which aphid colonisation could occur at a time early in the growth of the lupins. The row nearest the line of infected lupins was removed on August 4, to prevent shading of the lupins.

3.2.2.3 Initiation of colonies of *R. padi* on the oats in treatment VOA.

Colonies of *R. padi* were raised on 'NZ Cape' oats in aphid cages located outside at the Waite Institute. Oats seedlings, with 10-20 aphids, were placed on the oats in treatment VOA on June 1 and the aphids were allowed to move at will as the seedling wilted. Four colonies, evenly spaced along the oats strip, were established. Initially the young colonies were protected by single plant cages (plastic tubes), which were removed after 2 weeks.

3.2.2.4 Insecticide treatment of the oats in treatment VO.

Fig. 3.1: Design of the 1988 field trial.

Treatment V: rows of infected lupins were planted along two edges of the plot.

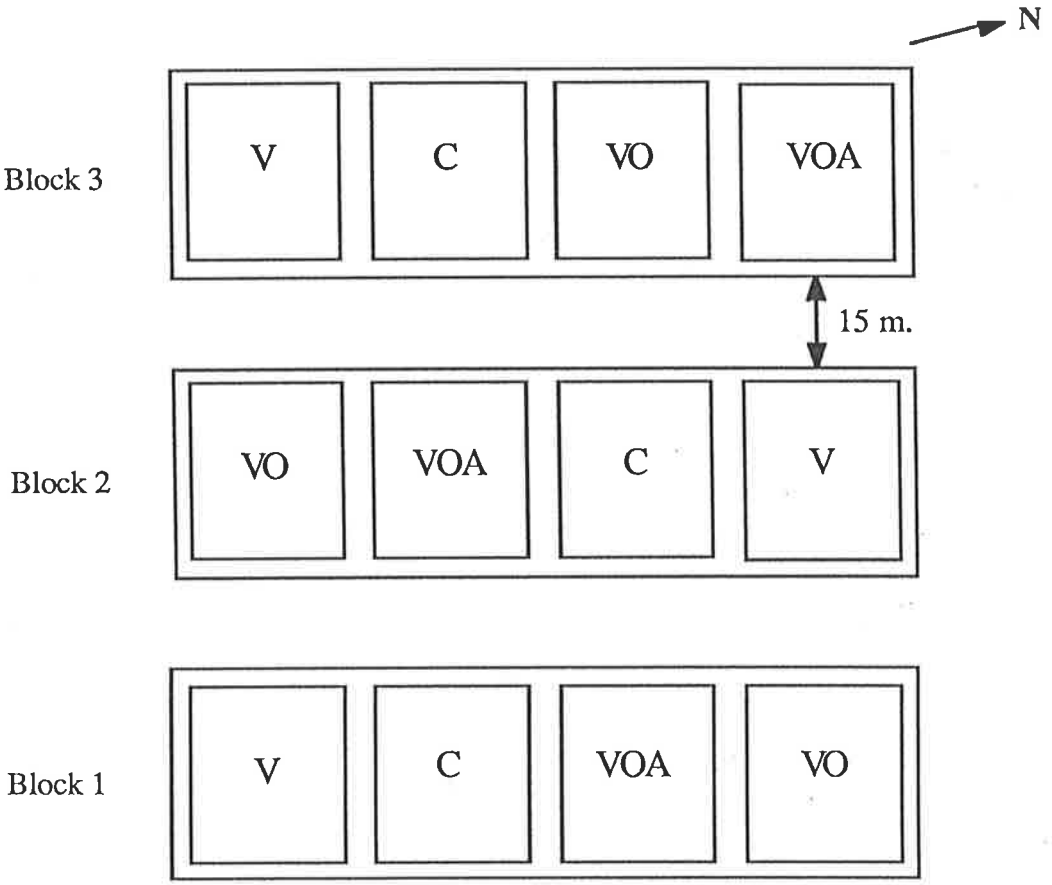
Treatment VO: as with treatment V, but 3 rows of oats were planted outside the lines of infected lupins.

Treatment VOA: as with treatment VO, but *Rhopalosiphum padi* were released onto the oats.

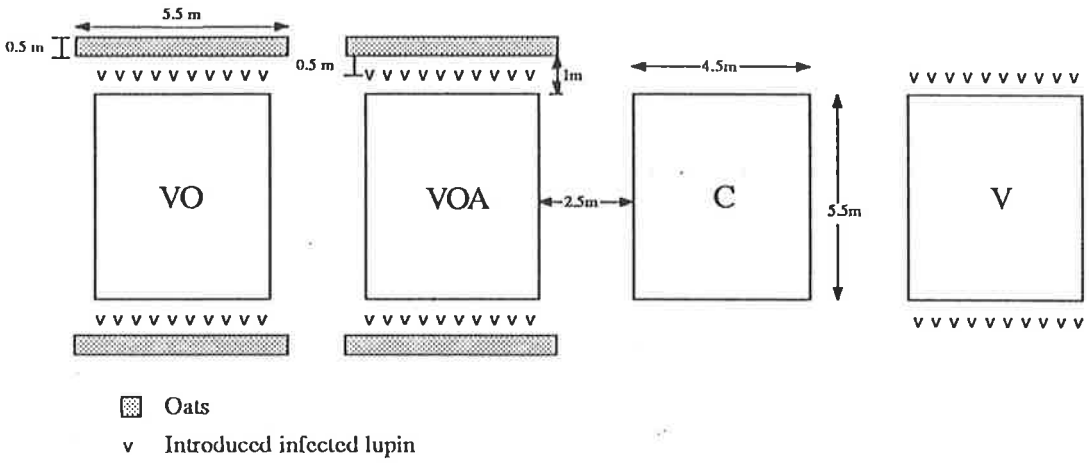
Treatment C: no introduced sources of virus inoculum or aphids.

Positions of infected lupins and oats are shown in the detailed map of block 2.

Layout of the plots



Detailed map of block 2



To control aphid colonisation, a band of Disyston 5 was placed 2 cm. below the oat seeds at sowing, and on June 28, July 27, August 17 and the September 8, the sward of oats was sprayed with Perfekthion.

3.2.2.5 Sampling of the oats for aphids.

To count aphid numbers, tillers were randomly selected, clipped at the crown and collected in paper bags for aphid counting (see section 4.2.5) on June 13, August 3, August 25 and September 14.

3.2.2.6 Surveying for virus infection

To estimate the incidence of infection, plants in every second column (rows perpendicular to the line source of inoculum) were sampled every 2 weeks and tested by ELISA. On September 7, all plants were tested by ELISA.

3.2.3 The 1989 field trial

A field trial was conducted in 1989 with the following objectives:

(a) To investigate a CMV epidemic in a plot with management closely resembling a commercial cropping situation.

(b) To compare trapping methods using yellow pan, green tile and suction traps and to correlate epidemic progress with aphid landing rates.

(c) To use trap plants to determine infection pressure.

3.2.3.1 Design

The field trial, with a design as illustrated in Fig. 3.2, was sown on May 18 with *L. angustifolius* 'Warrah'. Prior to sowing, a sample of 500 seeds was tested and shown to be free of detectable CMV. Seeds were sown 5 cm. apart in furrows spaced 25 cm. apart. Lupin seedlings inoculated at the cotyledon stage were transplanted into the plot on June 6. These infected seedlings were transplanted in groups of four in the positions shown in Fig. 3.2.

3.2.3.2 Surveying for virus infection

To estimate the incidence of infection, single plants were sampled every 2 weeks at 1 m. intervals along the same four rows. These rows ran lengthwise through each of the introduced infection foci. All samples were tested by ELISA.

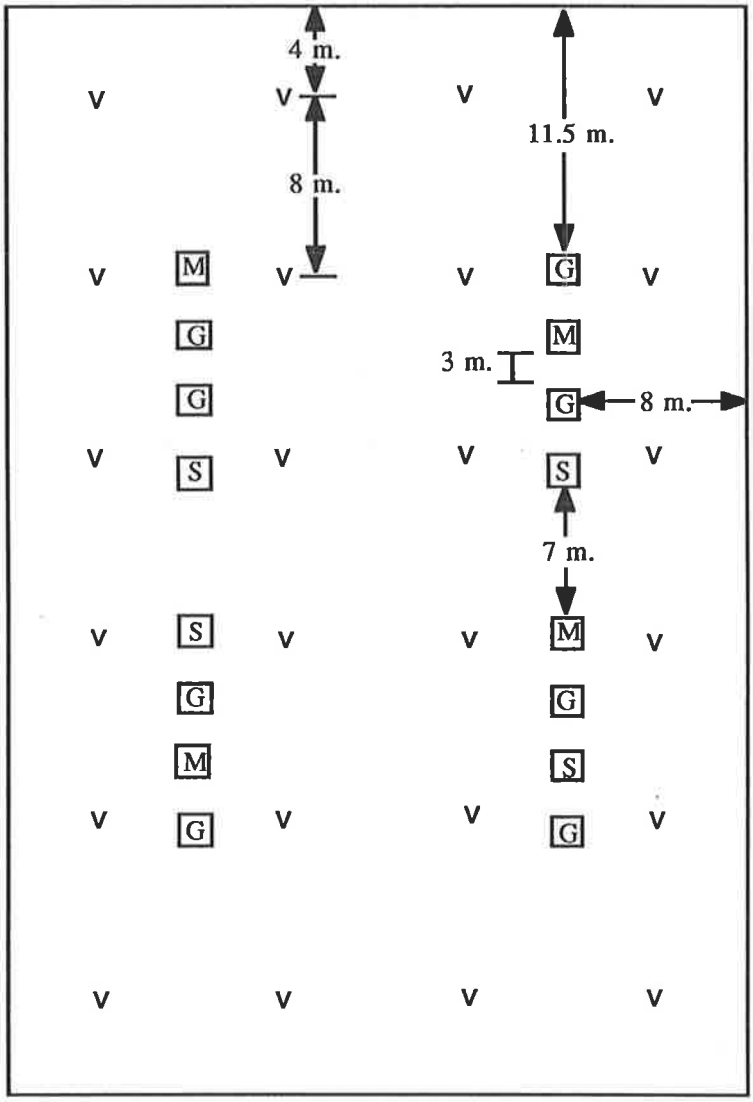
3.2.3.3 Use of trap plants

To assess infection pressure and to determine rates of infection by direct measurement, 6 pots of trap plants were arranged in a circle of 1 m. radius around each introduced infection focus. Each pot contained 3 fourteen day old *L. angustifolius* 'Warrah' seedlings. The trap plants were raised to the height of the canopy by suspending the pots in metal hoops, which could be moved up and down a wooden rod. Trap plants were exposed for 2 weeks and then transported back to the glasshouse where they were sprayed with the insecticide Pirimor, and grown on for at least 3 more weeks. Plants were then tested serologically for CMV.

3.2.3.4 Analysis of the spatial pattern of infected plants

To assess the importance of the introduced infected lupins as primary sources of inoculum, the number of plants with symptoms of CMV infection in a circle of 1.0 m. radius (3.14 m^2) around each group of four infected plants, was counted on September 26. These

Fig. 3.2: Design of the 1989 field trial.



S Suction trap.

M Horizontal mosaic green pan trap (Cambridge 815 ceramic tile)

G Horizontal green pan trap (VIP Verde tile)

V Group of four infected lupins transplanted into the plot

levels were compared with the number of infected plants in subplots also of 3 m^2 in area, which were located midway between the introduced primary sources of inoculum.

To produce a map of the distribution of infected plants, plants with symptoms of CMV infection were tagged with pieces of brown adhesive tap and the survey dates recorded on these tags with waterproof pen. The positions of single plants or clumps of infected plants were also marked with wooden pegs. At crop maturity, in December, the plot was divided into 8 x 8 metre sections and the relative positions of infected plants in each section recorded on graph paper.

To quantify the degree of aggregation of infected plants, Lloyds patchiness index was calculated (Lloyd, 1967; Pielou, 1974; Madden *et al.*, 1987). Lloyds patchiness index is the ratio of mean crowding (m^*) to density (m) of infected plants i.e. m^*/m. m is the mean number of infected plants per quadrat and V is the variance of m; m^* is the mean number of additional infected plants found in the same quadrat as each infected plant and is given by $m + (V/m - 1)$) (Madden *et al.*, 1987).

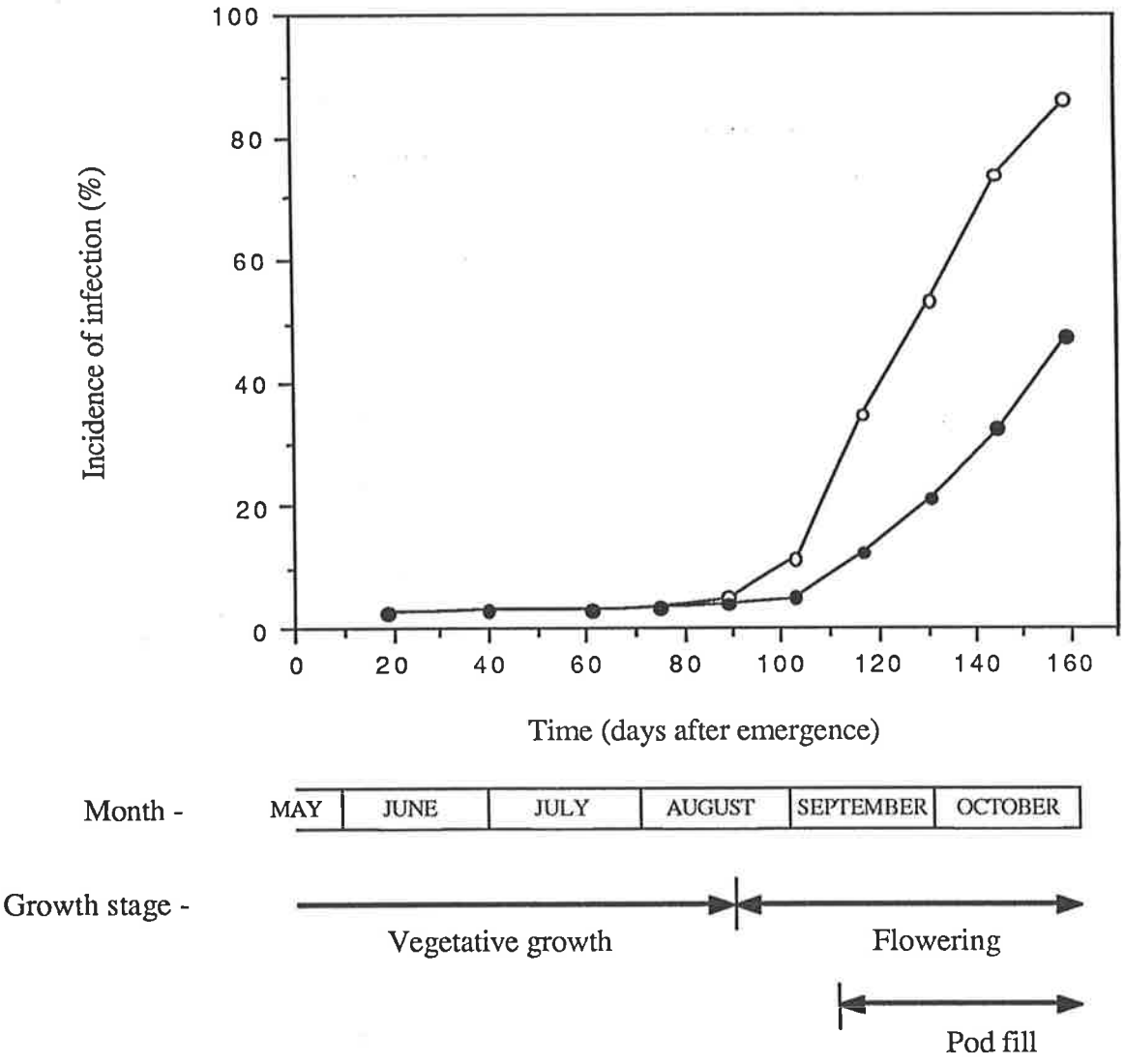
3.3 Results

3.3.1 Comparison of diagnosis by ELISA and by symptoms

Plants with symptoms of stunting, leaf epinasty and distortion of the leaflets, were found, by ELISA, to be infected with CMV. The time period between detection of CMV infection by ELISA and development of symptoms, shown in Table 3.1, was variable, but symptoms were most frequently seen 2 to 4 weeks after CMV infection was detected by ELISA. Some plants that became infected after the commencement of flowering, remained symptomless. Progress of the epidemic, monitored by either symptom appearance or ELISA, is illustrated in Fig. 3.3. The curve of increase in incidence of infection was delayed when symptoms were used for diagnosis, due to the time lag between development

Fig. 3.3: Temporal progress of the 1987 epidemic in treatment C.

Diagnosis of infection was by both ELISA (○) and symptoms (●). Incidence is the mean of 3 replicates. 5 rows were surveyed to determine incidence of infection in each replicate.


Incidence of infection (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Time (days after emergence)
Month -
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
Growth stage -
Vegetative growth
Flowering
Pod fill

of systemic infection and symptom appearance. Incidence of infection, based on symptoms, was underestimated at crop maturity in 1987 due to the occurrence of asymptomatic plant infections.

Table 3.1: Frequency distribution of the time intervals between detection of CMV by ELISA and the first recognition of symptoms of infection.

[1] Date	Time (weeks) 0	2	4	6	Number without symptoms
Aug. 20	0	2	2	0	0
Sept. 3	0	5	10	3	0
Sept. 17	5	8	11	5	7
Oct. 1	0	3	5	0	8
Total	5	18	28	8	15

[1] Refers to the survey date when CMV infection was first detected by ELISA.

3.3.2 Analysis of the temporal development of the epidemics

3.3.2.1 Epidemic development in relation to crop growth

The temporal development of the epidemics in 1987 and 1988, in relation to crop growth, are shown in Figs. 3.3 and 3.5. In both these years, the first period of rapid increase in incidence of plant infection in mid to late August coincided with the commencement of flowering. Further spread of CMV continued during the crop growth stage of pod filling. These observations of continued development of the epidemic during

the reproductive stages of the crop would indicate a long period of plant susceptibility to CMV infection.

3.3.2.2 Effects of treatments VV and V on development of the 1987 epidemic

The increase in incidence of diseased plants with time in treatments C, V and VV, is shown in Fig. 3.4. No significant difference (P=0.193) was found between the treatments and it was therefore concluded that the introduction of the infected lupins, with or without colonies of *A craccivora*, had no effect on the development of the epidemic.

Birds caused a high rate of mortality in the introduced infected lupins. In one replicate of treatment V and in 2 replicates of treatment VV, all the introduced infected seedlings were nipped below the cotyledons within 2 weeks of their introduction into the plots. The incidence of infection in the control plots, recorded on June 10, was 2.33 %. These infected seedlings were randomly distributed in the plots and are considered to have arisen from seed transmission. Both the high mortality of the introduced infected seedlings and the occurrence of seed transmission would be expected to mask any effects of the treatments.

3.3.2.3 Effect of treatments VOA, VO and V on development of the 1988 epidemic

Table 3.2 shows the numbers of aphids collected from the oats in treatment VO and VOA. Colonies of *R. padi* became established in treatment VOA, whereas in treatment VO, aphid colonisation was successfully prevented by the regular application of insecticides. The total numbers of alates collected from the oats in treatments VO and VOA were 12 and 14 respectively. This suggests that the alates collected in treatment VOA were not produced by the colonies on the oats, but were immigrants. Large numbers of *Metapolophium dirhodum* were also collected, a result of natural colonisation of the oats by this aphid.

Fig 3.4: Comparisons of the temporal progress of the 1987 epidemic for treatments C (○), V (●) and VV (Δ).

Infection was diagnosed by symptoms. Incidence is the mean of 3 replicates. All plants in each replicate were surveyed to determine incidence of disease.

Treatment V: a group of 9 infected lupins was introduced to the centre of the plot.

Treatment VV: as with treatment V, but *Aphis craccivora* were released onto the infected lupins.

Treatment C: no introduced sources of virus inoculum or aphids.

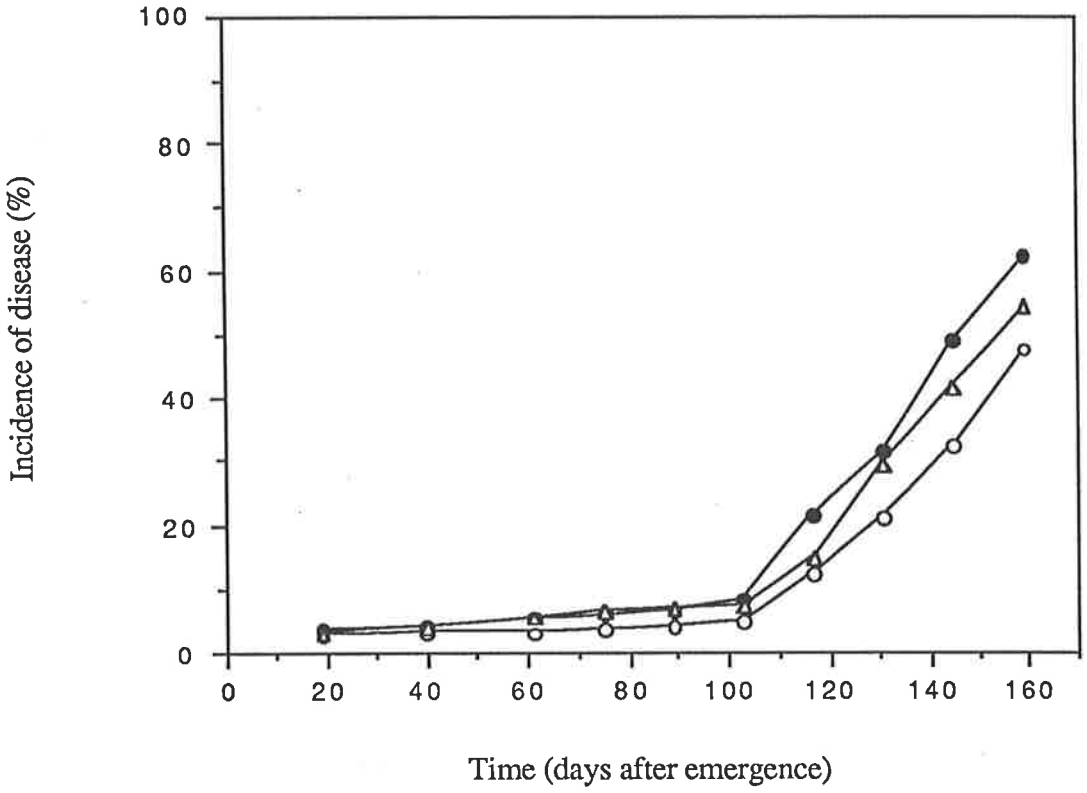

Incidence of disease (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Time (days after emergence)

Table 3.2: Aphids collected from oats in treatments VOA and VO.

Total aphid numbers on 180 randomly sampled tillers (30 from each oats strip) collected on July 13 and August 3, and 120 randomly sampled tillers (20 from each oats strip) collected on August 25 and September 14, are shown.

Refer to Fig. 3.1 for descriptions of the treatments.

		[1] Number of aphids collected											
		Rhopalosiphum padi				*Metapolophium dirhodum*				Other species			
		VOA		VO		VOA		VO		VOA		VO	
Date	Block	Apterae	Alatae	Apterae	Alatae	Apterae	Alatae	Apterae	Alatae	Apterae	Alatae	Apterae	Alatae
Jul.	1	17	1	4	1	2	0	0	0	0	0	0	0
13	2	7	1	0	0	0	1	0	0	0	0	0	0
	3	17	2	0	0	0	0	0	0	0	0	0	0
Aug.	1	29	0	2	4	28	0	0	1	0	0	0	0
3	2	56	2	1	1	56(2)	0	1	1	0	0	0	0
	3	36(2)	2	0	0	53(1)	0	0	0	0	0	0	0
Aug.	1	3	1	1	0	137(16)	7	2	2	0	0	0	1
25	2	10	0	0	1	76(13)	3	6	1	0	0	0	0
	3	9	1	0	3	76(5)	3	4	3	0	0	0	1
Sep.	1	0	4	0	0	187(29)	4	0	2	0	1	0	0
14	2	3(1)	0	0	1	194(26)	7	4	4	0	0	0	0
	3	6	0	0	1	40(7)	4	10	4	0	0	0	0

[1] The figure in brackets refers to the proportion of aphids collected that were alatoid nymphs.

Fig. 3.5 illustrates the temporal development of the epidemic in 1988. The progress of the epidemic was rapid in the 4 week period between August 24 and September 21, with the incidence of infection in treatments VOA, VO and V increasing from less than 5% to greater than 90%. On September 7, there were no significant differences (P=0.495) in incidence of infection between treatments VOA, VO and V. Incidence of infection in treatment C was, however, significantly different (P<0.001) from that found in the other three treatments. Therefore, it is concluded that the only treatment variable which had an effect on the incidence of infection was the presence or absence of introduced sources of inoculum. The onset of the epidemic in the treatment C was delayed because of the absence of introduced sources of inoculum.

3.3.2.4 Epidemic development in the 1989 trial and the use of trap plants to measure infection pressure

The epidemic in 1989 was minor compared to the epidemics in 1987 and 1988. Average plant density was 23.6 $\pm$ 2.0 plants/m^2 and the estimated number of plants in the plot was 36250. If each of the clusters of 4 introduced infected plants is considered a single infection focus, then the incidence of plant infection at the beginning of the epidemic was 0.07 % (24/36250 x 100). Using symptoms for diagnosis, incidence of plant infection on October 24 was 0.76 %. The incidence of infection on October 31, determined by serological testing of a sample of 172 plants, was 4.7 %.

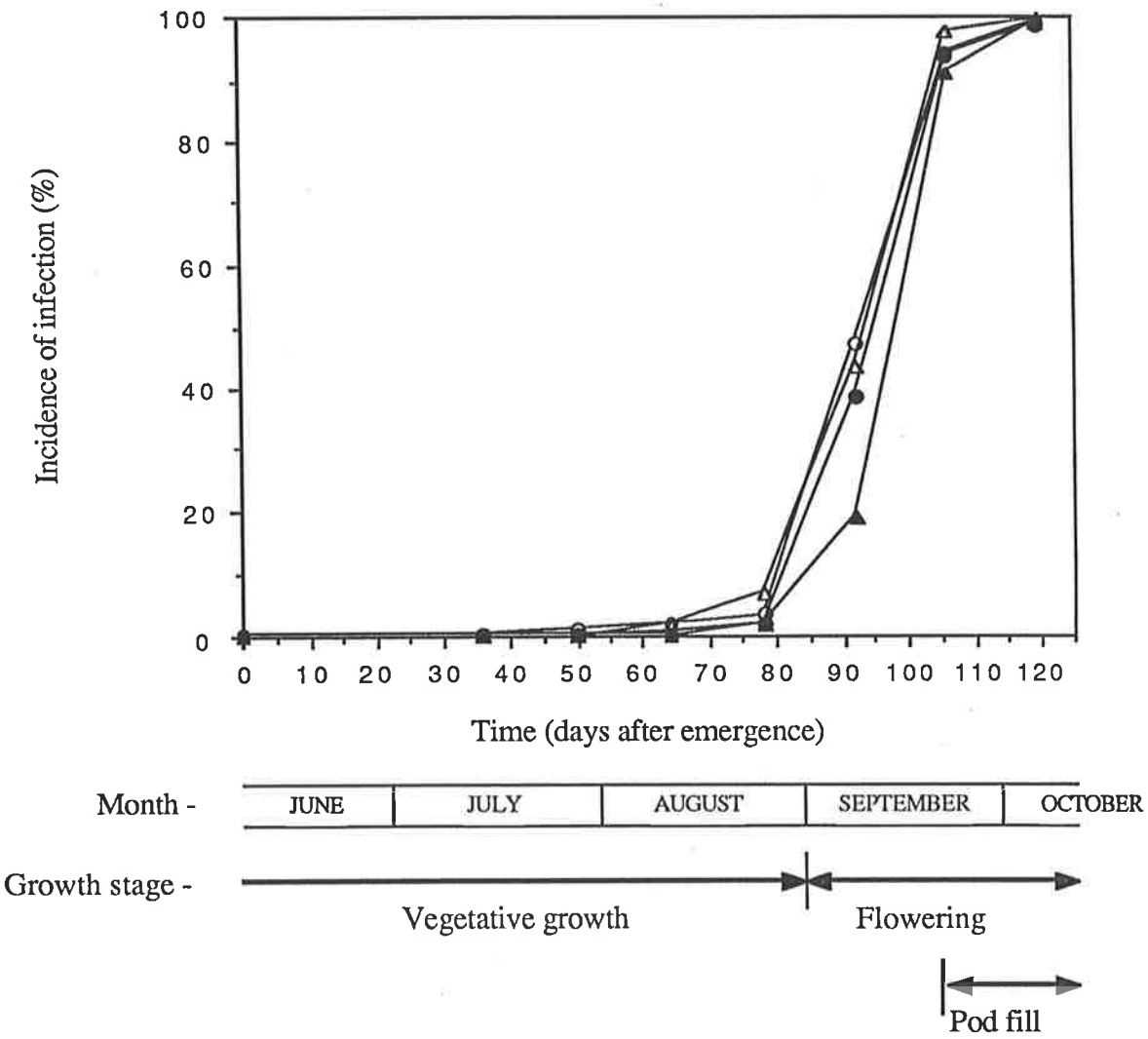
The trap plants were ineffective in measuring infection pressure. Only on two occasions were trap plants infected and the highest number infected in a two week period was 2 out of a total of 432 plants. It is considered that the number of of trap plants was too small to accurately estimate the infection pressure in this epidemic.

3.3.3 Analysis of the spatial development of the epidemics

Fig. 3.5: Comparisons of the temporal progress of the 1988 epidemic for treatments VOA (○), VO (●), V (Δ) and C (▲).

Infection was diagnosed by ELISA. Incidence is the mean of 3 replicates. 5 columns (rows perpendicular to the linear source of inoculum) were surveyed to determine incidence of infection in each replicate.

Refer to Fig. 3.1 for descriptions of the treatments.


Incidence of infection (%)
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Time (days after emergence)
Month -
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
Growth stage -
Vegetative growth
Flowering
Pod fill

3.3.3.1 Analysis of the spatial distribution of infected plants in treatment C of the 1987 field trial

The spatial pattern of diseased plants in treatment C of the 1987 field trial is shown in Fig. 3.6. Incidence data were categorised according to the time of appearance of symptoms and to the times when aphid flights occurred (see section 4.3.1). Time categories used were: June 10 - first survey date; June 10 to August 20 - the winter period when few aphids were trapped and little virus spread occurred; August 20 to September 9 - the period covering the first aphid flight; and September 9 to October 29 - the remaining period when aphid flights occurred. The sizes of the clumps of diseased plants at these times are given in Table 3.3. Initially, most infected plants occurred singly. On October 29, following the aphid flights, 59.4 % of the infected plants were in clumps of size greater than or equal to 6 plants.

Table 3.3: Frequency distribution of the size of clumps of adjacent diseased plants in treatment C of the 1987 field trial.

	[1] Size of the clump													
Time period	1	2	3	4	5	6	7	8	9	10	11	12	>12	[2] Total
Jun. 10	20	2												24
Jun. 10 - Aug. 20	34	2	1											41
Aug. 20 - Sept. 9	25	14	7	4	1				1		1	1		127
Sept. 9 - Oct. 29	28	10	6	4		1	2	1					5	202

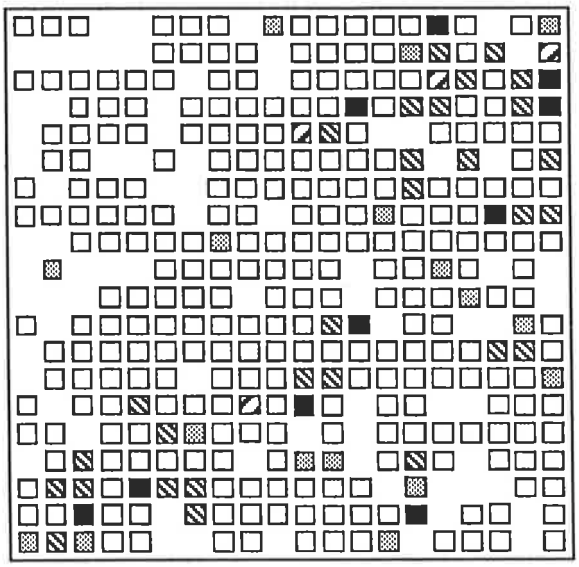
[1] Diseased plants were considered as part of a clump if they were immediately or diagonally adjacent to another infected plant.

[2] Total number of plants with symptoms.

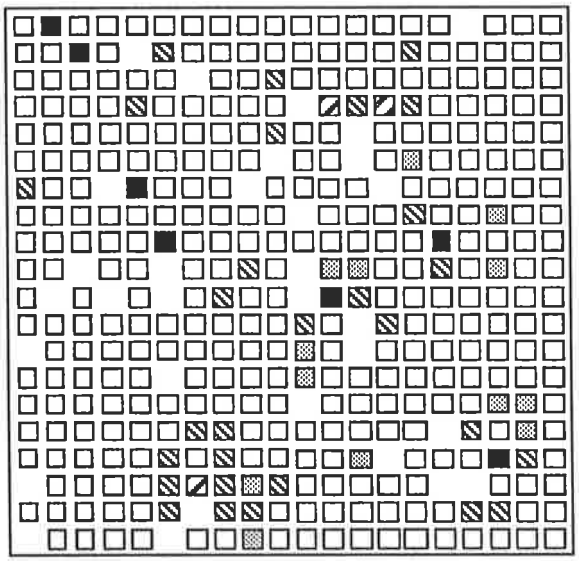
Fig. 3.6: Pattern of infected plants in treatment C of the 1987 field trial.

Diagnosis of infection was by ELISA on June 10, and by symptoms in all other surveys. (□) are healthy plants; (■) are plants which were infected on June 10; (◪) are plants which became diseased in the period from June 10 to August 19; (▧) are plants which became diseased in the period from August 20 to September 9; (▩) are plants which became diseased in the period from September 9 to October 28; a missing square is where a plant failed to establish.

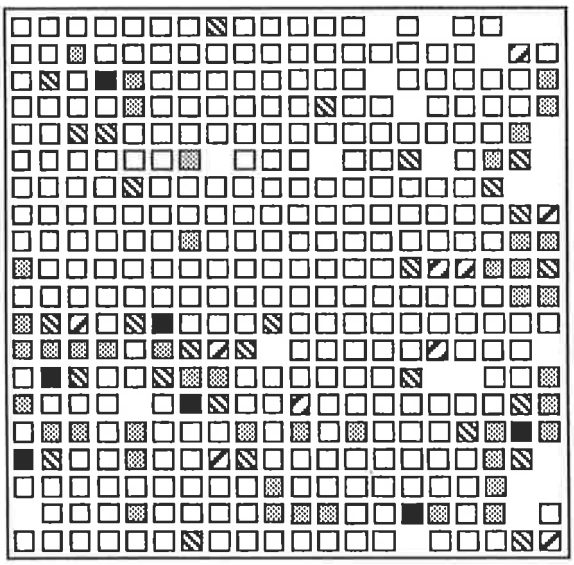
Replicate 1



Replicate 2



Replicate 3



3.3.3.2 Analysis of the spatial distribution of infected plants in the 1988 field trial

(a) Tests for infection gradients arising from the linear source of inoculum

The distribution of infected plants in the 1988 field trial on September 7 is shown in Fig. 3.7. When the incidence of infection in rows at increasing distances from the linear source of inoculum was plotted, as is shown in Fig. 3.8, there was a gradient of decreasing infection with increasing distance from the source. For treatments VOA, VO and V, the apparent gradients were real as the differences in incidence of infection between rows of varying distance from the focus were significant ($P<0.001$). No significant differences ($P=626$) were found between the treatments in incidence of infection at each given distance and therefore the shape and height of infection gradients in treatment VOA, VO and V were not different.

(b) Tests for differences between the infection gradients on each side of the plot

Treatments VOA, VO and V had linear sources of inoculum on both outside borders from both of which infection gradients developed. The incidence of infection was significantly different ($P<0.001$) between the north-west and south-east half-plots, with a higher incidence being found in the north-west half-plots (columns 7-12 in Fig. 3.5). The shapes of the gradients in opposing half-plots were not significantly different ($P=0.538$), indicating that direction had no effect on the steepness of the gradient.

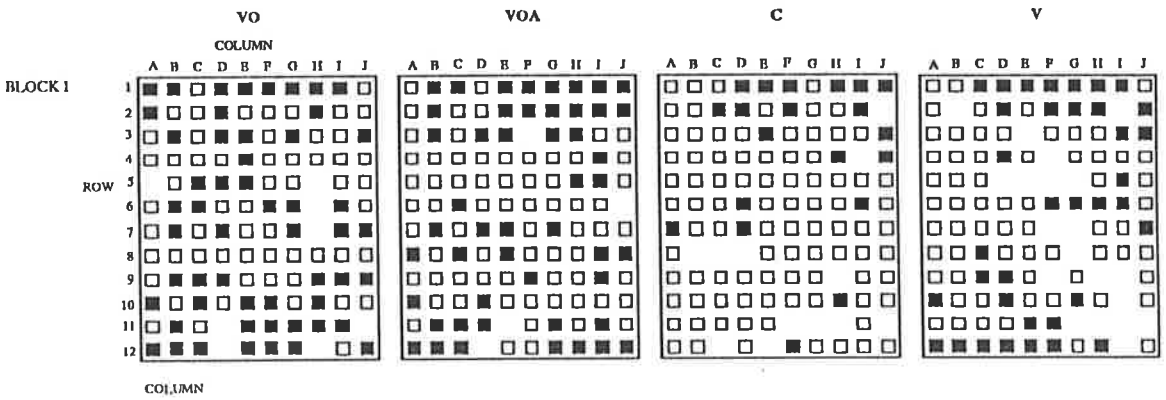
(c) Test for infection gradients arising from cross spread between treatments

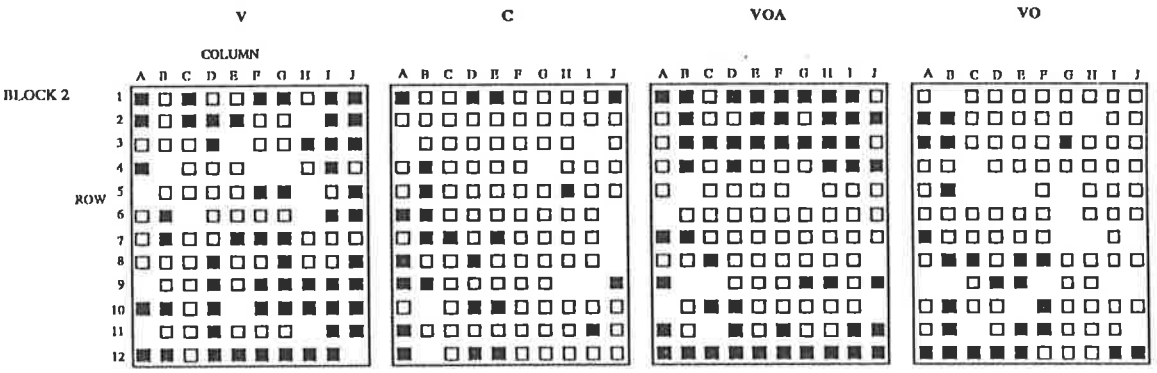
The incidence of infection in treatment C on September 7 was 19.8 %, which indicates that cross-spread had occurred between the treatments and/or from sources outside the field trial. To test for infection gradients resulting from lateral spread between neighbouring plots, incidence of infection in columns of plants (rows running perpendicular

Fig. 3.7: The pattern of infected plants in the 1988 field trial on September 7.

Diagnosis of infection was by ELISA. (■) are infected plants; (□) are healthy plants; a missing square is where a plant failed to establish. There were 4 guard rows between each treatment replicate.

Refer to Fig. 3.1 for descriptions of the treatments.


VO
VOA
C
V
COLUMN
A B C D E F G H I J
BLOCK 1
ROW
1 2 3 4 5 6 7 8 9 10 11 12
COLUMN


V
C
VOA
VO
COLUMN
A B C D E F G H I J
BLOCK 2
ROW
1 2 3 4 5 6 7 8 9 10 11 12

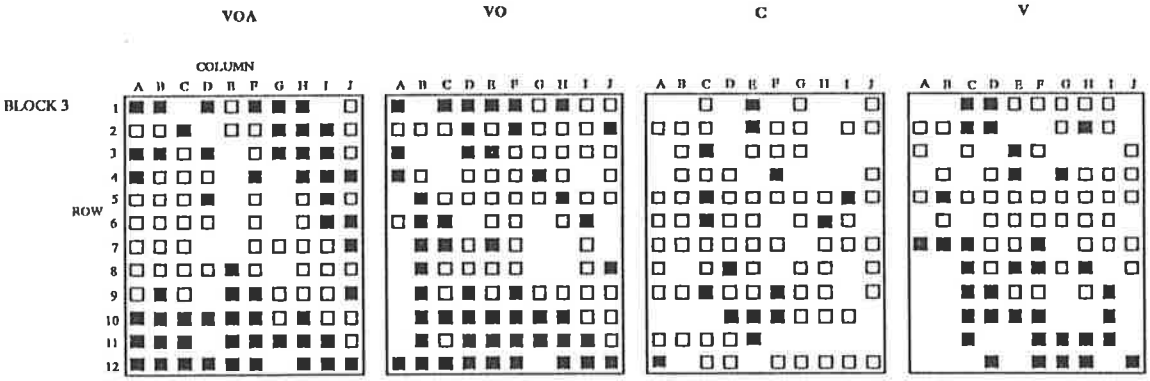
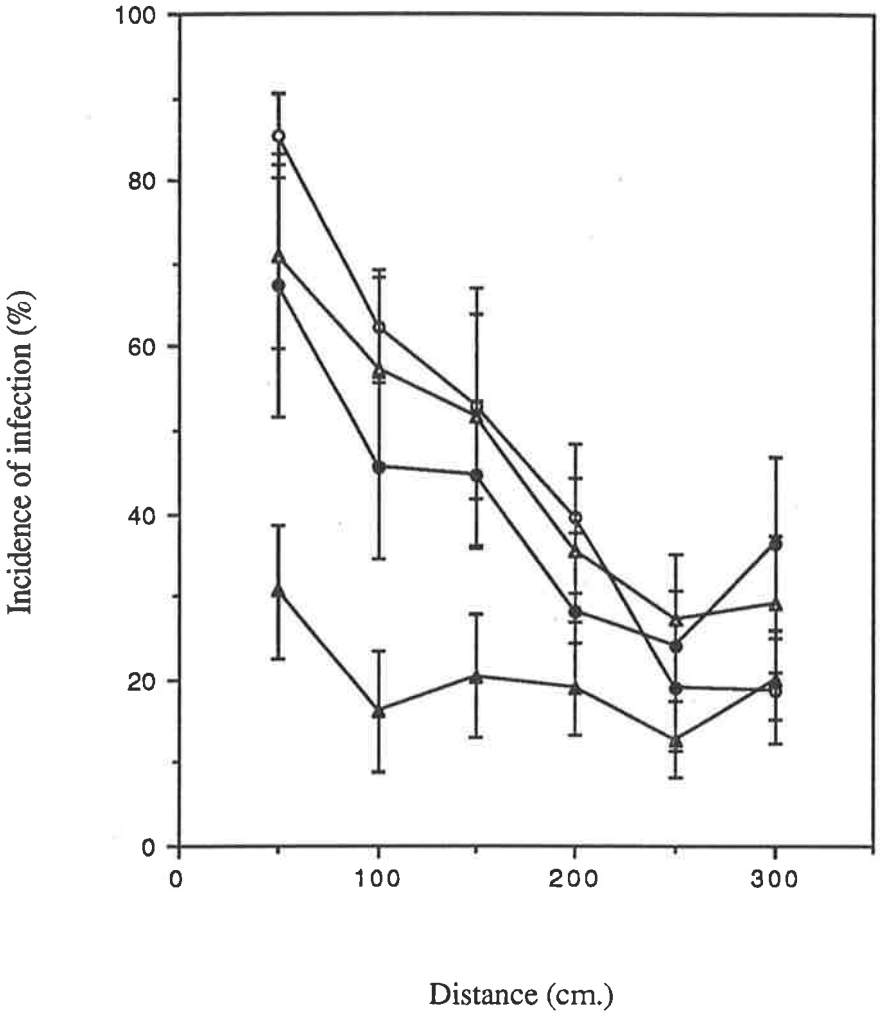

VOA
VO
C
V
COLUMN
A B C D E F G H I J
BLOCK 3
ROW
1 2 3 4 5 6 7 8 9 10 11 12

Fig 3.8: Infection gradients observed in the 1988 field trial on September 7.

(○) is treatment VOA; (●) is treatment VO; (△) is treatment V; (▲) is treatment C. Diagnosis of infection was by ELISA. The linear source of inoculum, comprising 10 infected lupins which were inoculated at the cotyledon stage, is at zero distance. Treatment C was initially all healthy. Bars represent the standard errors of the mean (6 replicates).

Refer to Fig. 3.1 for descriptions of the treatments.


Incidence of infection (%)
100
80
60
40
20
0
0
100
200
300
Distance (cm.)

to the line source of inoculum) at varying distances from the edge of the plot were compared. No significant differences (P=0.535) in incidence of infection were found between the columns and therefore it is concluded that no infection gradients formed in the direction parallel to the linear source of inoculum.

(d) Tests for infection gradients in treatment C

As previously (see c), incidence of infection was compared between rows and columns at different distances from the edge of the plot. No significant differences in incidence of infection were found between either the rows (P=0.478) or columns (P=0.846), indicating that no infection gradients existed in this treatment in the directions that were parallel or perpendicular to the linear sources of inoculum.

3.3.3.3 Analysis of the spatial distribution of infected plants in the 1989 field trial

On September 26, 3 weeks after the first spring flight of aphids (see chapter 4), infected plants were found within a circle of 1 metre radius around 19 of the 24 introduced groups of infected plants, at an average density of 0.85 infected plants/m^2, whereas infected plants were found only in 6 of the 24 equivalent areas surveyed midway between the introduced groups of infected plants, and at a much lower density of 0.13 plants/m^2. This shows that the introduced groups of infected plants were important primary sources of inoculum.

The spatial pattern of plants with symptoms of CMV infection is illustrated in Fig. 3.9. To calculate Lloyds patchiness index, the plot was divided into 4 m^2 quadrats and the number of infected plants in each quadrat counted, as shown in Fig. 3.10. The quadrat size chosen was the approximate size of the clusters of infected plants, at which size the variance is maximised in most cases (Madden *et al.*, 1987).

Fig. 3.9: Map showing the distribution of infected plants in the 1989 field trial.

Diagnosis of infection was by symptoms. (+) are the infected lupins introduced as artificial infection foci; (●) were found to be infected on October 5; (▲) were additional infected plants found on October 24.

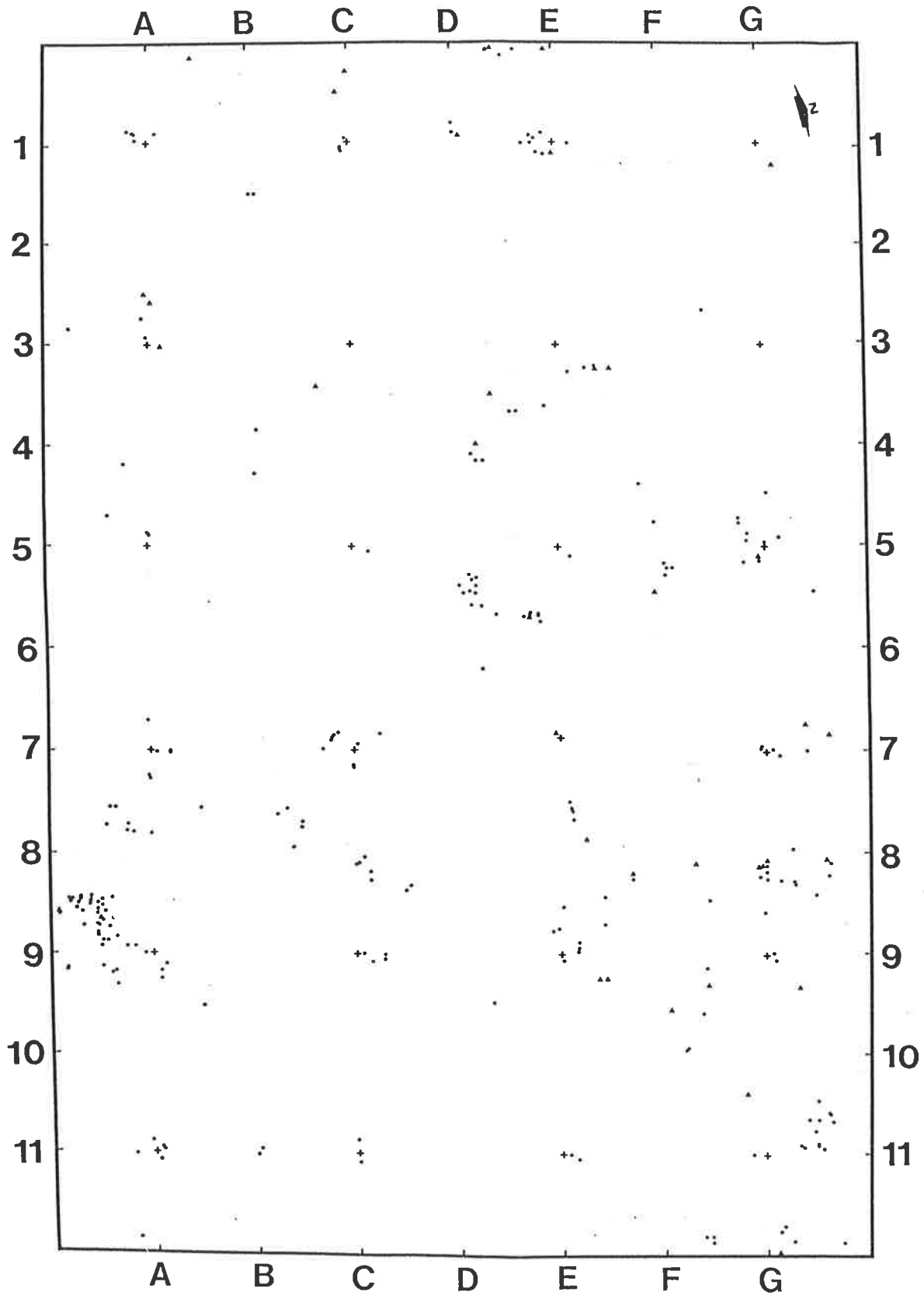
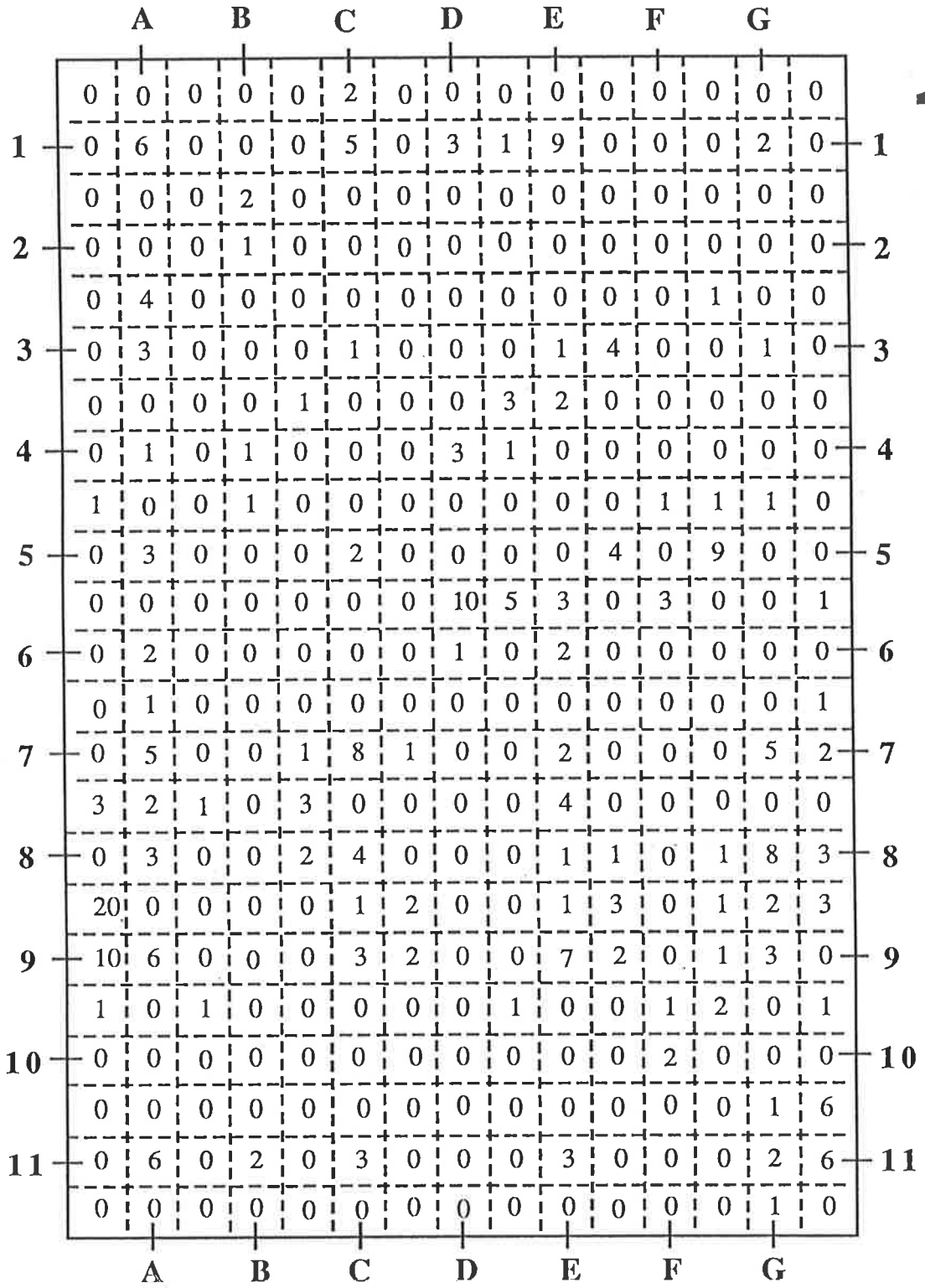

A
B
C
D
E
F
G
1
2
3
4
5
6
7
8
9
10
11

Fig 3.10: Diagram showing the distribution of infected plants in the 1989 field trial (see Fig. 3.9).

The plot, excluding a 1 m. wide border, was divided into 4 m^2 quadrats of 2 m. x 2 m. The artificial infection foci (see Fig. 3.9 for the appropriate coordinates), comprised four infected lupins, and were considered as one infection point when counting infected plants in quadrats. The numbers of infected plants, determined by symptoms, are shown at crop maturity for each quadrat.


A B C D E F G
N
1 2 3 4 5 6 7 8 9 10 11
0 0 0 0 0 2 0 0 0 0 0 0 0 0 0
0 6 0 0 0 5 0 3 1 9 0 0 0 2 0
0 0 0 2 0 0 0 0 0 0 0 0 0 0 0
0 0 0 1 0 0 0 0 0 0 0 0 0 0 0
0 4 0 0 0 0 0 0 0 0 0 0 1 0 0
0 3 0 0 0 1 0 0 0 1 4 0 0 1 0
0 0 0 0 1 0 0 0 3 2 0 0 0 0 0
0 1 0 1 0 0 0 3 1 0 0 0 0 0 0
1 0 0 1 0 0 0 0 0 0 0 1 1 1 0
0 3 0 0 0 2 0 0 0 0 4 0 9 0 0
0 0 0 0 0 0 0 10 5 3 0 3 0 0 1
0 2 0 0 0 0 0 1 0 2 0 0 0 0 0
0 1 0 0 0 0 0 0 0 0 0 0 0 0 1
0 5 0 0 1 8 1 0 0 2 0 0 0 5 2
3 2 1 0 3 0 0 0 0 4 0 0 0 0 0
0 3 0 0 2 4 0 0 0 1 1 0 1 8 3
20 0 0 0 0 1 2 0 0 1 3 0 1 2 3
10 6 0 0 0 3 2 0 0 7 2 0 1 3 0
1 0 1 0 0 0 0 0 1 0 0 1 2 0 1
0 0 0 0 0 0 0 0 0 0 0 2 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 1 6
0 6 0 2 0 3 0 0 0 3 0 0 0 2 6
0 0 0 0 0 0 0 0 0 0 0 0 0 1 0

Lloyds patchiness index (m^*/m) for the distribution of infected plants was 5.67 ± 1.07. To determine the significance of the clustering, a chi-square test of the variance to mean ratio was performed (Madden *et al.*, 1987). For the pattern analysed, χ^2_{observed} =1592 and $\chi^2_{(0.001)}$ = 413.77 (329 df). Thus, the probability that this pattern arose by chance alone is less than 1% and it can be concluded that the clustering of infected plants was highly significant. For a completely random pattern, $m^*/m = 1$, therefore the infected plants in the plot are 5.7 times as crowded as they would be if they were randomly distributed.

3.4 Discussion

3.4.1 Diagnosis of CMV infection of field plants using symptoms

The incidence of CMV infection in lupins was underestimated when symptoms were used for diagnosis, due both to the period between the development of systemic infection and symptom appearance in plants, and to an increased incidence of symptomless infections as the crop aged. The time delay between systemic infection of the plant and the development of symptoms such as stunting would depend on the growth rate of the plant. In phenotypic studies of *L. angustifolius* 'Unicrop' and 'Uniharvest', Perry and Poole (1975) found that the growth (increase in height) of lupins followed a sigmoidal path, with most rapid growth occurring in the period between initiation of the primary floral bud and the beginning of flowering. Initial growth of seedlings during winter is slow and symptoms of virus infection would not develop as quickly as for plants which are growing rapidly. Similarly, symptomless plant infections may arise when the plant becomes infected during flowering, as at this stage, further vegetative growth is limited. Symptoms of virus infection were also not apparent when plants were stunted from root rotting.

3.4.2 Effect of treatment, 1987 trial

Treatment C in the 1987 trial had an initial virus incidence arising from the use of infected seed. Seedlings that were infected from the seed were the dominant sources of inoculum and there was no measurable input of the infected seedlings introduced to the centre of the plots of treatments V and VV as sources of inoculum. The effectiveness of the introduced sources of inoculum was reduced by high mortality from bird damage.

There was no effect of early colonisation of the lupin plots with *A. craccivora* on the progress of the epidemic. The colonies of *A. craccivora* on the infectors failed to develop. The spread of CMV in the plots coincided with migratory flights of aphids and results shown in chapter 4 provide evidence that alates of the non-colonising aphid *R. padi* were the most important vectors at the beginning of the epidemic. Furthermore, the results presented in Fig. 4.2 show that *A. craccivora* was one of the last of the common aphid species to peak in migratory activity in Spring, 1987, and the epidemic was well developed by this stage.

3.4.3 Effect of treatment, 1988 trial

The onset of the epidemic in the control plots in 1988 was delayed compared with the other treatments where inoculum was introduced. This, and the observation of steep infection gradients arising from the linear sources of inoculum, suggests that the lupin source plants, which were infected by inoculation at the seedling stage, were the primary sources of inoculum of the epidemic in the spring.

Another main objective of the experiment, which was to provide further evidence of the role of *R. padi* as a vector of CMV in lupins, by looking for differences in the progress of the epidemic as a result of superimposing a higher local density of this aphid, was not achieved. This does not show that *R. padi* is not a vector, as a failure to detect differences between treatments VOA and VO may be a consequence of any of the following -

(a) There may be insufficient replication to detect small differences between the treatments; the amount of replication is obviously a compromise as there are labour, space and cost limitations to the size of experiments.

(b) The number of *R. padi* produced on the oats may be insignificant compared to the numbers migrating from other localities. The results of the surveys for aphid colonisation of the oats do support this conclusion.

(c) Alate aphids emigrating from the oats may disperse over all treatments and may thus not show a gradient of distribution.

(d) Alate aphids leaving the oats may require a period of migratory flight before they actively seek new hosts and may not have alighted on the lupins planted in this experiment.

3.4.4 Temporal progress of the epidemics

In 1987 and 1988, incidence of CMV increased rapidly in the period from late August to October. Rapid spread of CMV was correlated with peaks in the migratory activity of aphids. The flight patterns of common aphid species during the time of the field trials are described in chapter 4 and the role of alate aphids as field vectors is shown.

The epidemic in 1989 was small in comparison. Possible differences between this epidemic and the epidemics in 1987 and 1988, which resulted in less virus spread, include -

(a) lower initial levels of inoculum.

(b) reduced aphid landing rates due to the higher plant density (Maelzer, 1986).

(c) increased shading of the introduced infected lupins caused by the higher plant density, which could have reduced their effectiveness as sources of inoculum (Jones, 1988).

3.4.5 Spatial progress of the epidemics

The spatial distribution of infected plants in the three field trials suggest that the lupins which were infected at the seedling stage were the primary sources of inoculum from which secondary infections arose in spring. The maps of the distribution of infected plants in the 1987 and 1989 trials showed the formation of clumps of infected plants around plants that were infected through seed or inoculated at the seedling stage. Clumps of infected plants were also found around secondary infection foci (see Fig. 3.9), and these were smaller than those around the primary infection foci.

The infection gradients observed in the 1988 field trial were steep with incidence of infection decreasing from 100 % to about 25 % in a distance of 2.5 metres or 5 rows from the source. This indicates a rapid dilution of inoculum with increasing distance from the source, and assuming that transmission is due to aphid alatae (see chapter 4), this could have resulted from:

(a) Aphids preferentially flying short distances between host plants, and

(b) Viruliferous aphids rapidly losing infectivity between sequential probes.

At a distance of 2.5 metres from the linear source of inoculum, incidence of infection had decreased to the level found in the control plots. The occurrence of infection in treatment C on September 7 suggests that spread occurred between the plots or from sources outside the field trial. No infection gradients were found in treatment C, suggesting that the infection was not a continuation of gradients originating from the linear source of inoculum in the adjacent plots. The pattern observed in the control plots is better explained by long

distance movement of infective aphids which would have provided secondary foci of infection. These foci occurred at random in the plots.

Chapter 4

Vector Studies

4.1 Introduction

CMV is transmitted in a non-persistent manner by more than 60 aphid species (Kennedy *et al.*, 1962). Epidemics of non-persistently transmitted viruses are often correlated with migratory flights of aphids. Alate aphids when seeking new hosts, assess the suitability of a plant for food by brief investigatory probes and during these probes, virus acquisition and transmission can occur (Francki *et al.*, 1979). Consequently, an aphid species can be an important vector of a non-persistently transmitted virus but be incapable of colonising the crop plant (van Hoof, 1977, 1980; Halbert *et al.*, 1981; Raccah *et al.*, 1985).

Yellow pan, suction and tile traps have been used to monitor aphid flights. Yellow pan traps (Moericke, 1951), are selective in that they collect aphids attracted to the colour yellow. They are easy and inexpensive to set up and maintain and provide an adequate estimate of the relative abundance of a species through the season (Irwin, 1980; Robert, 1988). A disadvantage of this type of trap is that aphids differ in the extent to which they are attracted to yellow and therefore these traps are poor for the estimation of the comparative abundance of species (Eastop, 1955; Heathcote, 1957). Suction traps, first used by Johnson (1950) and Taylor (1951), actively filter aphids from the air and hence give a good estimate of the relative aerial densities of different aphid species (Irwin, 1980; Robert, 1988). However, they require an electricity source and are expensive to run. Green tile traps have been designed to mimic the leaf and are considered to provide an accurate estimate of the landing rates of aphids, an important parameter which is needed for the formulation of accurate epidemic models (Irwin, 1980; Irwin and Ruesink, 1986).

To provide information on which species of aphids were important as vectors of CMV, aphid flights were monitored using yellow pan traps during the periods of the field trials at Strathalbyn. In 1989, the yellow pans, suction traps and green tiles were compared for their ability to trap aphids. Commonly trapped aphids were tested for their ability to transmit CMV. To evaluate factors affecting the timing of aphid flights, daily patterns of aphid flights were monitored in 1989, and compared with climatic factors.

4.2 Materials and methods

4.2.1 Descriptions of aphid traps

(a) Yellow pan traps

Yellow pan traps (see Fig. 4.1) were yellow plastic trays (35 x 31 x 14 cm.), supported at a height of 40 cm. in a square steel frame mounted to a steel post (Jayasena, 1984). All traps were suspended over bare ground. Water was added to a depth of *ca.* 9cm. and a few drops of formalin and non-ionic detergent were added.

(b) Suction traps

Suction traps (see Fig. 4.1) were mounted so that the opening was 45 cm. above ground. Engine power was 60 W and fan blade width was 20 cm. The traps were run continually.

(c) Green tile traps

Green tile traps (see Fig. 4.1) were constructed using two types of tiles; either a Cambridge 815 ceramic tile (12 x 12 cm.), supplied by Dr. M.E. Irwin, or a Eurotile VIP Verde (10 x 10 cm) tile. The VIP tile was selected from locally available tiles because it

Fig. 4.1: Aphid traps used in the field trials.

(A) Yellow pan trap.

(B) Suction trap.

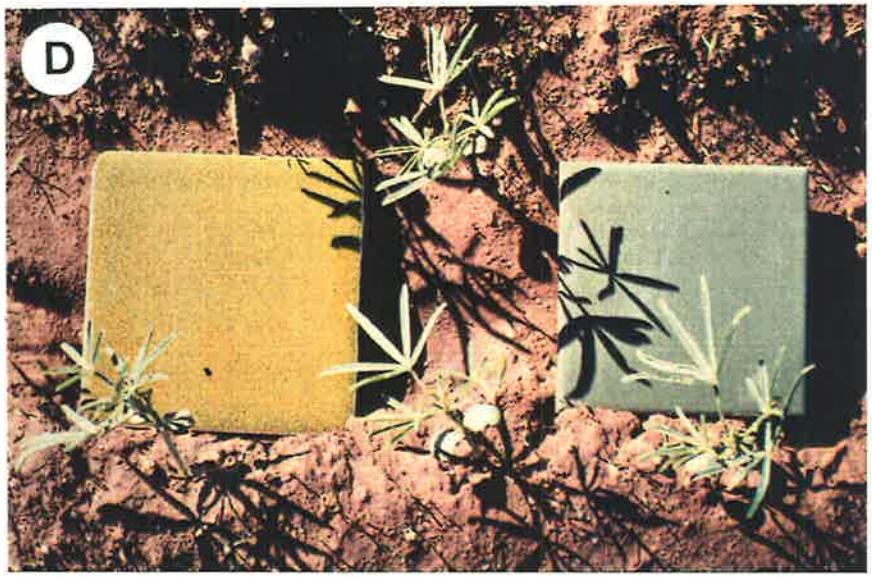
(C) Green tile trap.

(D) Colour comparison of the Cambridge 815 (left side of photograph) and VIP (right side of photograph) tiles with the foliage of 'Warrah' lupins.


A


B


C


D

most closely resembled the lupin leaves in colour and texture (see Fig. 4.1). These tiles were placed in small plastic containers, which were filled with 50% ethylene glycol. The traps were then suspended at canopy level on a steel rod, using a retort clamp. The heights of the tiles were adjusted as the plants grew.

4.2.2 Collection, storage and identification of aphids

Aphids were collected from the aphid traps once a week and stored in 90% ethanol. Apterae and alatae were identified with the aid of keys described by Cottier (1953) and Taylor (1984). All aphid identifications were confirmed by Dr. M. Carver (CSIRO Division of Entomology, Canberra). Nymphs collected fom lupins in the 1988 field trial were identified by Dr. M. Carver. Nymphs collected from lupins in the 1989 trial were classified into instars using size and number of antennal segments as a guide.

4.2.3 Monitoring of aphid flights

To monitor aphid flights during the lupin growing season, yellow pan traps were placed adjacent to the field trials in 1987, 1988 and 1989.

In 1989, 4 suction traps and 12 green tile traps (4 Cambridge tiles and 8 Eurotiles) were also placed within the plot of lupins. The arrangement of the traps within the plot is shown in Fig. 3.2.

4.2.4 Daily flight patterns

To study their daily flight patterns, aphids were collected from the yellow pan and suction traps on an hourly basis, on 6 days in September 1989. To investigate climatic factors affecting flight patterns, hourly records of wind distance (at a height of 2 m.), wind

direction and temperature (inside a Stevenson Screen) were obtained from the metereological station at Charlick Experimental Station.

4.2.5 Aphid colonisation

To identify the species of aphids alighting on the lupins in the 1988 field trial, alates were collected from plants using a camel-hair brush. To identify the aphid species colonising the lupins in 1989, shoots (*ca.* 30 cm. long) were clipped from plants which were spaced 1 m. apart along 4 randomly selected transects across the plot. These shoots were bagged, transported back to the laboratory and stored for no longer than 3 days at 4 C prior to counting. Aphids were dislodged by heating the shoots to 50 C for 1 hour (Hussein, 1982; Jayasena, 1984). The contents of the bag were then knocked onto a white piece of cardboard and aphids collected.

4.2.6 Aphid transmission experiments

The ability of a range of aphid species to transmit the CMV-B_{SA} was tested in glasshouse experiments. The aphid species used in the transmission tests and the hosts on which they were raised is given in Table 4.1. Aphid colonies were kept in a glasshouse compartment at 20 ± 2 C.

Apterae and final instar apterous nymphs were used. Aphids were starved for 3-8 hours, then allowed to probe briefly on systemically infected leaves of *L. angustifolius*. Aphids were allowed 60-90 seconds acquisition access period. Single aphids were then transferred, using a camel-hair brush, to 8-10 day old healthy test seedlings (*L. angustifolius*) and caged for at least 60 minutes inoculation access, then sprayed with the aphicide Pirimor. The incidence of plants with symptoms of CMV infection was recorded 3 weeks later and samples from these plants were tested by ELISA.

Table 4.1: List of the aphid species used in the transmission tests and their glasshouse hosts.

Aphid species	Glasshouse host
Aphis craccivora	*Vicia faba* 'Aquadulce'
Brachycaudus rumexicolens	*Rumex crispus*
Dysaphis aucupariae	*Plantago lanceolata*
Hyperomyzus lactucae	*Sonchus oleraceus*
Lipaphis erysimi	*Brassica napus*
Macrosiphum euphorbiae	*Pisum sativum* 'Greenfeast'
Metapolophium dirhod um	*Avena sativa* 'NZ Cape'
Myzus persicae	*Brassica pekinensis*
Rhopalosiphum padi	*Avena sativa* 'NZ Cape'

4.2.7 Correlation between aphid flights and the field spread of CMV

To investigate their role as field vectors of CMV, numbers of alates trapped in the yellow pans were compared with the rates of infection in the 1987 and 1988 field trials (see chapter 3). Rate of infection was calculated using the following formula -

Rate of infection (%/day) = $100 \times \{(y_2 - y_1)/[(1 - y_1) \times 14]\}$,

where y is the proportion infected at two times (1 and 2), and time 2 is 14 days after time 1.

4.3 Results

4.3.1 Aphid species trapped in yellow pan traps and their seasonal flight pattern.

Table 4.2 shows the species composition of the yellow pan catch in the years 1987 to 1989. In the three year period, *M. persicae*, *R. padi* and *L. erysimi* were the most abundantly trapped species. *B. rumexicolens* was the dominant species trapped in 1987, but was trapped in much smaller numbers in 1988 and 1989.

Table 4.2: Aphid species trapped in the yellow pans.

	Composition of yellow pan catch (% of season total)		
Aphid species	[1] 1987	[2] 1988	[3] 1989
A. craccivora	11.3	3.2	0.4
B. rumexicolens	39.4	0.9	0.9
L. erysimi	7.5	18.4	18.2
M. persicae	19.0	68.2	53.1
R. padi	13.7	4.8	21.5
Other species	9.0	4.6	6.0

[1] Trapping period between June 3 and October 28.

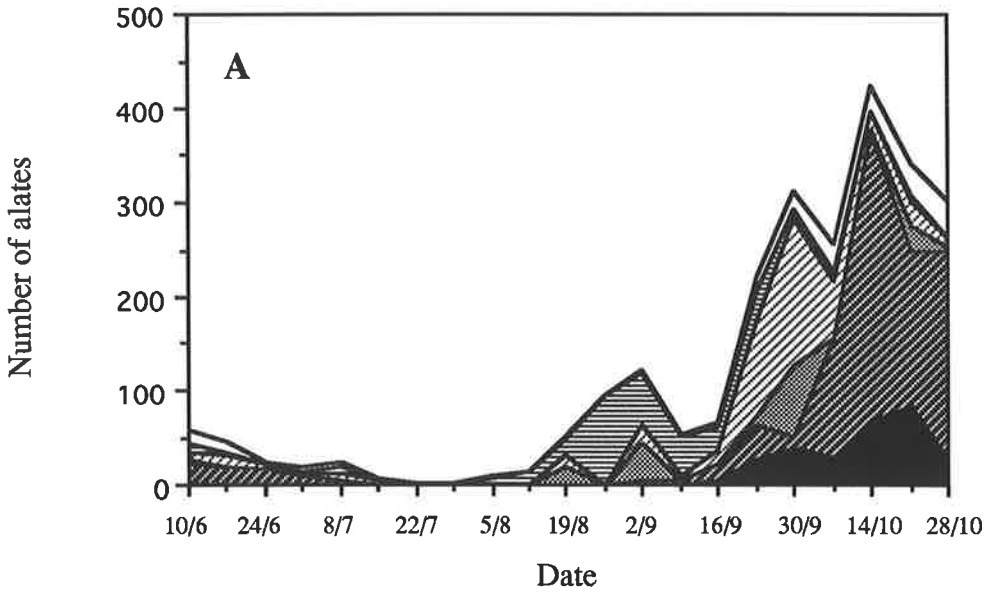
[2] Trapping period between June 15 and September 21.

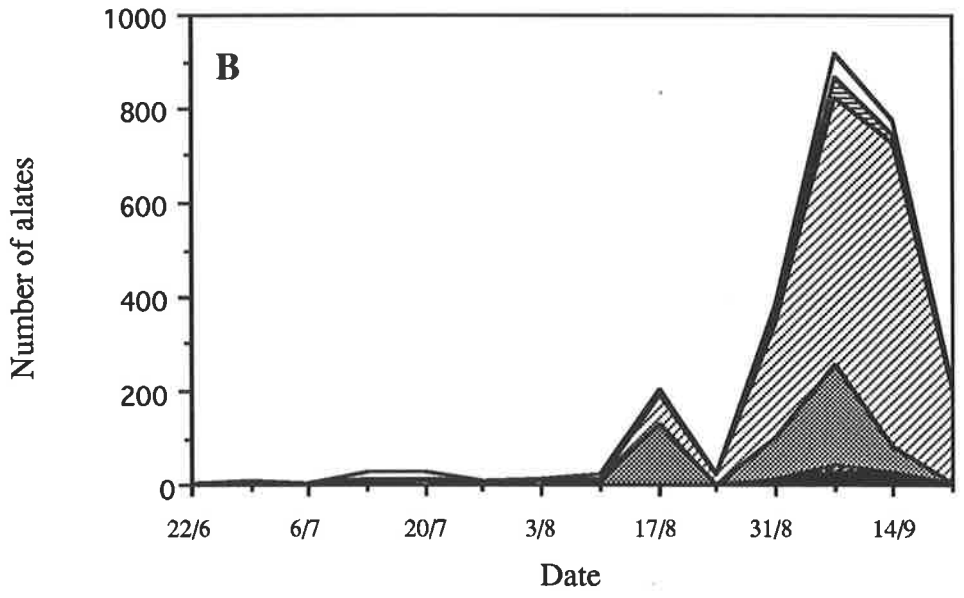
[3] Trapping period between June 6 and October 31.

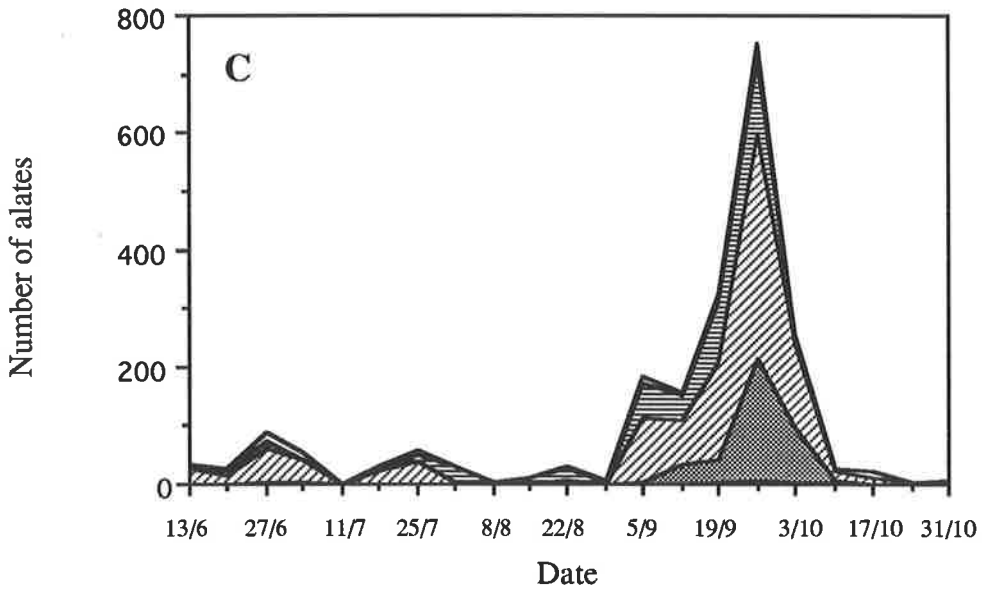
The seasonal patterns of trap collections in 1987, 1988 and 1989 are shown in Fig. 4.2. For all species, the largest collections were made in late winter and spring. In 1987, *R. padi* numbers showed the earliest peak in late August, followed by peaks of *L. erysimi* and *M. persicae* in late September, and of *B. rumexicolens* and *A. craccivora* in October. In 1988 and 1989, collections of *M. persicae*, *R. padi* and *L. erysimi* all peaked in September.

Fig. 4.2: Patterns of aphid flights during the lupin growing season in 1987, 1988 and 1989.

(■) is *A. craccivora*; (▨) is *B. rumexicolens*; (■) is *L. erysimi*; (▨) is *M. persicae*; (▤) is *R. padi*; (□) is other aphid species. Total numbers of alates trapped in 2 yellow pans in 1987 and 1989, and 1 yellow pan in 1988, are shown.


A
500
400
300
200
100
0
Number of alates
10/6
24/6
8/7
22/7
5/8
19/8
2/9
16/9
30/9
14/10
28/10
Date


B
1000
800
600
400
200
0
Number of alates
22/6
6/7
20/7
3/8
17/8
31/8
14/9
Date


C
800
600
400
200
0
Number of alates
13/6
27/6
11/7
25/7
8/8
22/8
5/9
19/9
3/10
17/10
31/10
Date

Flight of *R. padi* and *M. persicae* was detected regularly through winter (see Table 4.3). Of the common aphid species, *R. padi* was most abundantly trapped (relative to the year total) during winter (see Table 4.3).

Table 4.3: Relative abundance of common aphid species during the winter months (*a* proportion of weeks that the aphid species was trapped in the yellow pans; *b* percent of total that was trapped during the winter period in the yellow pans).

Aphid species	1987 (Jun. 3 - Aug. 26)		1988 (Jun. 15 - Aug. 31)		1989 (June 6 - Aug.29)	
	a	*b*	*a*	*b*	*a*	*b*
A. craccivora	1/12	1.1	3/11	24.1	1/12	12.5
B. rumexicolens	6/12	7.22	2/11	17.4	3/12	27.8
L. erysimi	3/12	14.1	3/11	45.7	0/12	0
M. persicae	9/12	10.7	11/11	21.3	10/12	19.6
R. padi	11/12	47.6	8/11	43.2	12/12	22.7

4.3.2 Daily flight pattern of aphids

Aphid flights were monitored in 1989 on September 5, 7, 8, 20, 21 and 28. *M. persicae*, *L. erysimi* and *R. padi* were the most common aphids trapped in the yellow pan and suction traps on these days. The daily patterns of aphid flights and corresponding fluctuations in wind speed and temperature, are illustrated in Fig. 4.3.

The effects of temperature and wind on aphid migration are illustrated in Figs. 4.4 and 4.5. On windy days, the largest aphid flights occurred during lulls. High winds delayed but did not completely inhibit aphid flight. Some aphids were still trapped when the hourly average wind speed was greater than 10 km/hour. The minimum hourly average

Fig 4.3: Daily patterns of aphid flights on six days in September 1989 and corresponding changes in wind speed and temperature.

Aphid flight was monitored using either (A) 2 yellow pans or (B) 4 suction traps. Total numbers of alates trapped each hour are shown. On September 8, aphid flights were monitored hourly after 14.00 hours; prior to 14.00 hours, the mean number trapped each hour between sunrise (6.30 hours) and 14.00 hours is shown.

Fig. 4.3.1: September 5, 1991.

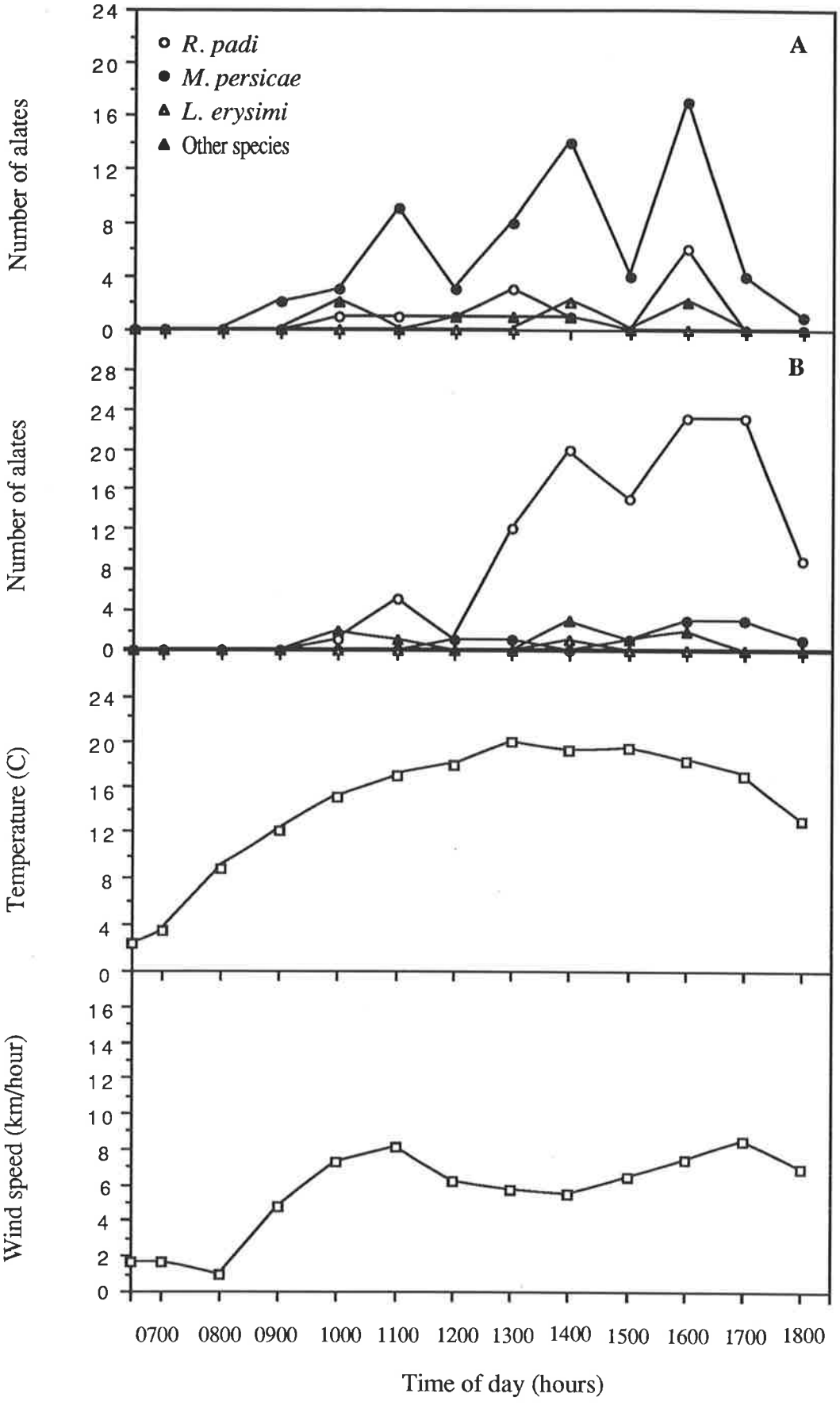


Fig. 4.3.2: September 7, 1989.

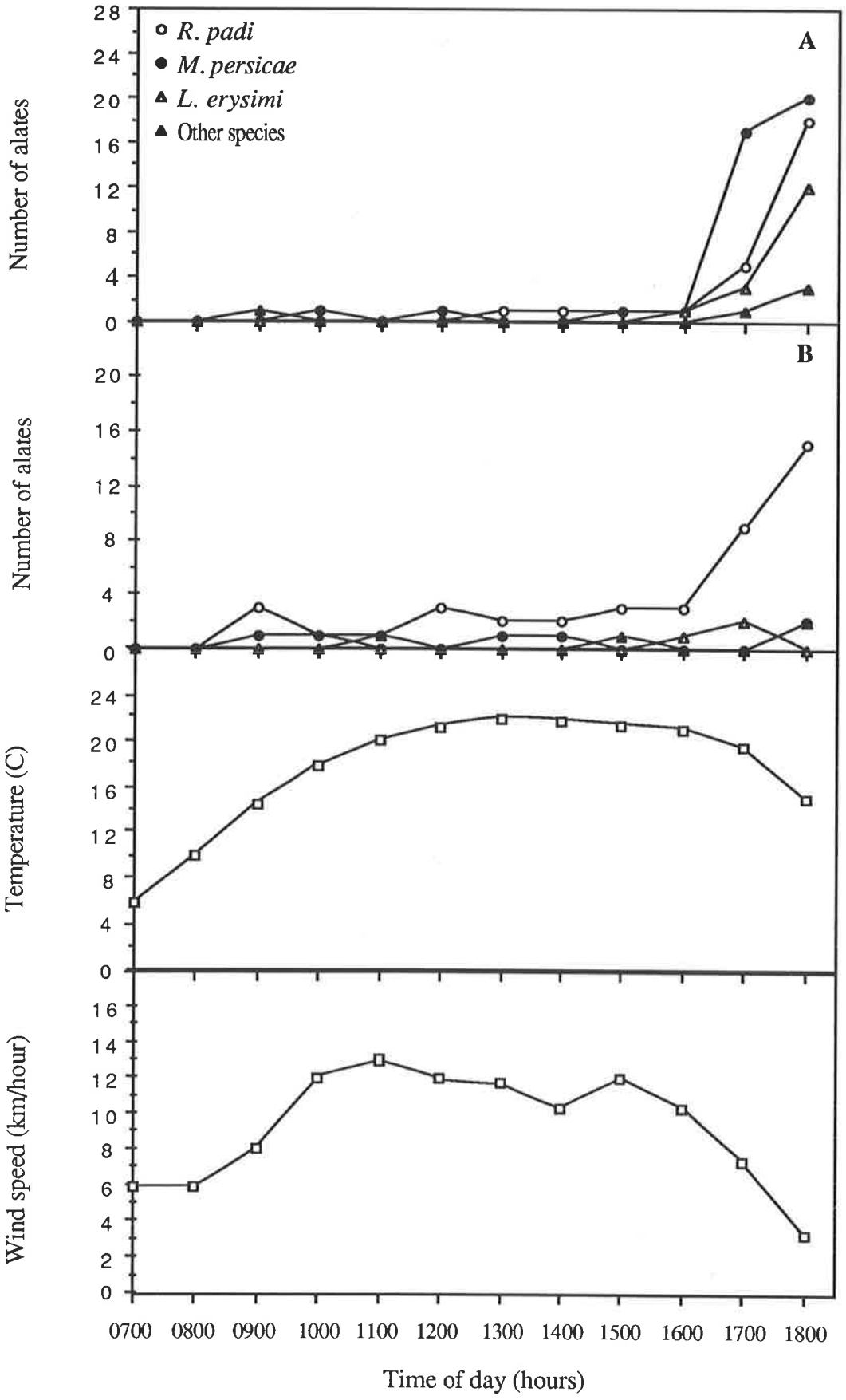


Fig. 4.3.3: September 8, 1991.

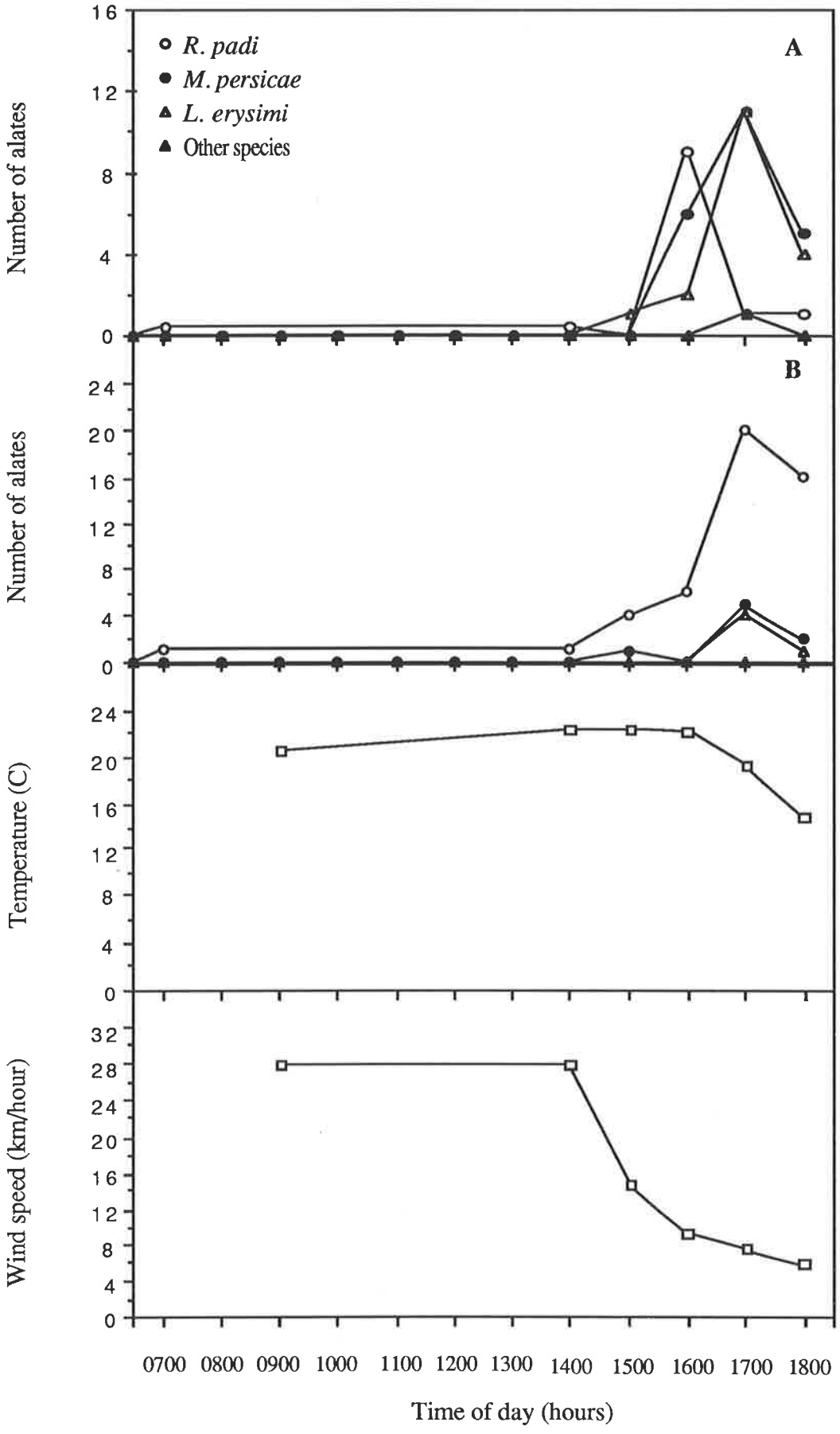


Fig. 4.3.4: September 20, 1989.

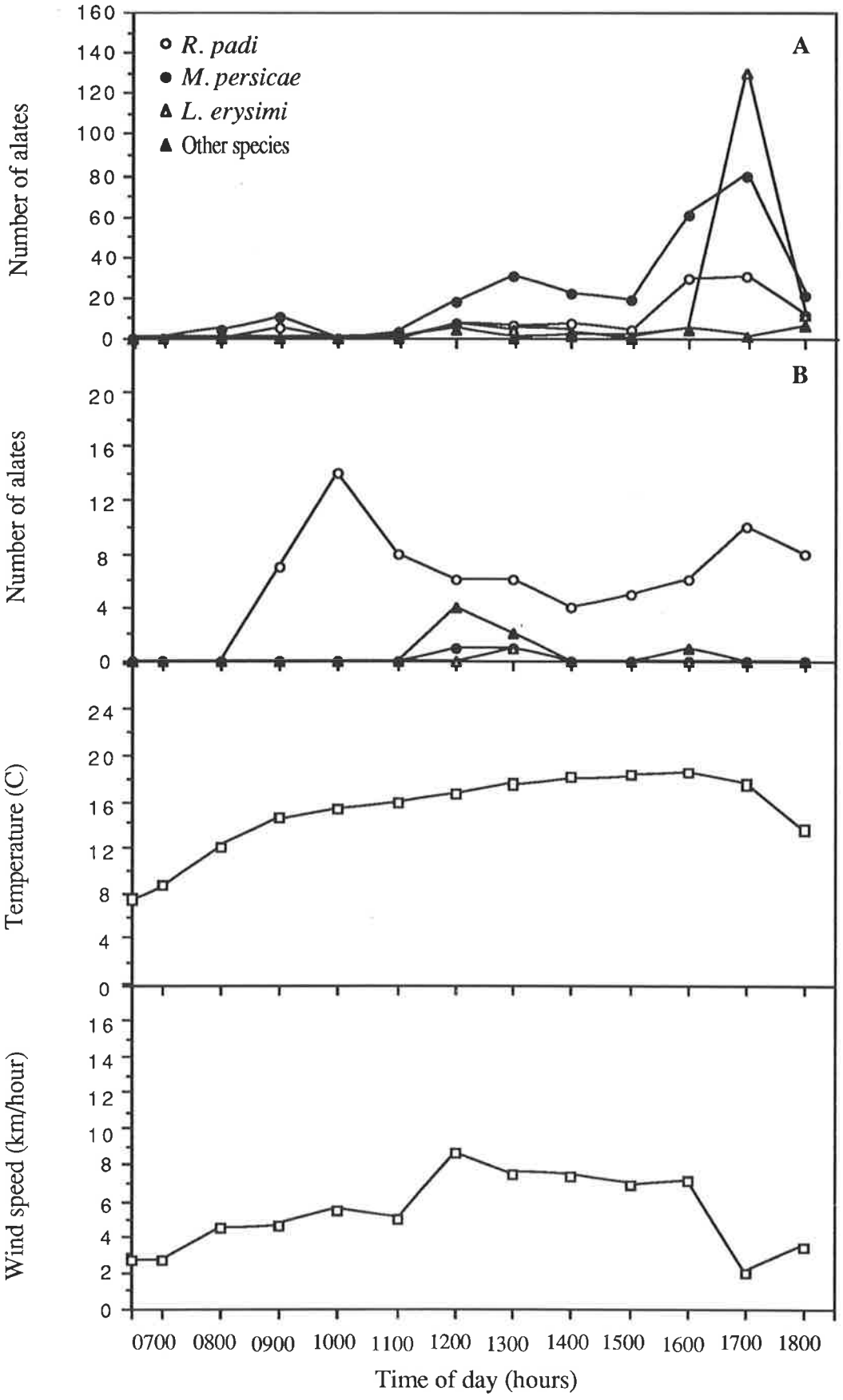


Fig. 4.3.5: September 21, 1989.

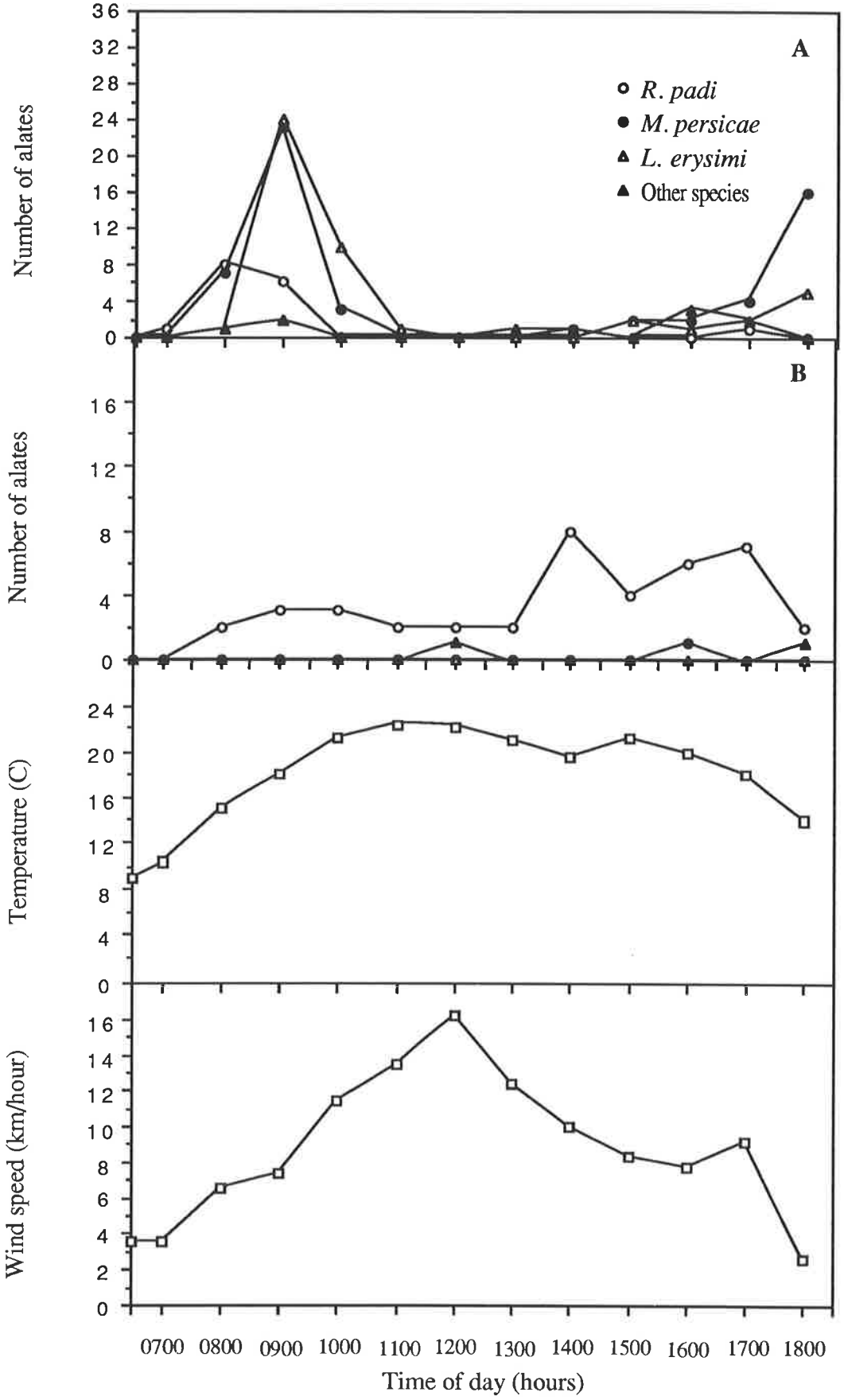


Fig. 4.3.6: September 29, 1989.

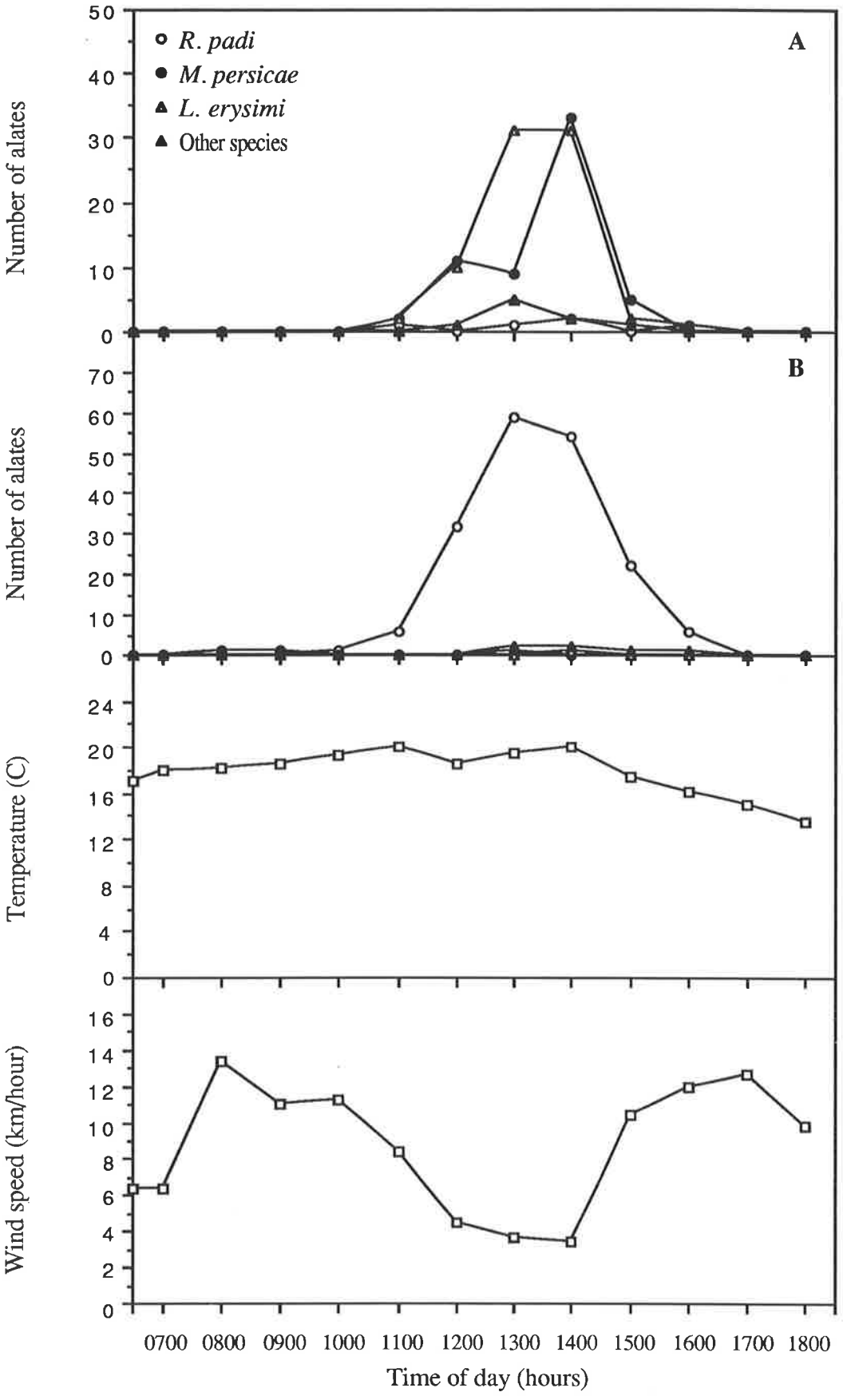
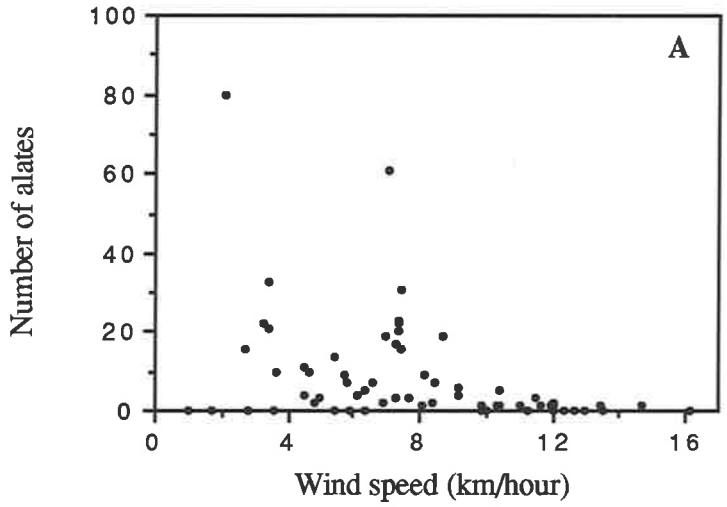
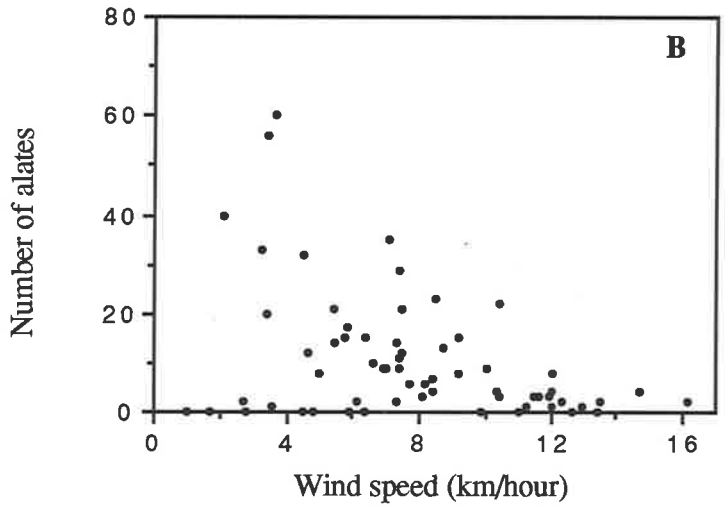
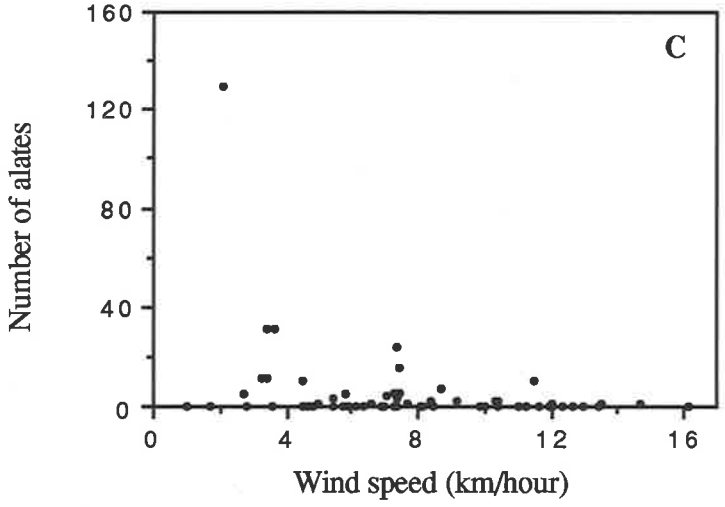


Fig. 4.4: Relationship between wind speed and flight of (A) *M. persicae* (B) *R. padi* (C) *L. erysimi* and (D) other aphid species.

Wind speed is the wind distance travelled in one hour. The combined totals of alates trapped in two yellow pan traps and four suction traps are shown.


A
Number of alates
Wind speed (km/hour)
0
20
40
60
80
100
0
4
8
12
16


B
Number of alates
Wind speed (km/hour)
0
20
40
60
80
0
4
8
12
16


C
Number of alates
Wind speed (km/hour)
0
40
80
120
160
0
4
8
12
16

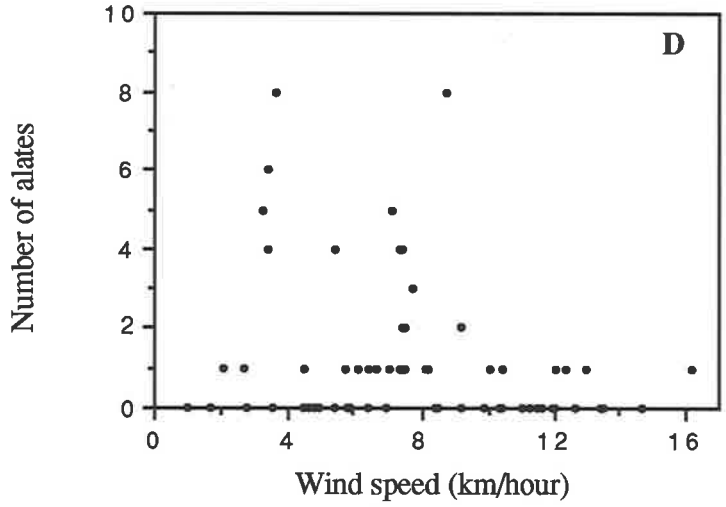
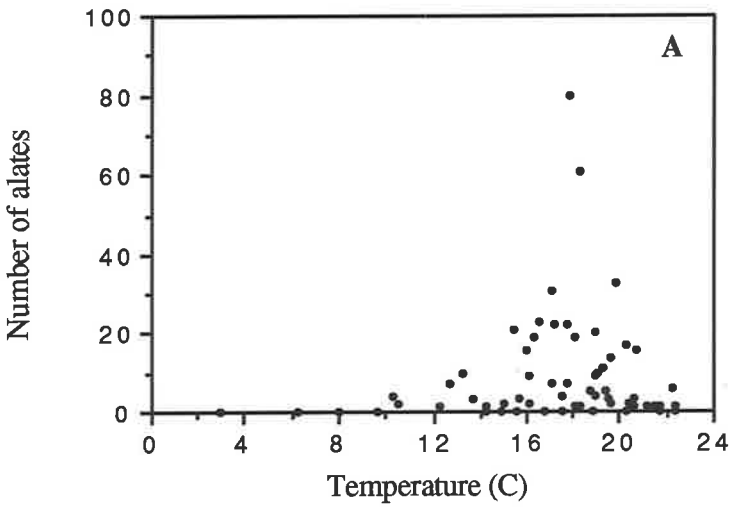
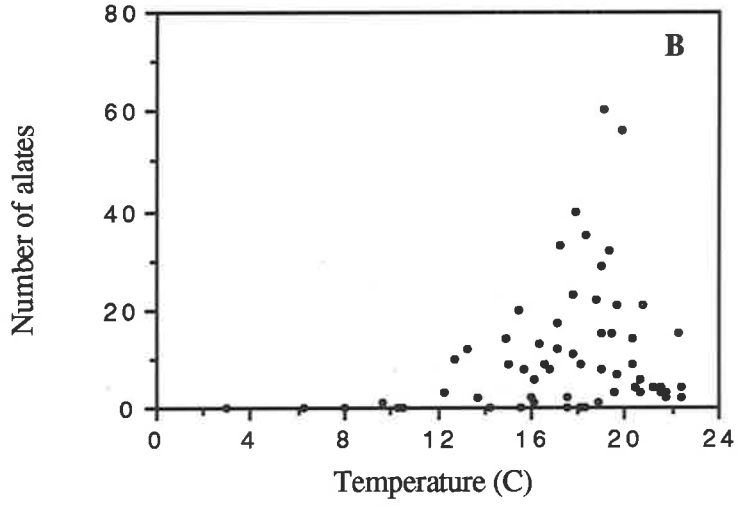
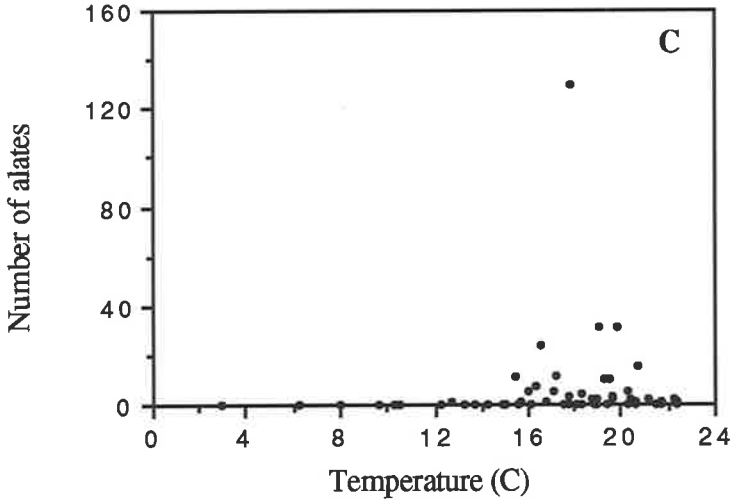

D
Number of alates
Wind speed (km/hour)
0
2
4
6
8
10
0
4
8
12
16

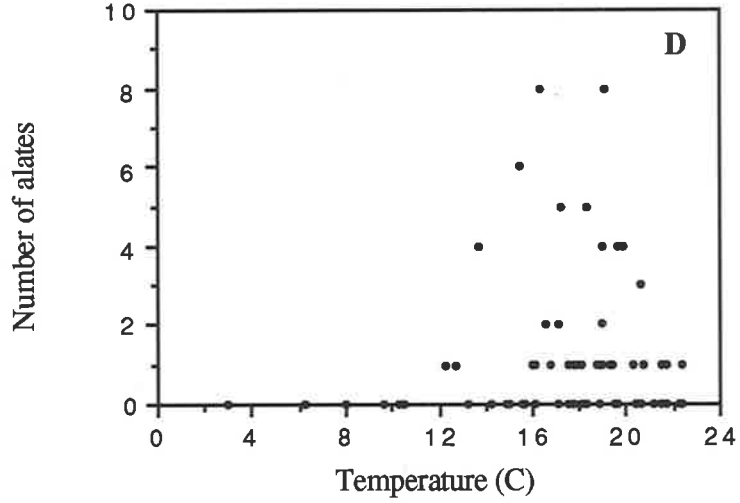
Fig. 4.5: Relationship between air temperature and flight of (A) *M. persicae* (B) *R. padi* (C) *L. erysimi* and (D) other aphid species.

Temperature is the mean of that found at the beginning and end of each hour. The combined totals of alates trapped in 2 yellow pans and 4 suction traps are shown.


A
Number of alates
Temperature (C)
0
20
40
60
80
100
0
4
8
12
16
20
24


B
Number of alates
Temperature (C)
0
20
40
60
80
0
4
8
12
16
20
24


C
Number of alates
Temperature (C)
0
40
80
120
160
0
4
8
12
16
20
24


D
Number of alates
Temperature (C)
0
2
4
6
8
10
0
4
8
12
16
20
24

temperatures at which *R. padi*, *M. persicae* and *L. ersimi* were trapped were 9.7, 10.6 and 12.7 C repectively.

On September 5, flights of *M. persicae* and *R. padi* occurred over most of the day, except for the early morning period (see Fig. 4.3.1). The cool morning temperatures (overnight minimum of 2.5 C and 9.00 a.m. temperature of 12.2 C), are considered to be the reason for the delay in aphid migration. Average wind speed was 2.7 km/hour between 6.30 and 9.00 a.m. and unlikely to limit aphid flight.

In comparison to September 5, large numbers of aphids were trapped prior to 9.00 a.m. on September 21 (see Fig. 4.3.5). Between 6.30 and 9.00 a.m., 56.2 % of the days yellow pan catch was obtained. Early morning temperatures were higher (9.0 to 18.0 C between 6.30 and 9.00 a.m.) and wind speeds were similarly low (average of 6.3 km/hour between 6.30 a.m. and 9.00 a.m.). Sunrise on September 21 was at 6.10 a.m. and only 20 minutes earlier than on September 5. Both days were cloudless and it is therefore considered that low light intensity could not have accounted for the delay in aphid flight on September 5.

The effect of wind in inhibiting aphid flights was most pronounced on September 29 (see Fig. 4.3.6). Not many aphids were trapped between 6.30 and 11.00 a.m., when wind speed and temperature averaged 10.5 km/hour and 18.5 C repectively. In the following 3 hour period, the wind decreased (average 3.8 km/hour), and the temperature increased only marginally (average 19.4 C). In this period of calm, large numbers of *M. persicae, R. padi* and *L. erysimi* were trapped.

4.3.3 A comparison of the species composition of the aphid catches from yellow pans and suction traps.

Table 4.4 lists the relative abundance of the 5 most common aphid species in the yellow pan and suction traps. *M. persicae* and *L. erysimi* were trapped in greater abundance in the yellow pan traps and conversely, *R. padi* were found in greater abundance in the suction traps.

Table 4.4: Comparison of the species composition of the aphid catch from the yellow pan and suction traps.

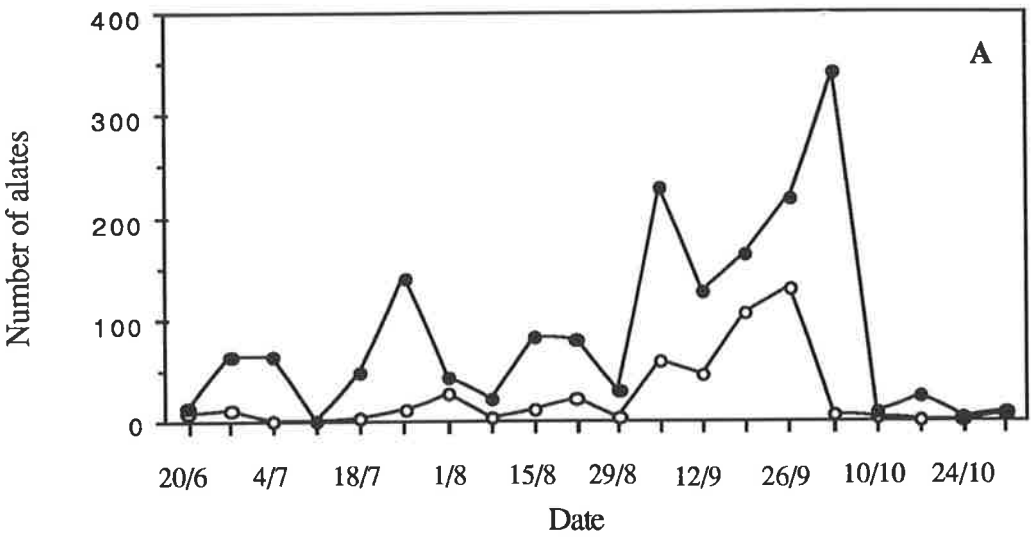
Aphid species	Species composition (% of season total)	
	Yellow pan	Suction
B. rumexicolens	0.9	1.5
Dysaphis species	2.5	1.4
L. erysimi	18.2	0.7
M. persicae	53.1	14.8
R. padi	21.5	77.7

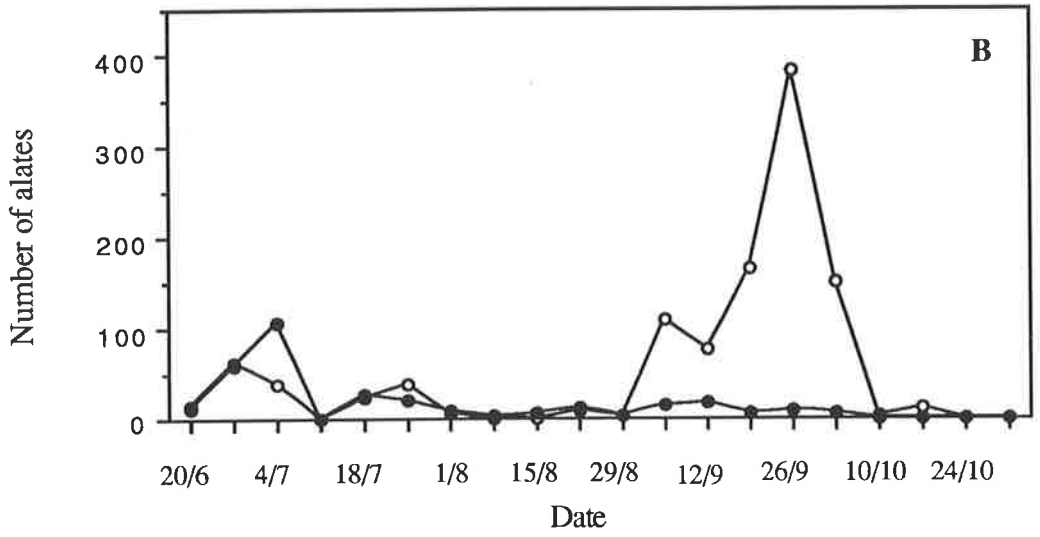
Fig. 4.6 illustrates the seasonal pattern of migration of *M. persicae*, *R. padi* and *L. erysimi*, as measured by yellow pan and suction traps. Flights of *M. persicae* in late June/early July were represented by peaks in numbers trapped in both the yellow and suction traps. In spring, large numbers of *M. persicae* were trapped in the yellow pan traps, whereas only few were trapped in the suction traps at the same time. This indicates that the efficiency of trapping of *M. persicae* in the spring differed from that in winter.

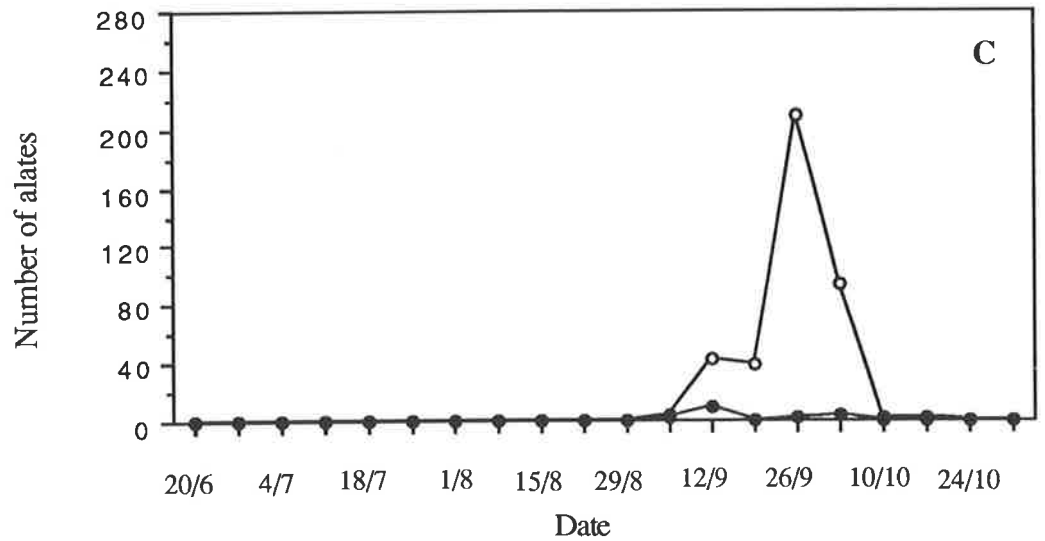
Peaks in the numbers of *R. padi* trapped in the yellow pan traps were commonly correlated with peaks in numbers trapped in the suction traps. This indicates that the yellow pan traps provided a good estimate of the relative abundance of *R. padi* throughout the

Fig. 4.6: Comparison of numbers of (A) *M. persicae* (B) *R. padi* and (C) *L. erysimi*, trapped in the yellow pans (○) and suction traps (●).

Total numbers of alates trapped in either 2 yellow pans or 4 suction traps are shown.


A
Number of alates
400
300
200
100
0
20/6
4/7
18/7
1/8
15/8
29/8
12/9
26/9
10/10
24/10
Date


B
Number of alates
400
300
200
100
0
20/6
4/7
18/7
1/8
15/8
29/8
12/9
26/9
10/10
24/10
Date


C
Number of alates
280
240
200
160
120
80
40
0
20/6
4/7
18/7
1/8
15/8
29/8
12/9
26/9
10/10
24/10
Date

season and that migrating *R. padi* flew actively in the zone immediately above the lupin canopy.

L. erysimi was rarely trapped in the suction traps, yet large numbers were trapped in the yellow pan traps in September. This result shows that although large numbers of *L. erysimi* were migrating over the lupin crop, they rarely flew in the zone above the lupin canopy where they would have been subject to the air flow into the suction trap.

4.3.4 Aphids trapped in green tile traps in 1989

Both types of tile traps trapped inefficiently, with a total of 15 aphids being trapped in the 4 Cambridge tile traps and 20 in the 8 VIP tile traps in the period from June 13 to October 31. Table 4.5 lists the aphid species trapped.

Table 4.5: Aphid species and their numbers trapped in the green tile traps.

	Aphid species						
Type of tile	*A. kondoi*	*D. aucupariae*	*H. lactucae*	*L. erysimi*	*M. euphorbiae*	*M. persicae*	*R. padi*
[1] Cambridge	1	1	1	0	1	4	7
[2] VIP	4	1	1	1	0	5	8

[1] Season total for 4 traps with Cambridge 815 tiles.

[2] Season total for 8 traps with VIP tiles.

4.3.5 Aphid colonisation of the lupins

Table 4.6 lists aphids collected from the lupins during Spring, 1988. Alates of *A. craccivora*, *M. persicae*, *M. euphorbiae*, *R. padi* and *B. rumexicolens* were found on the lupins.

Table 4.6: Number and species of alates collected from *L. angustifolius* 'Illyarrie' plants during Spring 1988. Aphids were collected from *ca.* 500 shoots.

Aphid species	Number of alates		
	August 24	September 7	September 21
A. craccivora	47	104	5
Aulacorthum solani	-	-	1
B. rumexicolens	1	1	1
M. euphorbiae	12	-	-
M. persicae	35	33	2
R. padi	1	3	-

Nymphs of *A. craccivora*, *M. persicae* and *M. euphorbiae*, which were mostly first and second instar, were also collected on August 24 and September 7. No adult apterae were collected, and visual inspection showed that colonies did not develop.

Table 4.7 lists aphids found on the lupin shoots in 1989. Alatae, apterae and nymphs of *M. persicae* and *Acyrthosiphon kondoi* were found in small numbers on the lupins. The majority of nymphs of these two species were first or second instar, suggesting that although larviposition occurred, nymphs failed to mature to reproductive age on the lupins. Only alatae of *R. padi* were collected from the lupins.

Table 4.7: Aphid colonisation of *L. angustifolius* 'Warrah' in the 1989 field trial.

Date	Aphid species	Details of colonisation				
		[1] Shoots with aphids	Number and developmental stage of aphid			
			Instar 1-2	Instar 3-4	Aptera(e)	Alata(e)
Aug. 15	*M. persicae*	13/132	8	8		1
	A. kondoi	1/132	1			
	R. padi	1/132				1
Aug. 28	*M. persicae*	12/132	8	4	1	4
	A. kondoi	24/132	50			6
Sept. 12	*M. persicae*	16/132	18	4	1	4
	A. kondoi	9/132	9	2		1
Sept. 31	No aphids found.	-	-	-	-	-

[1] Ratio indicates the proportion of shoots sampled with aphids.

4.3.6 Aphid transmission of CMV

Table 4.8 describes the results of aphid transmission tests using nine commonly trapped aphid species. *M. persicae*, *R. padi*, *B. rumexicolens*, *A. craccivora*, *D. aucupariae* and *H. lactucae* all transmitted, whereas *L. erysimi*, *M. dirhod um* and *M. euphorbiae* did not.

4.3.7 Aphid activity in relation to virus spread

In 1987 and 1988, changes in the rate of infection correlated with changes in the

Table 4.8: Transmissibility of CMV-B_{SA} by a range of common aphid species (*a* proportion of test seedlings infected; *b* percentage of test seedlings infected).

Aphid species	Rate of CMV transmission									
	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
M. persicae	8/50	16.0	10/40	25.0	12/50	26.0	16/50	32.0	8/40	20.0
R. padi	5/50	10.0	5/40	12.5	3/50	6.0				
L. erysimi	0/50	0	0/40	0	0/50	0				
M. dirhod um	0/50	0								
B. rumexicolens	9/50	18.0								
A. craccivora							8/49	16.3		
D. aucupariae					2/50	4.0				
M. euphorbiae									0/40	0
H. lactucae									8/39	20.5

number of aphids trapped (see Fig. 4.7), assuming a 2 week delay between plant inoculation and detection of systemic infection.

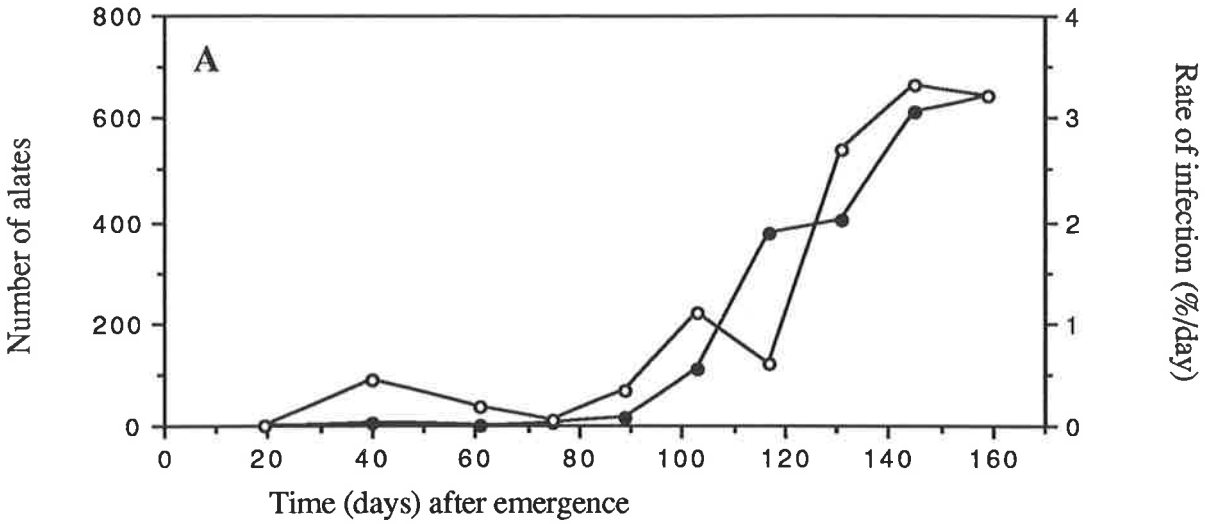
In 1987, incidence of infection increased rapidly following abundant aphid flights that occurred between August 12 and September 2. The dominant species trapped in the yellow pans during this period was *R. padi* (see Fig. 4.2), which consisted 63.0 % of the catch.

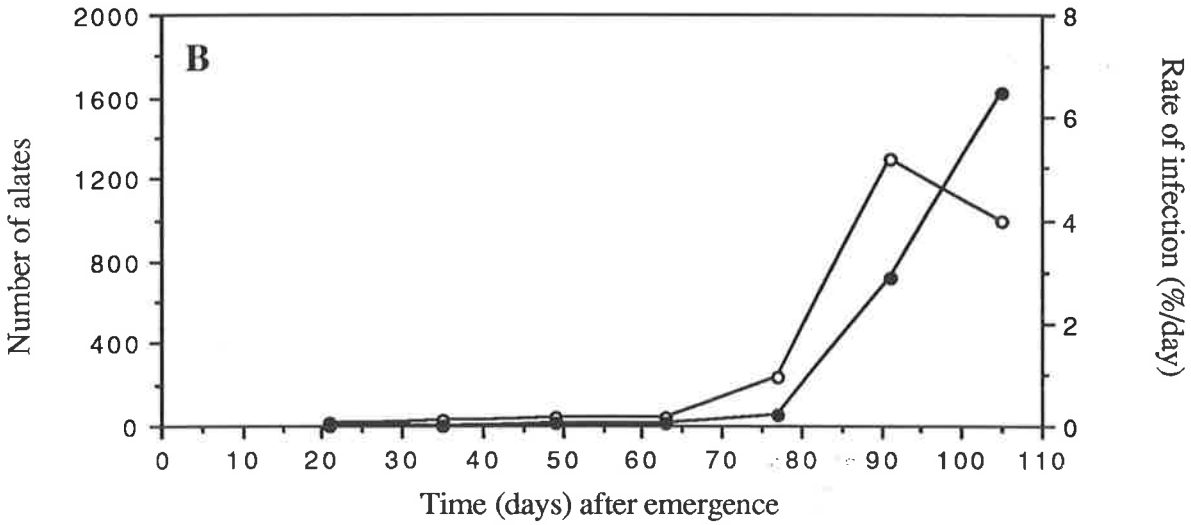
4.4 Discussion

4.4.1 Seasonal patterns of aphid flights

Fig. 4.7: Comparison of the number of alates trapped (○) with the rate of infection (●) in (A) the 1987 field trial and (B) the 1988 field trial.

Total numbers of all species trapped in 2 yellow pans in 1987, and 1 yellow pan in 1988, are shown. For the 1987 field trial, rate of infection was calculated for treatment C (see section 3.2.1). For the 1988 field trial, rate of infection is the mean of the rates calculated for treatments VOA, VO and V (see section 3.2.2).


A
Number of alates
Rate of infection (%/day)
Time (days) after emergence
800
600
400
200
0
4
3
2
1
0
0
20
40
60
80
100
120
140
160


B
Number of alates
Rate of infection (%/day)
Time (days) after emergence
2000
1600
1200
800
400
0
8
6
4
2
0
0
10
20
30
40
50
60
70
80
90
100
110

Aphid flights, measured during the growth period of the lupin crop, peaked in abundance in the late winter/spring period, with a smaller peak in early winter and a lull in mid-winter. This pattern of aphid migration is similar to that observed by O'Loughlin (1962), Hughes *et al.* (1965) and Jayasena (1984) in other trapping programmes in south-east Australia. Maelzer (1981) concluded that many species reached maximum abundance in September and October in south-east Australia because at this time, predators are frequently scarce and plant growth is both abundant and rapid and senescence of the new plant tissue is slow.

In the three year period of the study, *M. persicae*, *R. padi*, *L. erysimi*, *A. craccivora* and *B. rumexicolens* were most commonly trapped. All but *B. rumexicolens* are cosmopolitan in distribution and common in southern Australia, with *M. persicae* colonising a wide range of dicotyledonous plants, *R. padi* colonising a wide range of grasses, *L. erysimi* colonising cruciferous crop and weed plants and *A. craccivora* mainly colonising legumes (O'Loughlin, 1962; Hughes *et al.*, 1965; Eastop 1983).

B. rumexicolens was recently introduced to Australia in 1985 (Carver 1989) and had not been observed in South Australia prior to 1987 (M. Carver, pers. comm.). In 1987, *B. rumexicolens* dominated yellow pan catches, but in the following two years, it was trapped much less frequently. The abundance of this species in yellow pans in 1987 suggests rapid multiplication and dispersal of this aphid since its introduction, similar to that observed when *Therioaphis trifolii f. maculata* and *Acyrthosiphon kondoi* first arrived in Australia (Carver 1989). Carver (1989) has speculated that the early rapid increase in population of an aphid invader may be due to an initial void of parasites and predators and a failure of natural plant populations to resist the new pest. *B. rumexicolens* has been observed to colonise *Rumex crispus* in South Australia (Geering, unpublished results).

4.4.2 Daily patterns of aphid flights

The daily pattern of aphid flights in spring, 1989, was variable and affected by changes in temperature and wind. This is consistent with previous studies which have shown that changes in environmental factors such as light intensity, wind speed, temperature and character of the alighting surface, will either activate or suppress flight (Dixon, 1985; Robert, 1987).

Broadbent (1949) reported the minimum temperature threshold for flight initiation to be 12.8 C for *M. persicae*. On four occasions, *M. persicae* was trapped when the average hourly temperature was below this temperature. Walters and Dixon (1984), in laboratory experiments on behaviour of *R. padi*, found that the minimum temperature for flight initiation to be 15.5 C for alates that fly between secondary grass hosts (alate exules). On eight occasions, flight of *R. padi* was recorded when average hourly temperature was below this temperature.

Although most abundant flights occurred when wind speed was low, some aphids were trapped when wind speed was greater than 10 km/hour. This is consistent with previous work which has shown that strong winds may delay, but not inhibit flight, as when the migratory urge is strong, aphids may take-off in winds faster than their active flying speed (Haine, 1955; Walters and Dixon, 1984; Robert, 1987).

It is difficult to deduce from the evidence presented, the minimum temperature and maximum wind speed thresholds for initiation of flight of the three aphid species studied. Temperature and humidity in a crop canopy may differ markedly from that recorded at a meteorological station, even when it is located as little as 100 metres from the crop (Burrage, 1978). The microclimate of the aphid prior to take-off was not studied. In addition, the flight duration prior to trapping is not known and only the weather conditions when aphids were first trapped were measured.

The close correlation between change in the weather conditions to those that favour initiation of flight and large increases in the number of aphids trapped, suggests that the source of aphids was close to the traps. For example, on September 29 (see Fig. 4.3.6), abundant aphid flights occurred after 11.00 a.m when wind speed decreased to below 5 km/hour. Between 11.00 and 12.00 a.m., aphids would have migrated no further than 4.5 km, if it is assumed that the maximum flight speed of the aphid was that of the wind.

4.4.3 Comparison of aphid trapping methods

The species composition of aphid trap catches differed between the yellow pan and suction traps. These differences may have resulted from -

(a) differences in the attractiveness of yellow to aphid species. *A. craccivora*, *L. erysimi*, and *M. persicae* are highly attracted to yellow compared with *R. padi* (Eastop,1955; Heathcote, 1957; O'Loughlin, 1962).

(b) differences between the aphid populations sampled by the traps. The yellow pans were situated outside the lupin crop, over bare ground, whereas the suction traps were located within the plot of lupins and mounted at the height of the canopy. The suction traps are considered to have only collected aphids flying near the canopy, presumably whilst attempting to find new hosts. The yellow pans may have attracted aphids flying at greater heights.

Flights of *R. padi* were simultaneously detected in the yellow and suction traps. This result suggests that migrating *R. padi* were seeking new hosts in the lupin crop.

Large numbers of *L. erysimi* were trapped in the yellow pans in spring, but they were rarely trapped in the suction traps. This difference in trapping efficiency could partially be explained by the large attraction of *L. erysimi* to yellow (O'Loughlin, 1962). Numbers

trapped in the suction traps could also have been limited if the aphid could appraise host suitability at a distance and did not descend to the canopy height, or if it alighted for an exploratory probe, then flew on and left the crop.

The efficiency of collection of *M. persicae* by the suction traps was lower in spring than in early winter. Assuming that the extent to which *M. persicae* was attracted to yellow did not change at different times of the year, change in trapping efficiency may have resulted from changes in the settling behaviour of the aphid. Increased settling in spring may have reduced the number of aphids flying above the canopy.

4.4.4 Aphid colonisation

Aphid colonies did not develop on the lupins. *A. kondoi*, *A. craccivora*, *M. euphorbiae* and *M. persicae* larviposited, but most nymphs collected were first or second instar, which suggests that nymphs did not reach maturity and consequently colonies did not develop. These results are in contrast to those from Western Australia, where large infestations of the same four aphid species occur in lupins (Sandow, 1987).

Alates of non-colonising species, such as *R. padi* and *B. rumexicolens*, were collected from the lupins. In 1988 and 1989, most alatae collected were those of colonising aphids such as *M. persicae*, *A. kondoi* and *A. craccivora*. These aphids may not have had greater landing rates than other non-colonising species, but slower rates of departure. This conclusion is supported by the results of the suction and tile traps in 1989, which most frequently trapped *R. padi*.

4.4.5 Transmissibility of CMV-B_{SA} by different aphid species

Common aphid species, including *M. persicae* and *R. padi*, were shown to be capable of transmitting CMV-B_{SA}. *L. erysimi*, *M. euphorbiae* and *M. dirhodum* did not

transmit CMV in the glasshouse transmission tests. They may transmit at a very low efficiency and the number of aphids used in these experiments was too small to detect transmission. Results may also differ if other aphid biotypes or CMV strains are tested. In contrast to the results presented in this chapter, *M. euphorbiae* transmitted CMV between gladioli more efficiently than *M. persicae* (Aly *et al.*, 1986). Jones (1991) reported *L. erysimi* to be capable of transmitting CMV between lupins. The clone of *L. erysimi* used in the transmission tests described in this chapter is capable of transmitting lettuce isolates of CMV between lettuces (D. Graetz, pers. comm.).

4.4.6 Relationship between aphid flights and field spread of CMV

Field spread of CMV was correlated with the spring flights of aphids. This, and the absence of evidence for aphid colonisation of the lupins, suggests that migratory alates are the vectors of CMV.

In 1987, spread of CMV in early spring followed a flight of aphids that consisted primarily of *R. padi*. This, and other results that show that *R. padi* alights on the lupins and is capable of transmitting CMV, suggests that this species is important as a vector.

The relationship between aphid flights and epidemic progress is further investigated in chapter 5.

Chapter 5

Modelling of epidemic progress

5.1 Introduction

Quantification of the temporal and spatial progression of an epidemic provides a means by which epidemics can be compared, a theoretical framework by which factors affecting the rate of disease increase may be better understood and a capacity to predict the outcome of epidemics (Madden and Campbell, 1986; Campbell and Madden, 1990).

Models have been proposed to describe changes in incidence of infectious agents within the single dimensions of either time or space (Madden and Campbell, 1986; Minogue, 1986; Waggoner, 1986; Campbell and Madden, 1990). More recently, progress has been made in the development of models that incorporate both these dimensions (Jeger, 1983). Two simple models describing the temporal development of epidemics are the monomolecular and the logistic models. The biological interpretations of these models, as provided by Vanderplanck (1963), are that the monomolecular model applies to epidemics in which all new infections arising in a season originate from the single inoculum source, whereas the logistic model applies to epidemics in which new infections in turn act as sources of inoculum and many cycles of infection occur within a season.

The logistic model predicts an epidemic in which incidence of infection increases with time in a sigmoidal manner, with an inflection point at 50 % infection (Campbell and Madden, 1990). In practice, the absolute growth rate of many epidemics is observed to slow at a rate faster than that predicted by the logistic model, due to factors such as the development of mature age resistance in the crop. In such circumstances, other models such as the Gompertz model or models with variable shape parameters have been found to be

more suitable to describe the temporal progress of the epidemic (Berger, 1981; Madden *et al.*, 1987).

Epidemic growth models such as the logistic model are too simplistic in that they assume that immediately a plant becomes infectious, inoculum will be dispersed. Most plant viruses are dispersed via the action of vectors (Matthews, 1991). In the absence of vectors, virus spread will not occur, regardless of the number of sources of inoculum. This characteristic of plant virus epidemics has led to the introduction of models that describe changes in incidence of infection as a function of vector numbers, rather than of time (Madden *et al.*, 1990b)

Models have also been proposed to quantify infection gradients. These models describe the rate of decrease in incidence of infection with increasing distance from the inoculum source. Jeger (1983) has provided 4 gradient models, which correspond with models advocated by Gregory (1968) and Kiyosawa and Shiyomi (1972). When biological interpretations are made of these models, they differ both in the effect of distance on dilution of inoculum and on the contribution of secondary plant infections as sources of inoculum (Jeger, 1983; Campbell and Madden, 1990).

In this chapter, an attempt is made to quantify the temporal and spatial development of the CMV epidemics observed in the field trials. Simple models are used to provide further insight into factors that affect the rate of epidemic progress.

5.2 Methods

5.2.1 Frequently used symbols and their definitions

ln - Natural logarithm.

t - Time, measured in days.

y - Incidence of CMV infection, measured as a proportion.

A - Cumulative number of vectors.

s - Distance, measured in centimetres.

dy/dt - Absolute growth rate, which is the change in y with an infinitesimal change in time t (Campbell and Madden, 1990).

5.2.2 Modelling increases in incidence of infection as a function of numbers of vectors

The vector models listed in Table 5.1 were fitted to experimental data obtained from the epidemic investigated in the 1987 field trial (see chapters 3 and 4).

Table 5.1: Four alternative models to describe epidemic progress as a function of vector numbers (Madden *et al.*, 1990b) A is the cumulative number of vectors; B is the constant of integration and k is the rate parameter. The parameters of k for each model are not directly comparable.

Model	dy/dA =	y =	Linear form
1	$k(1 - y)$	$1 - Bexp(-kA)$	$\ln[1/(1 - y)] = -\ln(B) + kA$
2	$k(1 - y)/A$	$1 - BA^{-k}$	$\ln[1/(1 - y)] = -\ln(B) + k\ln(A)$
3	$ky(1 - y)$	$1/[1 + Bexp(-kA)]$	$\ln[y/(1 - y)] = -\ln(B) + kA$
4	$ky(1 - y)/A$	$1/(1 + BA^{-k})$	$\ln[y/(1 - y)] = -\ln(B) + k\ln(A)$

Vector numbers were the combined total of alate aphids trapped in two yellow pans positioned adjacent to the plots. A latent period of 2 weeks was assumed and therefore at time t, the cumulative number of vectors was the number of alates trapped on the day t-2 weeks. Incidence of infection was taken as the proportion of infected plants in treatment C, as determined by ELISA (see section 3.2.1.3). Only values of y for which corresponding

information on A was available were used for modelling. The designated beginning of the epidemic (when $A = 0$) was June 17. The incidence of infection at $A = 0$, which is the incidence of infection found on July 1, was greater than zero, due to the occurrence of seed transmission. The following adjustment was made to y values to allow for maximum possible increase in incidence of infection being less than one -

$$y' = (y-y_0)/(1-y_0)$$

where y' is the adjusted y value and y_0 is the value of y when $A = 0$.

Two approaches were taken to determine cumulative vector numbers-

(1) The five most abundant aphid species trapped that were shown to be capable of transmitting CMV (see section 4.3.6), namely *A. craccivora*, *B. rumexicolens* and *D. aucupariae*, *M. persicae* and *R. padi*, were considered to have equal vector propensity and the total number of these species was used for calculations. These 5 aphid species, and the non-vector *L. erysimi* (see sections 4.3.3 and 4.3.6), comprised 94 % of the total numbers of aphids trapped in the yellow pans in the period between June 17 and October 14.

(2) The number of *R. padi* trapped was tallied. For the period of June 17 to September 16, *R. padi* was considered to be the most important vector, as it was the dominant species trapped in the yellow pans (see Table 5.2).

To determine the model parameters k and B, incidence of infection was transformed using either $\ln[1/(1-y)]$ for models 1 and 3, or $\ln [y/(1-y)]$ for models 2 and 4, and plotted against either A (models 1 and 3) or $\ln A$ (models 2 and 4). Parameter estimates were found by linear regression analysis: k is the slope and $-\ln (B)$ is the y axis intercept of the line.

Table 5.2: Aphid species trapped in the yellow pans in the period from June 17 to September 16.

Aphid species	Number trapped	Trap composition (%)
R. padi	272	55.5
M. persicae	70	14.3
L. erysimi	60	12.2
B. rumexicolens	59	12.0
Other species	29	5.9
Total	490	

5.2.3 Modelling temporal progress of the epidemic

Monomolecular and logistic growth models (see Table 5.3) were fitted to the experimental data obtained from the 1987 field trial.

Table 5.3: Mathematical expressions for the monomolecular and logistic models (Campbell and Madden, 1990). B is the constant of integration and r is the rate parameter. The parameters of r for each model are not directly comparable.

Model	dy/dt =	y =	Linear form	Units of r
Monomolecular	$r(1 - y)$	$1 - B\exp(-rt)$	$\ln[1/(1-y)] = \ln(B) + rt$	time^{-1}
Logistic	$ry(1 - y)$	$1/[1 + B\exp(-rt)]$	$\ln[y/(1-y)] = \ln(B) + rt$	time^{-1}

To determine the model parameters r and B, incidence of infection was transformed using either $\ln[1/(1-y)]$ for the monomolecular model, or $\ln [y/(1-y)]$ for the logistic model, and plotted against t. Parameter estimates were found by linear regression analysis: r is the slope and $\ln B$ is the y axis intercept of the line.

5.2.4 Modelling infection gradients

The models listed in Table 5.4 were fitted to the gradient data obtained from the 1988 field trial on September 7 (see section 3.3.3.2). Incidence of infection was transformed using either $\ln[1/(1-y)]$ for models 1 and 3, or $\ln [y/(1-y)]$ for models 2 and 4, and plotted against either s, for models 1 and 3, or $\ln s$, for models 2 and 4. Estimates of b and a were found by linear regression analysis: b is the slope of the regression line and $\ln(a)$ is the y axis intercept. To test for differences in the infection gradients observed in treatments VOA, VO and V, the slope and y axis intercepts of the regression lines were compared and if no significant differences were found, then one regression line was calculated for data from the three treatments.

Table 5.4: Four alternative models to describe the shape of infection gradients (Jeger 1983). b is the parameter for steepness of the gradient and a is the constant of integration. The parameters of b for each model are not directly comparable.

Model	$dy/ds =$	$y =$	Linear form	Units of b
(1)	$-b(1 - y)$	$1 - a \exp(bs)$	$\ln\{1/(1- y)\} = \ln(a) - bs$	distance^{-1}
(2)	$-by(1 - y)$	$1/[1 + a \exp(bs)]$	$\ln\{y /(1- y)\} = \ln(a) - bs$	distance^{-1}
(3)	$- b(1 - y)/s$	$1 - as^b$	$\ln\{1/(1-y)\} = \ln(a) - b\ln(s)$	dimensionless
(4)	$- by(1 - y)/s$	$1/(1 + as^b)$	$\ln\{y/(1-y)\}=\ln(a) - b\ln(s)$	dimensionless

5.2.5 Transformation of y when $y = 0$ or 1

When $y = 1$, a corresponding transformed value cannot be defined using the transformation of ln $y/(1-y)$ or ln $1/(1-y)$. A similar problem exists when $y = 0$ with the transformation ln $y/(1-y)$. In cases where y could not be transformed, the data point was treated as a missing value.

5.2.6 General criteria for the selection of the most appropriate model

To select the most suitable model, the 'goodness of fit' of the linearised forms of the models fitted to the experimental data was assessed using the coefficient of determination (R^2) and the shape of the plot of residual errors. The incidence of infection predicted by the models was compared with that obtained experimentally, and the coefficients of determination (R^{*2}) for each model compared.

5.3 Results and Discussion

5.3.1 Modelling epidemic progress in 1987 as a function of the cumulative number of *M. persicae*, *R. padi*, *A. craccivora*, *D. aucupariae* and *B. rumexicolens*.

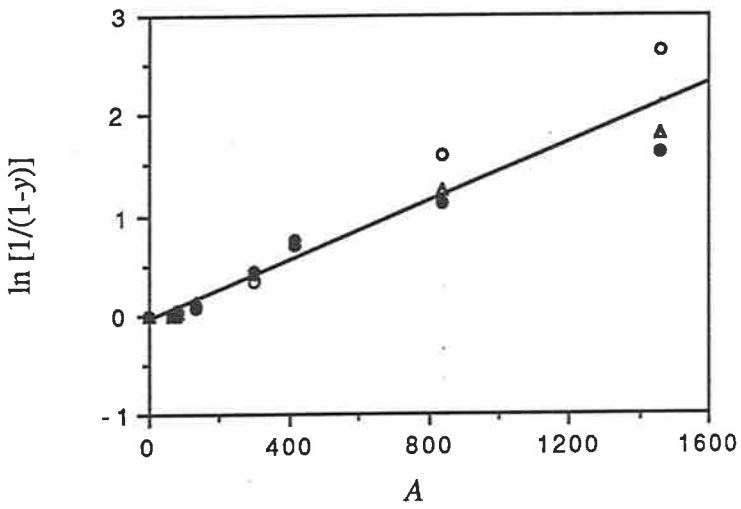
The linear forms of the vector models fitted to experimental data are illustrated in Fig. 5.1 and estimates of the model parameters are shown on the graphs. The progress of the epidemic predicted by each of the models is shown in Fig. 5.2. On the basis of the R^2 and R^{*2} values, models 1 and 4 are the best fitting models. A superior plot of residuals (see Appendix 3.5) was obtained for model 4, with the plot for model 1 showing greater heterogeneity of variance.

The appropriateness of the vector models was also assessed by considering the temporal progress of the epidemic. The epidemic was well described by the logistic model,

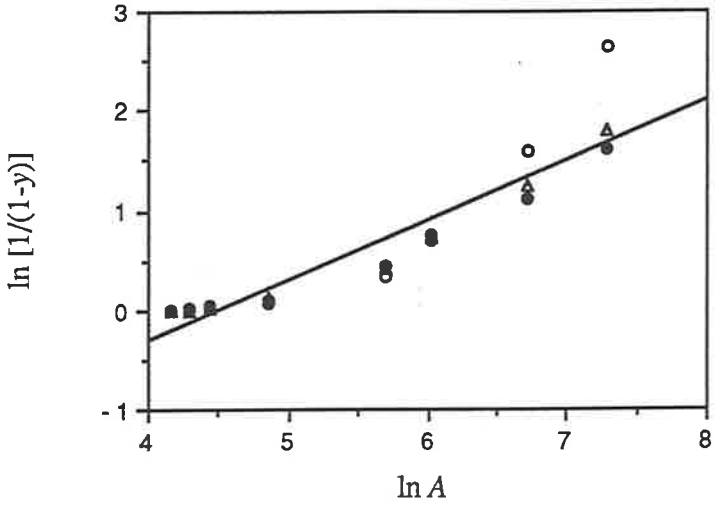
Fig. 5.1: Estimation of vector model parameters (see Table 5.1) by linear regression analysis.

A is the cumulative number of *A. craccivora*, *B. rumexicolens*, *D. aucupariae*, *M. persicae* and *R. padi* trapped in the yellow pans. Incidence of infection is that found in treatment C of the 1987 field trial. (○) is for replicate plot 1; (●) is for replicate plot 2; (△) is for replicate plot 3; (—) is the regression line.

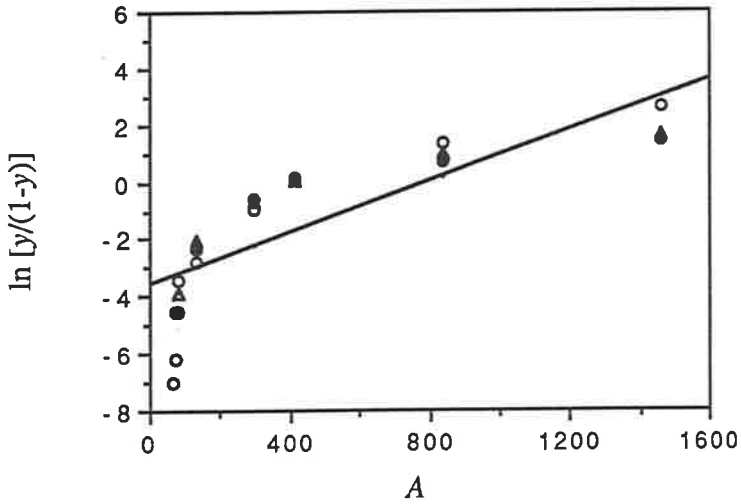
Model 1



Model 2



Model 3



Model 4

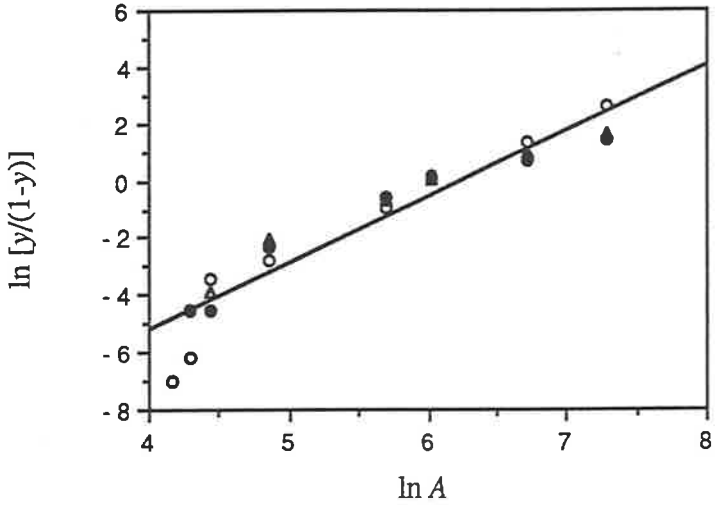
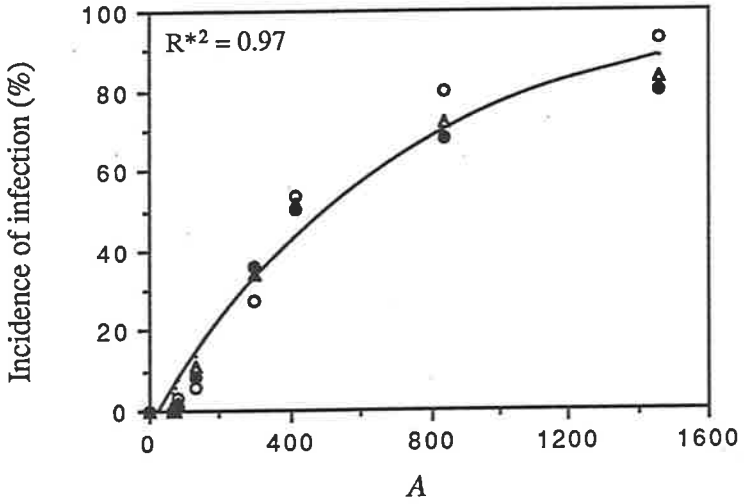


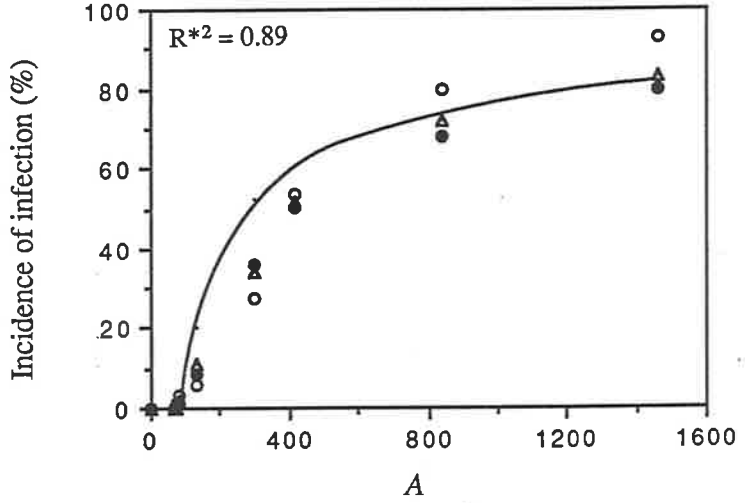
Fig. 5.2: Comparison of epidemic progress observed in treatment C of the 1987 field trial with that predicted by the vector models.

A is the cumulative number of *A. craccivora*, *B. rumexicolens*, *D. aucupariae*, *M. persicae* and *R. padi* trapped in the yellow pans. (○) is for replicate plot 1; (●) is for replicate plot 2; (Δ) is for replicate plot 3; (—) is the incidence of infection predicted by the vector models.

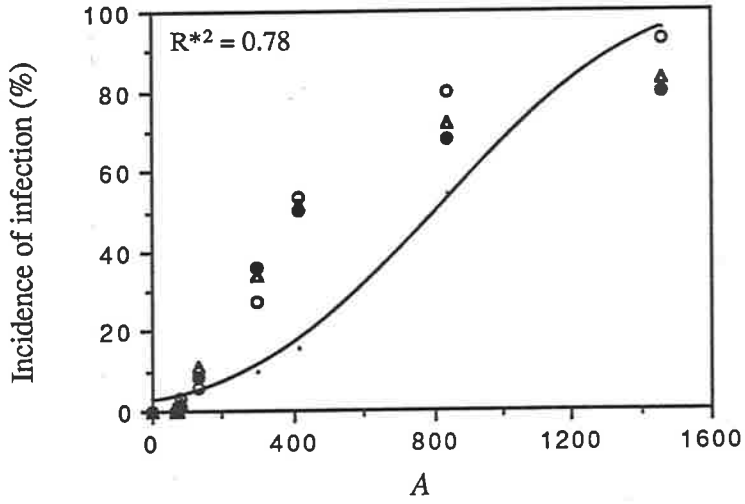
Model 1



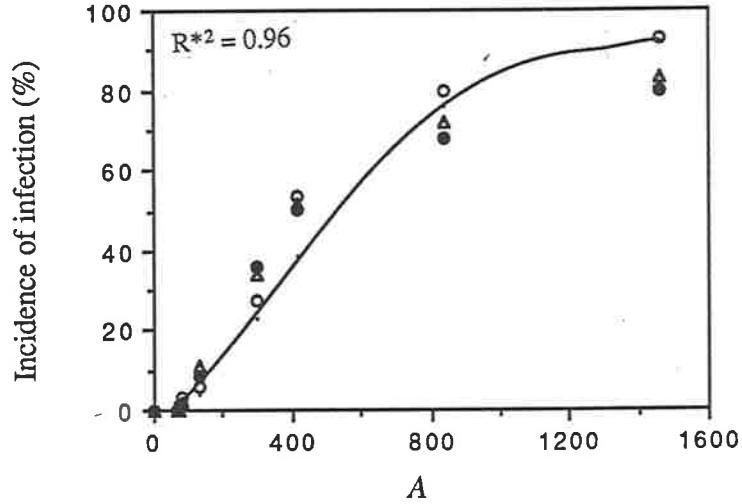
Model 2



Model 3



Model 4



as is shown in Fig. 5.3. Assuming logistic growth of the epidemic, then exponential increase in *A* with time is predicted if vector model 4 is the most appropriate model (see Table 5.6). In the period of the epidemic, the increase in *A* with time was in fact well described by the exponential model, as is shown in Fig. 5.4.

Table 5.6: Rate of increase in *A* with time when temporal progress of the epidemic is described by either the logistic or the monomolecular model and the functional relationship between *y* and *A* is described by either vector model 1 or 4 (Madden *et al.*,1990b) *r* is the rate parameter for the monomolecular and logistic models, and *k* is the rate parameter for the vector models.

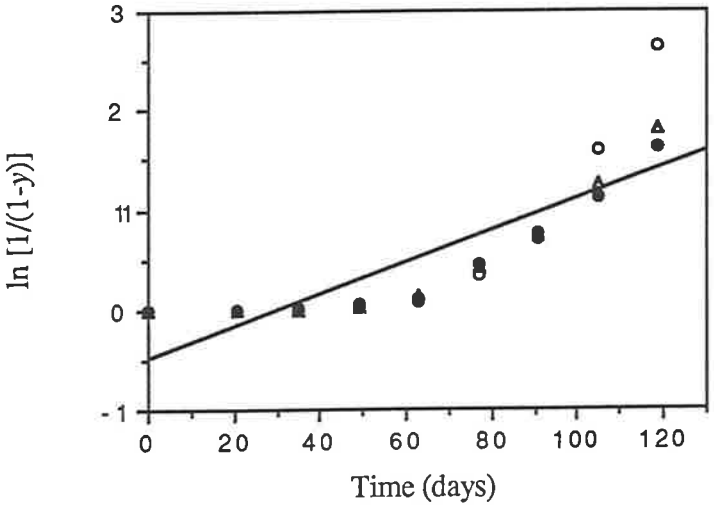
Vector model	dA/dt = Monomolecular	Logistic
1	$(r/k)y$	(r/k)
4	$(r/k)A$	$(r/k)A/y$

Vector model 1 predicts *dA/dt* to be proportional to *y* for logistic growth of *y* with time. This was considered to be illogical by Madden *et al.* (1990b) unless "the infected plants had a positive effect on aphid development". This is considered to be unlikely as *R. padi*, *B. rumexicolens* and *D. aucupariae* do not colonise lupins and colonisation by *M. persicae* and *A. craccivora* was insignificant (see section 4.3.5). Alternatively, if the temporal progress of the epidemic was described by the monomolecular model and vector model 1 was also appropriate, then *dA/dt* would be constant. Neither a linear relationship between *A* and *t* nor a monomolecular increase in *y* was observed (see Figs. 5.3 and 5.4). Therefore it can be concluded that vector model 4 is the most realistic biological model to describe the epidemic.

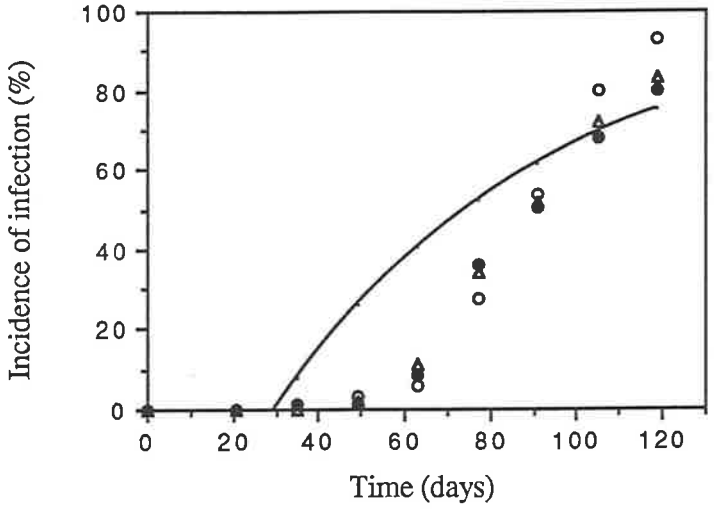
Fig. 5.3: (A) Estimation of monomolecular and logistic model parameters (see Table 5.3) by linear regression analysis and (B) comparison of the epidemic progress in treatment C of the 1987 field trial with that predicted by the two temporal models.

(○) is for replicate plot 1; (●) is for replicate plot 2; (Δ) is for replicate plot 3; (—) is the regression line (A) and the incidence of infection predicted by the temporal models (B).

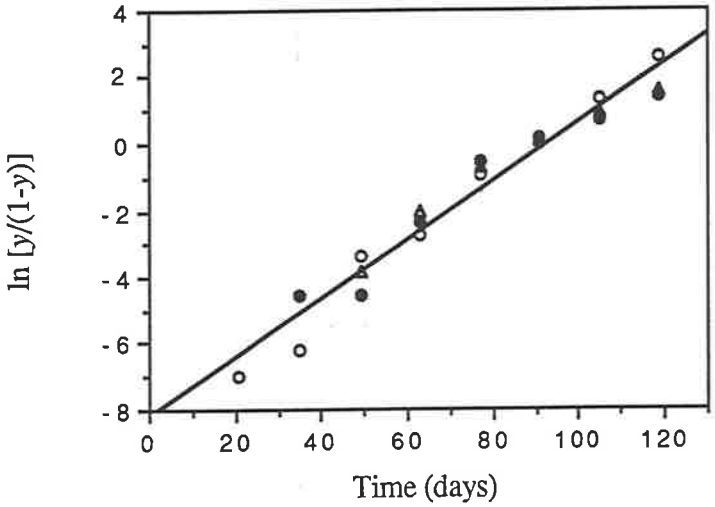
Monomolecular (A)



Monomolecular (B)



Logistic (A)



Logistic (B)

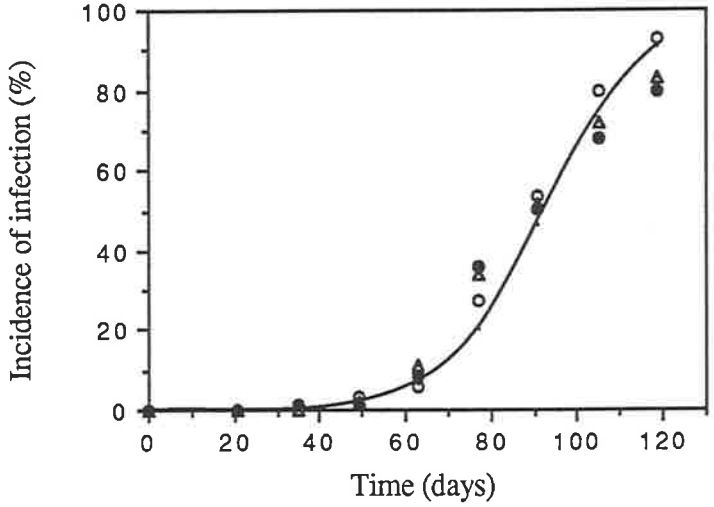
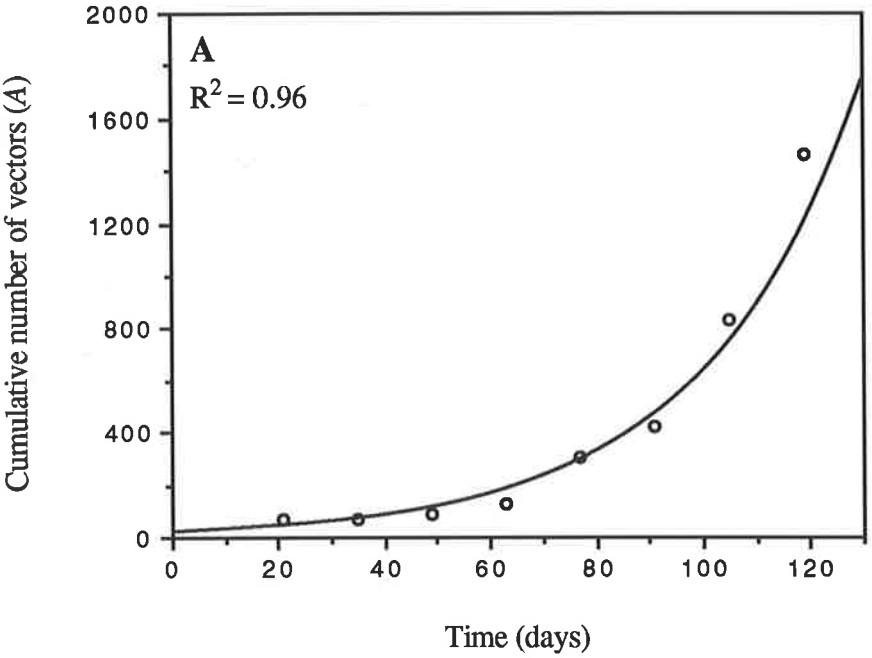
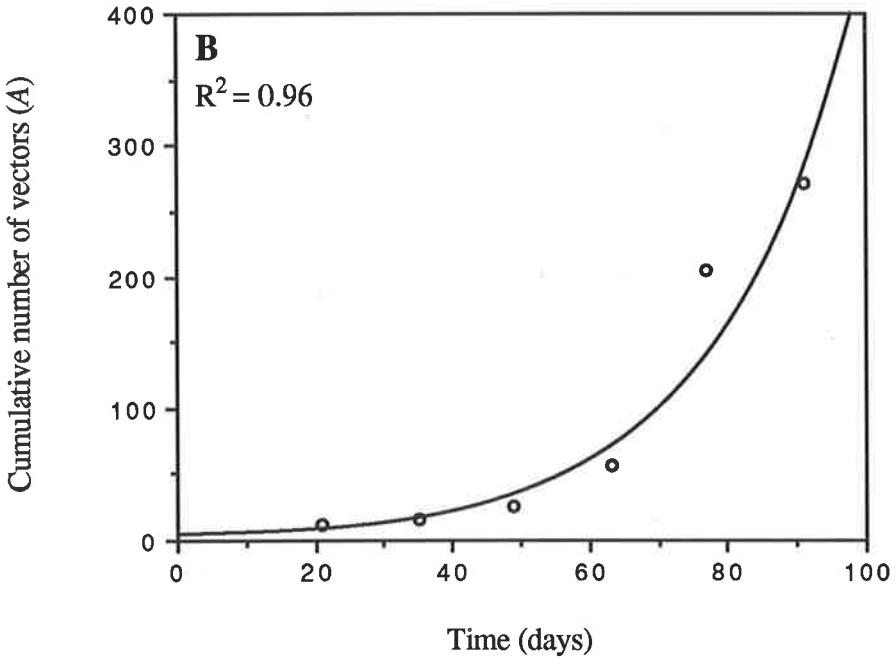


Fig 5.4: Increase with time in the cumulative number of aphid vectors (*A*).

Vector species are for (A) *M. persicae*, *R. padi*, *A. craccivora*, *B. rumexicolens* and *D. aucupariae* and (B) *R. padi*. (—) is the cumulative number of vectors predicted by the exponential model.


A
$R^2 = 0.96$
Cumulative number of vectors (A)
Time (days)
0
400
800
1200
1600
2000
0
20
40
60
80
100
120


B
$R^2 = 0.96$
Cumulative number of vectors (A)
Time (days)
0
100
200
300
400
0
20
40
60
80
100

5.3.2 Modelling epidemic progress in 1987 as a function of the cumulative number of *R. padi*

There are two limitations to the preceding approach to modelling in which the cumulative number of vectors was the total of 5 aphid species trapped in the yellow pans. These are -

(a) Yellow pan traps provide a poor estimate of the relative aerial density of aphid species and also of their landing rates on the lupins.

(b) Vector propensity may differ between species.

To account for these limitations, a second analysis was conducted in which an increase in y in the period July 1 to September 30 was modelled as a function of the cumulative number of *R. padi*. The linear forms of the vector models fitted to the experimental data are illustrated in Fig. 5.5 and estimates of the model parameters shown on the graphs. The progress of the epidemics predicted by each of the models is shown in Fig. 5.6.

Again, on the basis of R^2 and R^{*2} values, models 1 and 4 are the most appropriate models, but model 4 had a superior plot of residuals (see Appendix 3.6). The increase in cumulative number of *R. padi* with time was well described by the exponential model, as is shown in Fig. 5.4. This is predicted when increase in y with time is logistic (see Fig. 5.3), and vector model 4 is the most appropriate model.

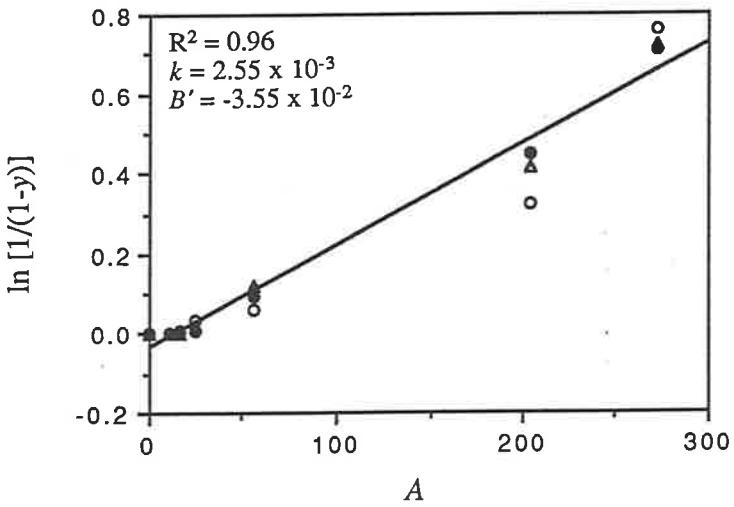
5.3.3 Biological interpretation of vector model 4.

Model 4 can be written as: $dy/dt = ky(dA/dt)(1-y)/A$ (Madden *et al.*, 1990b). The absolute growth rate of the epidemic is proportional to y, dA/dt and $1/A$. This can be

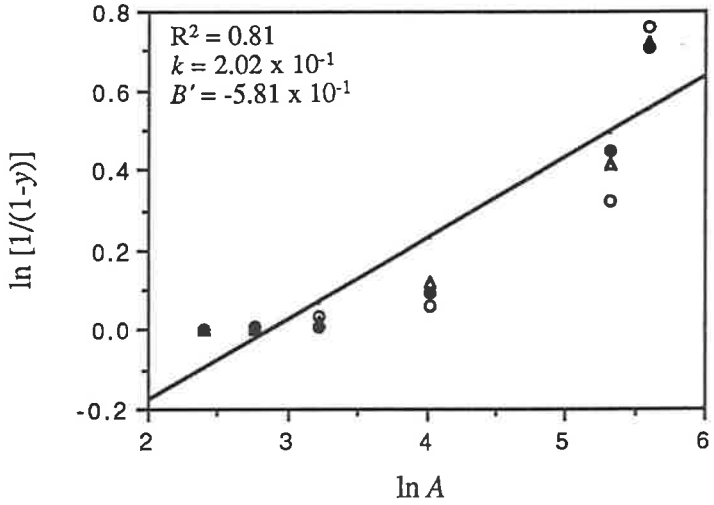
Fig. 5.5: Estimation of vector model parameters (see Table 5.1) by linear regression analysis.

A is the cumulative number of *R. padi* trapped in the yellow pans. Incidence of infection is that found in treatment C of the 1987 field trial. (○) is for replicate plot 1; (●) is for replicate plot 2; (Δ) is for replicate plot 3; (—) is the regression line.

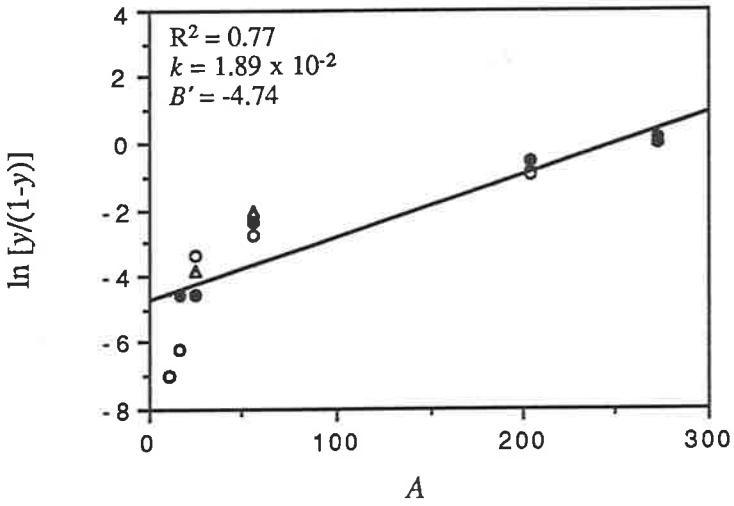
Model 1



Model 2



Model 3



Model 4

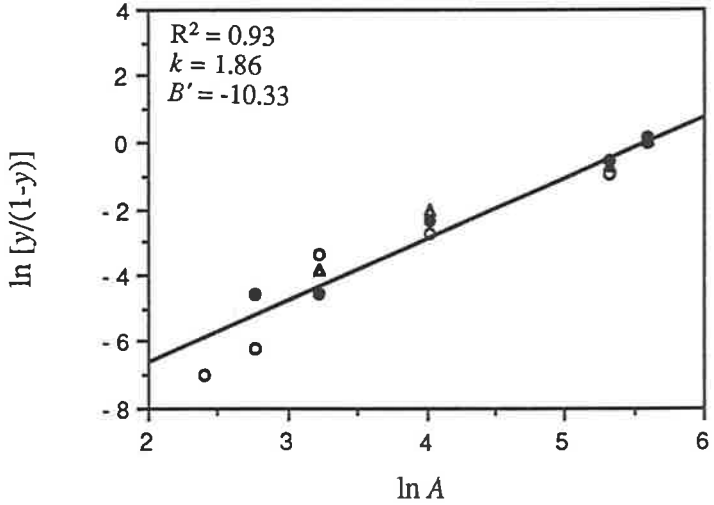
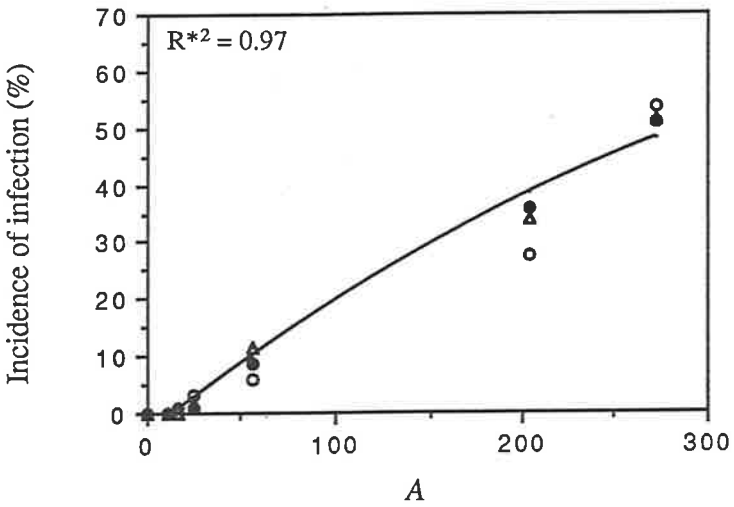


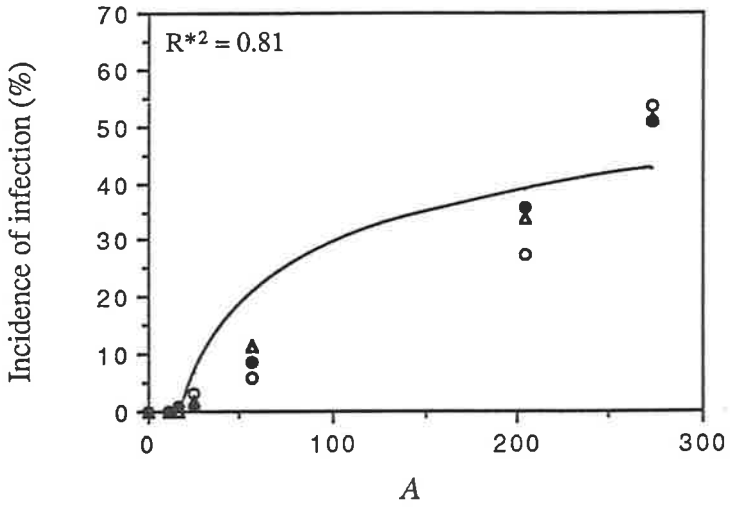
Fig. 5.6: Comparison of epidemic progress observed in treatment C of the 1987 field trial with that predicted by the vector models.

A is the cumulative number of *R. padi* trapped in the yellow pans. (○) is for replicate plot 1; (●) is for replicate plot 2; (Δ) is for replicate plot 3; (—) is the incidence of infection predicted by the vector models.

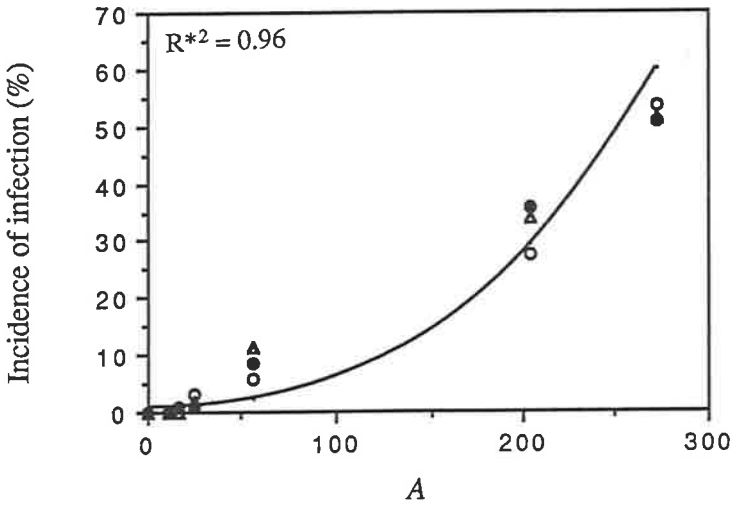
Model 1



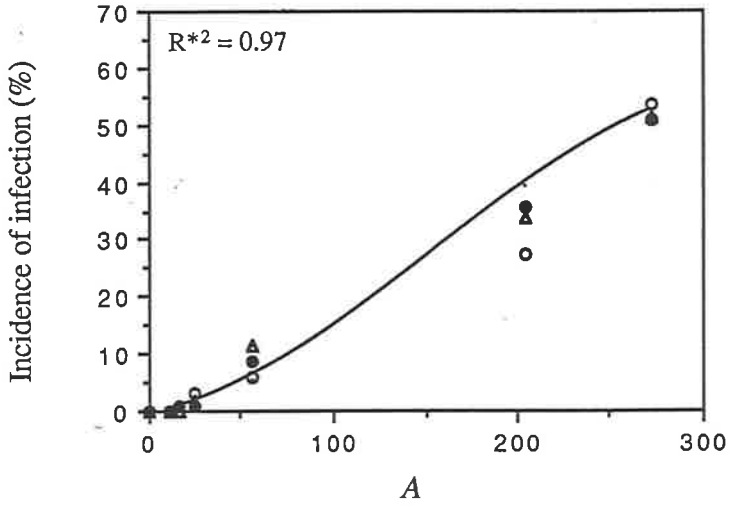
Model 2



Model 3



Model 4



interpreted as meaning that virus acquisition increases as the incidence of infection increases, that an increase in the number of aphids entering the crop in a given time period will cause an increase in the absolute growth rate of the epidemic and that the probability of transmission decreases as the cumulative number of vectors increases.

There are many factors that could contribute to the probability of transmission decreasing with plant age, such as an increasing difficulty of inoculation, decreasing effectiveness of infected lupins as sources of inoculum because of stunting or reduction in the virus concentration and the development of a non-random pattern of infected plants. Alternatively, the inverse relationship between dy/dt and A may be an artifact caused by the use of yellow pan traps to determine A and also an incorrect assumption of equal vector propensity amongst the aphid species. *R. padi* was most frequently caught at the beginning of the epidemic and its relative aerial density would have been underestimated compared with aphids such as *M. persicae* and *A. craccivora*, which were most common at the end of the epidemic (see section 4.3.2). For this reason, A may not have increased exponentially as indicated by the yellow pan trap results.

In the second modelling approach in which only *R. padi* was considered a vector, problems associated with use of the yellow pan traps to determine A and potential differences in vector propensity between species were avoided. The constant difference between number of *R. padi* landing on the crop and number being trapped in the yellow pans would have been incorporated in the rate parameter k.

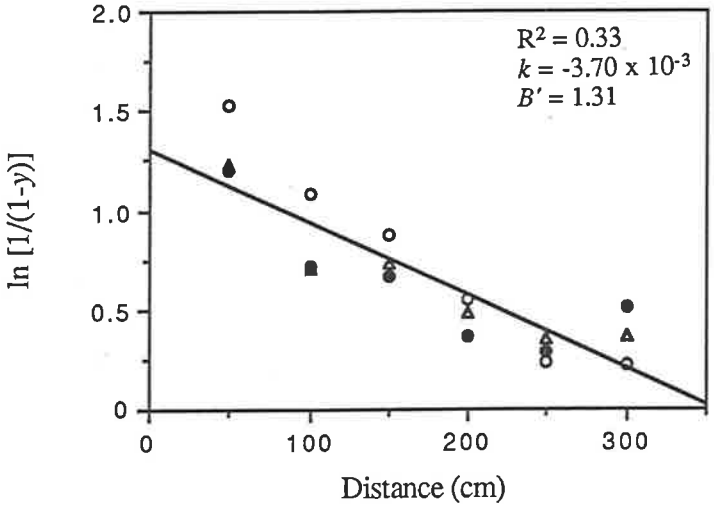
5.3.4 Modelling of the infection gradients observed in 1988

The linear forms of the gradient models fitted to the experimental data are illustrated in Fig. 5.7 and the estimates of the model parameters are shown on the graphs. The gradients predicted by each of the models are shown in Fig. 5.8. On the basis of R^2 and

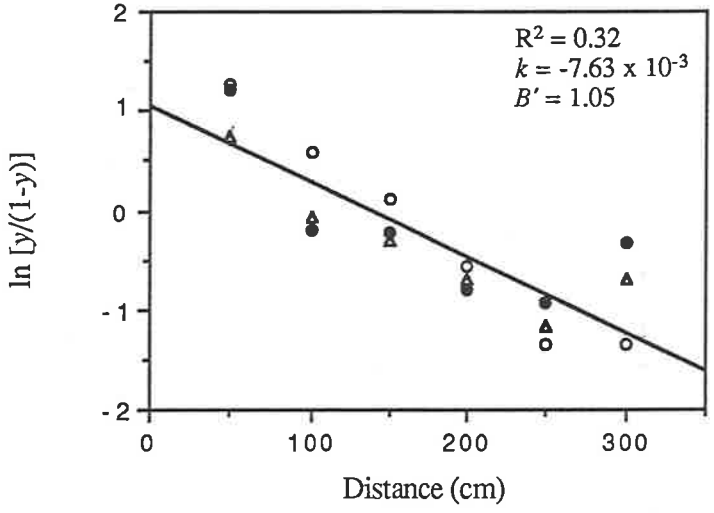
Fig. 5.7: Estimation of gradient model parameters (see Table 5.4) by linear regression analysis.

Incidence of infection is that found in the 1988 field trial on September 7. For all four models, no significant differences ($P>0.05$) were found between the slopes and y axis intercepts of the regression lines for treatments VOA (○), VO (●) and V (△). Therefore, one regression line (—) for data from all three treatments was fitted.

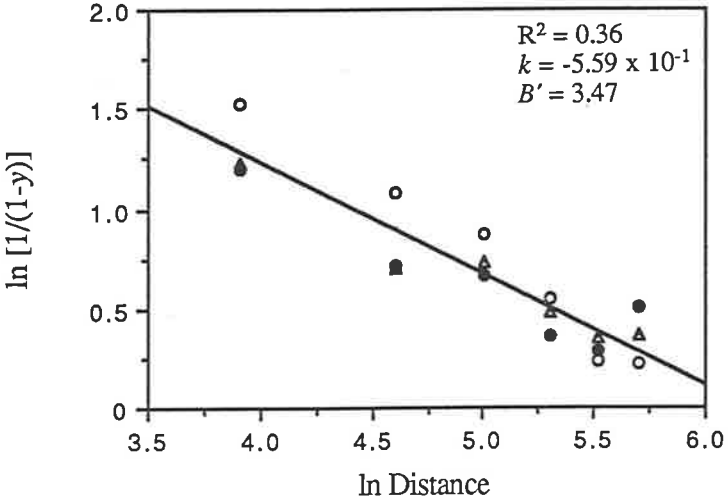
Model 1



Model 2



Model 3



Model 4

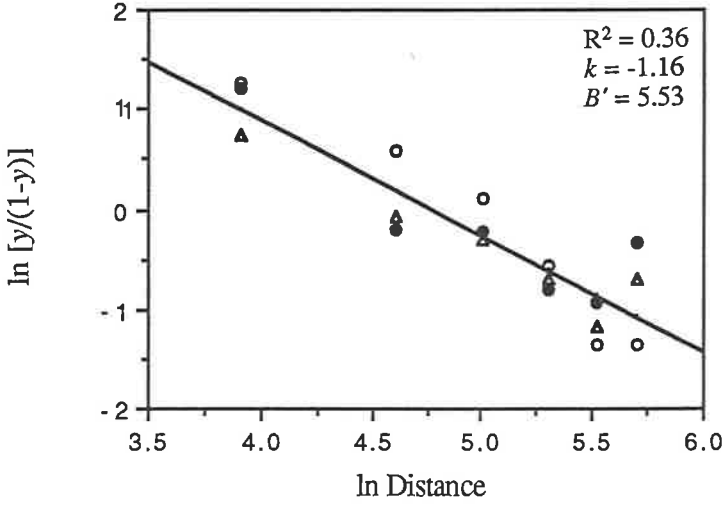
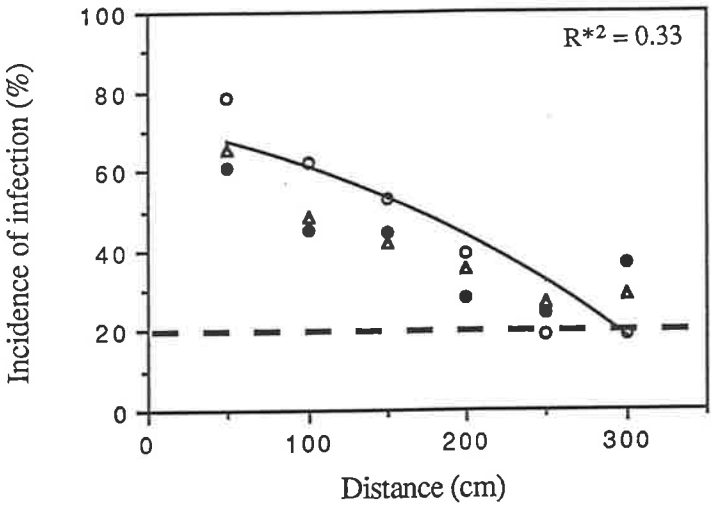


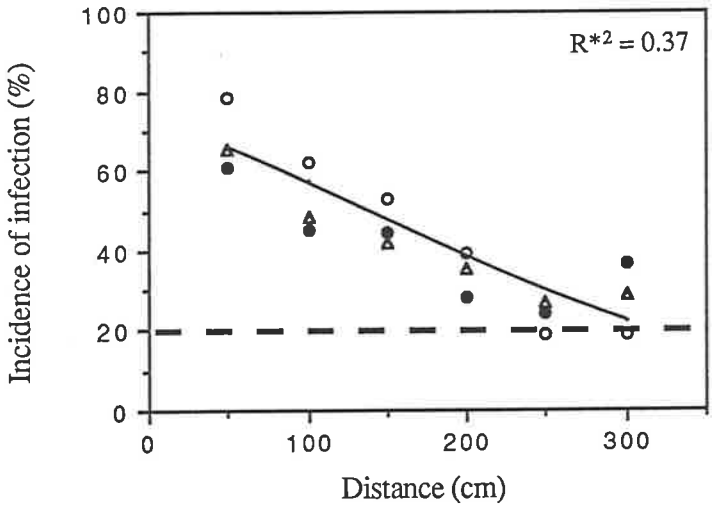
Fig. 5.8: Comparison of the infection gradients observed in the 1988 field trial on September 7, with that predicted by the gradient models.

(○) is for treatment VOA; (●) is for treatment VO; (Δ) is for treatment V; (—) is the incidence of infection predicted by the gradient models; (- -) is the incidence of infection in treatment C which is the level of background infection in treatments VOA, VO and V.

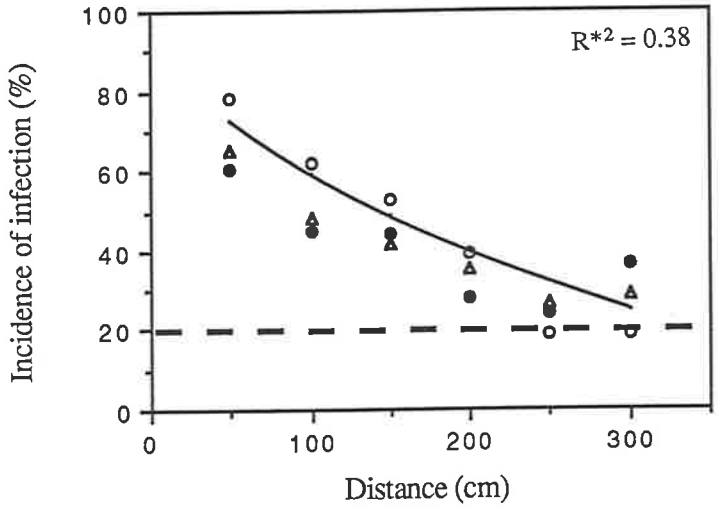
Model 1



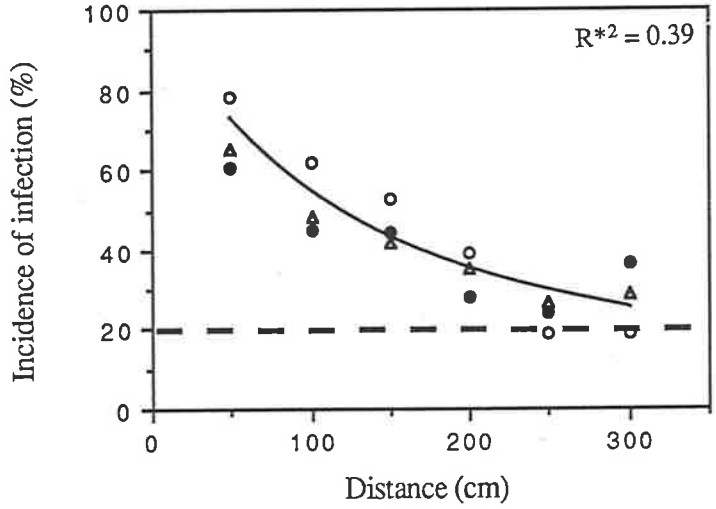
Model 2



Model 3



Model 4



3·7

R^{*2} values and the shapes of the plots of residuals (see Appendix), models 3 and 4 are the best fitting models.

5.3.5 Biological interpretation of the gradient models

Jeger (1983) suggests that model 3 applies to infection gradients that form during a monocyclic epidemic, in which all inoculum originates from a primary source, and inoculum is diluted with increasing distance from this source. Model 4 is considered to apply to infection gradients which form at the beginning of a polycyclic epidemic, where the majority of inoculum originates from the primary source (Jeger, 1983). Both these interpretations are biologically realistic. The infection gradients were observed 3 weeks after the first abundant flights of aphids in spring (see Fig. 4.2). Prior to these flights, little virus spread had occurred; incidence of infection was less than 2.1 % on August 10 (see Fig. 3.5). The linear sources of inoculum would have been the dominant sources of inoculum. If it is assumed that there is a 2 week latent period, not enough time would have elapsed to allow detection of two cycles of infection following the spring aphid flights.

Each plot had 2 linear sources of inoculum which were arranged along opposite sides of the plot. There is the possibility that the gradients arising from opposite sides of the plot have merged in the middle. At 300 cm. distance from the infection focus, incidence of infection had decreased to the level found in the control plots (see Fig. 5.6), which is the level of 'background' infection found in treatments VOA, VO and V.

The gradient models poorly fitted the experimental data and this is considered to have been a consequence of -

(a) the large variation in incidence of infection at each given distance from the infection focus. A source of this variation may have been from the random formation of secondary

infection foci from longer distance movement of inoculum between treatments (see section 3.6).

(b) the small number of distance measurements of incidence of infection used to fit the models. The gradient was very steep and a smaller row spacing may have provided a greater number of data points that could be used to fit the gradient. Changing the density of plants may, however, have had an unpredictable effect on the nature of the gradient. Decreasing the row spacing may increase the steepness of the gradient if aphid movement is merely a process of flitting between neighbouring plants. Increasing the plant density may have influenced the type of spread occurring. For example, a greater component of spread may result from aphids walking across canopy bridges. Alternatively to decreasing row spacing, plot size could be increased so that the infection gradient could be measured over a greater distance.

Chapter 6

Seed transmission of CMV and the effect of CMV infection on lupin productivity

6.1 Introduction

Annual hosts die following completion of reproduction and there is therefore strong selection either for variants of a virus that are transmitted vertically in seed or which are able to infect alternative hosts. In South Australia the summer period of drought prevents most annual plant growth in non-irrigated areas and seed transmission of viruses such as CMV would ensure reintroduction of randomly dispersed primary virus sources into the next season's crop (Stace-Smith and Hamilton, 1987). Seed transmission would also provide an efficient means of long distance dispersal of viruses through commercial trading of seed. If a virus also has a wide host range, such as CMV, there would be a high probability that several oversummering species could act as alternative hosts of the virus.

Epidemics of CMV occurred in lupins in New South Wales in 1978 (Bowyer and Keirnan, 1981) and in South Australia after 1982 (Alberts *et al.*, 1985) and the ability of the lupin infecting isolates of CMV to be seed transmitted (Alberts *et al.*, 1985; Jones 1988) is considered to be the main reason for the epidemics. Prior to these epidemics in lupins, CMV was rarely found in South Australia, principally infecting some ornamental and weed species (Warcup and Talbot, 1981). The role of alternative hosts was therefore considered to be unimportant and the main source for the introduction of CMV into the lupin crops was considered to be in lupin seed which was derived from the Western Australian breeding programmes. Jones (1988) found widespread CMV infection in the *L. angustifolius* germplasm collection, including the cultivars 'Illyarrie', 'Chittick' and 'Yandee' and newly released cultivars, such as 'Wandoo'. Furthermore, a crop of 'Wandoo' lupins growing near Padthaway, South Australia, in 1986, sown with seed released by the breeders for

certified seed production, had 44 % plant infection when inspected at maturity in December (Geering, unpublished results).

Seed transmission has been reported for viruses in 21 of the 28 plant virus taxonomic groups, although it is only considered economically important in 10 of these (Stace-Smith and Hamilton, 1987). Mandahar (1981) lists reports of seed transmission of CMV in 19 hosts, of which 8 are legumes. The rate of seed transmission of a virus is variable and depends on the interaction between the host plant, virus and the environment. For example, the ability of a virus to be seed transmitted varies between strains, and differences between plant cultivars affects rate of seed transmission (Adams and Kuhn, 1977; Goodman and Oard, 1980; Davis and Hampton, 1986). Also, temperature of plant growth (Frosheiser, 1974; Adams and Kuhn, 1977; Hanada and Harrison, 1977) and plant maturity at the time of inoculation (Owusu *et al.*, 1968; Bowers and Goodman, 1979; Davis and Hampton, 1986) affect rates of seed transmission. A seedborne virus may be self-eliminating if it causes severe disease and reduces the amount of seed produced by the plant (Timian, 1973; Garrett and McLean, 1983; Jones and McLean, 1989). The yield of seed from an infected plant, its viability and the rate of seed transmission of the virus must all be considered in assessing the overall rate of transmission in the crop seed.

This chapter describes experiments done to investigate incidence of CMV transmission in commercially traded seed in South Australia and the effect of plant age at time of inoculation on seed and dry matter production and rate of seed transmission.

6.2 Materials and methods

6.2.1 Seed source

Presowing and harvested seed samples of *L. angustifolius* 'Danja' and 'Gungurru' from experimental plots at Walpeup, Victoria, were provided by I. Mock, Victorian Department of Agriculture.

6.2.2 Virus source

CMV-B_{SA} was used throughout in the inoculation trials because symptoms of seedborne infection were recognisable.

6.2.3 Tests for seed transmission of CMV

Seeds were stored in paper bags at room temperature for no longer than 6 months prior to testing for seed transmission. Seed transmission was tested by either assay of germinated seedlings for virus, or serological testing of seed.

6.2.3.1 Testing of seed by ELISA

Seeds were tested for CMV by ELISA after soaking for 24 hours at 25 C. Seeds were batch tested in groups of 20. The proportion of uninfected seed (q) was calculated using the formula $q = Q^{1/N}$ (where N is the number of seeds per batch and Q is the proportion of uninfected batches) and from this, rate of seed transmission was derived (Moran *et al.*, 1983). a maximum likelyhood estimate can be used see Burrows 19__ in Phytopathology late 80s

6.2.3.2 Assay of germinated seedlings for virus (seedling assay)

Seeds were germinated in trays of a peat/sand mix in an aphid free glasshouse. The first true leaves of the seedling were sampled, 12-16 days after sowing, for testing by ELISA. Symptoms of seedborne infection with CMV-B_{SA} included shortening of the epicotyl and hypocotyl and distortion of the leaves. Seedlings with these symptoms and

others with abnormal germination were individually tested by ELISA. Samples from normal seedlings were batch tested in groups of 20 or less. For the 1989 trial, all seedling progeny from 19 plants (1732 seedlings in total), representative of all but the first treatment, were tested by ELISA. This test showed that there were no symptomless infections and in further studies, only plants with suspected symptoms were subjected to serological testing.

6.2.4 Screening for seed transmission of CMV in commercial seedlots

To determine the incidence and rates of CMV transmission in seed that was commercially traded in south-eastern Australia, samples of lupin seed, which were submitted to the South Australian Department of Agriculture for fungal disease testing, were also tested for CMV transmission. Transmission was tested by seedling assay, except for the 1988-89 harvest, for which transmission was tested by both seedling assay and testing of seed by ELISA.

6.2.5 Field experiments

Field experiments were conducted at Charlick Experimental Station, University of Adelaide, Strathalbyn. The lupins were raised using standard management practices, which are summarised in Table 2.2.

6.2.5.1 1988 experiment: effect of time of infection on rate of seed transmission of CMV

To test the effect of plant age at the time of inoculation on rate of seed transmission, a preliminary experiment was conducted in which seed was collected from plants in the 1988 field trial that had been naturally inoculated by aphids in the course of the epidemic and compared with plants that were introduced as primary sources of inoculum (see section 3.2.2). Mature pods were collected from individual plants in January 1989 and categorised according to the survey date on which systemic infection of the plant was first detected by

ELISA. Seeds from plants that became infected in the same two week period were pooled and a sample was removed from each batch for testing by the seedling assay.

6.2.5.2 1989 experiment: relationship between age of the plant at time of inoculation and seed weight, dry matter production and rate of transmission of CMV in the seed

To further evaluate the relationship between time of inoculation and rate of seed transmission, plants were inoculated at 5 specific developmental stages. The trial was sown on May 19, 1989. Table 6.1 gives the inoculation times relative to the stages of plant development. A randomised complete block design was used, with 3 blocks and the 5 treatments arranged randomly in each block. Each treatment replicate consisted of 44 plants arranged in 11 x 4 rows, with plants spaced 30 cm. apart. The distance between the treatment replicates in a block was 50 cm. Muslin covered cages were placed over each block on July 14 to exclude migratory aphids.

For treatment 1, the plants were mechanically inoculated. This inoculation was repeated 2 days later. All subsequent inoculations (treatments 2-5) were by patch grafting. Infection of plants was diagnosed using symptoms and testing by ELISA.

Mature pods were harvested from the plants and the remaining dried plant cut at ground level and weighed. Seeds were weighed and the rate of CMV transmission was determined by seedling assay using all seeds produced by a plant.

6.2.6 The distribution of infected seed on the lupin plant

Seeds from treatments 3 and 4 of the 1989 experiment (see section 6.2.5.2) were partitioned according to their position on the plant to investigate whether the order of emergence of the inflorescence, relative to the time of inoculation, affected the rate of seed transmission. The branching pattern of a typical lupin plant is shown in Fig. 6.1. Pods

Table 6.1: Time of inoculations in the 1989 experiment relative to the phenological development of *L. angustifolius* 'Illyarrie'.

Seed was sown on May 19 and most seedlings had emerged by May 29 (time = 0). L1 and L2 refers to the first and second order lateral branches respectively (see Fig. 6.1).

Treatment	1	2	3	4	5
Time (days after emergence)	2	58	94	114	135
Developmental stage	Seedling stage. Cotyledons and first pair of true leaves were present.	Mid-vegetative stage. Plant had single axis. Lateral shoots were beginning to arise from the basal nodes. Bud of primary inflorescence was not visible.	Beginning of flowering of the primary inflorescence.	L1 inflorescences were flowering (up to 6 flowers were opened). Top flowers of the primary inflorescence were opened. Pods were forming in the positions of lowest flowers in the primary inflorescence.	L2 inflorescences were flowering. Pods were forming in the positions of the lowest flowers in the L2 inflorescences.
Height (m) 1 0					

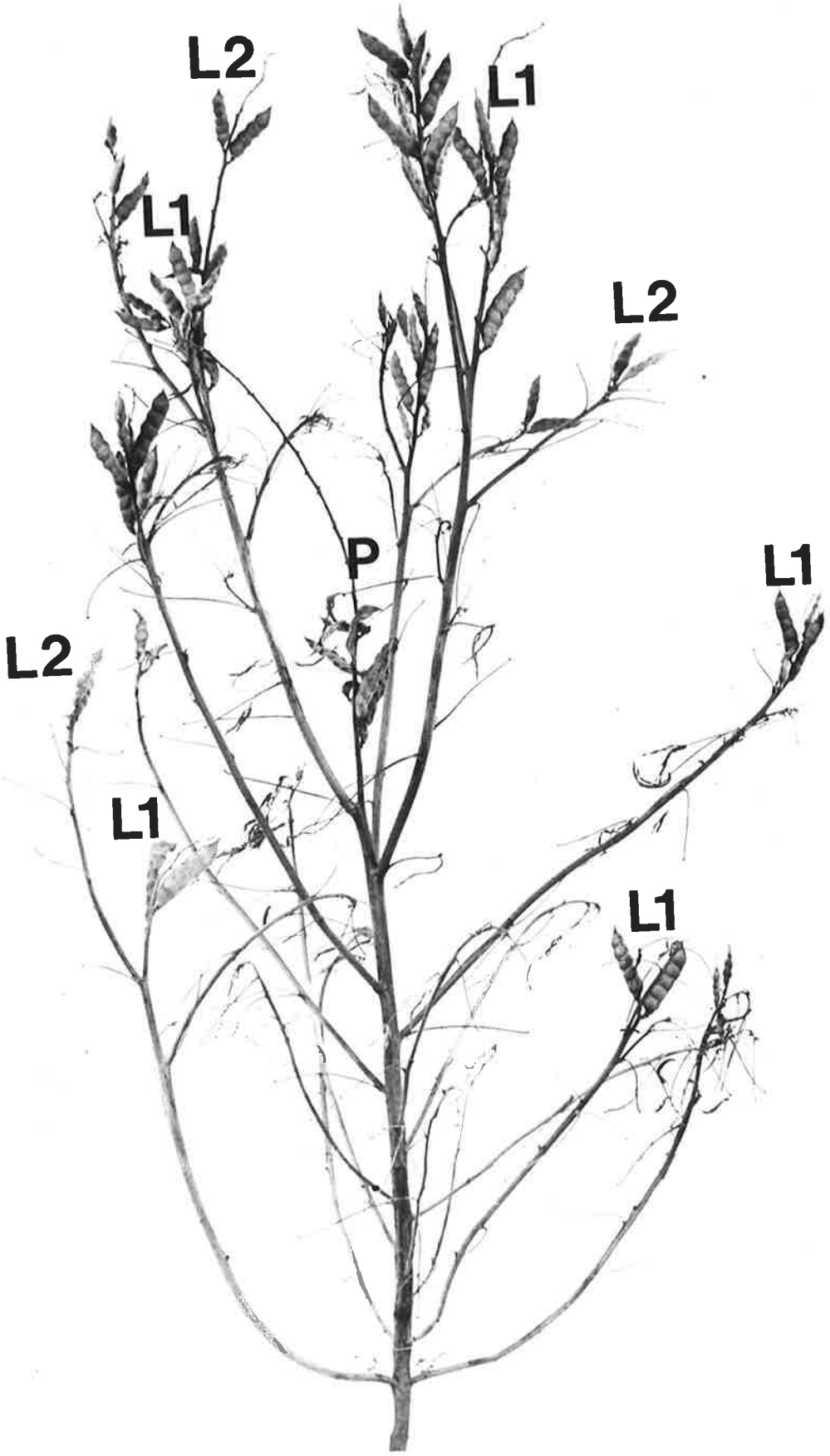
Fig. 6.1: Classification of pods according to their position on the plant (after Perry and Poole, 1975).

P Pods of the primary inflorescence

L1 Pods of a first order lateral inflorescence

L2 Pods of a second order lateral inflorescence

Lupin flowering is indeterminate. Initially, the plant has a single axis and growth of this shoot terminates with the primary inflorescence. Following the beginning of flowering of the primary inflorescence, new shoots, called first order laterals, arise from nodes on the primary axis and inflorescences are also borne terminally on these shoots. The lupin plant continues to ramify, with second order laterals elongating after the beginning of flowering of the first order lateral inflorescences. Normally, no more than three orders of laterals are produced and the third order lateral inflorescences may or may not set seed, depending on the availability of moisture (Lopez-Bellido and Fuentes, 1986). The end of flowering of one order of inflorescences coincides with the beginning of flowering of the following order of inflorescences. The primary inflorescence produces more than 25 flowers, though most abort, leaving scars on the rachis.


L2
L1
L1
L2
P
L1
L2
L1
L1

were classified according to their position in the hierarchy of orders of lateral branches, as either being from the primary inflorescence, or inflorescences on the first, second or third order laterals. The pods of the primary inflorescence were further divided according to their relative position on the rachis. The pod positions were numbered in order of increasing age, starting from the bottom of the inflorescence. Scars on the rachis marked the positions where pods had failed to develop and were also counted. Pods in positions 1-4 were pooled to form one sample, followed by pods in positions 5-8, 9-12 etc.

6.2.7 Distribution of CMV in the seed

To test whether CMV detected in seed was from embryonic or maternally derived seed tissues, seeds were harvested from plants in treatment 2 of the 1989 field trial (see section 6.2.5.2) and hydrated overnight (*c* 16 hours) at 4 C, under moistened tissue paper. They were dissected to separate the testa, cotyledons and embryo (consisting of the primordial radicle and plumule). To remove surface contamination with virus, these seedparts were immersed in 10 % Na_3PO_4 for one minute, followed by washing with running distilled water (Yang and Hamilton, 1974; Bowers and Goodman, 1979). The seedparts were then biologically indexed for CMV infection. Either single or pooled samples of seedparts were tested.

6.2.8 Relationship between seed weight and recovery of the virus from the seed

To investigate the relationship between weight of seed and presence of CMV in that seed, individual seed weights were recorded and CMV transmission tested by bioassay. Seeds harvested from plants in treatment 2 of the 1989 field experiment were used (see section 6.2.5.2).

6.2.9 Comparison of the growth rate of seedlings infected via seed with those inoculated at the cotyledon stage

To compare the growth rate of plants infected via seed with that of plants inoculated at the cotyledon stage, seed from plants in treatment 3 of the 1989 experiment were sown in trays and 112 healthy seedlings and 64 seedlings with seedborne infection selected at emergence, 10 days after sowing. Of these seedlings, 16 infected and 16 healthy were randomly harvested for the first growth measurements and the remainder transplanted into 5 inch pots of peat/sand soil mix. The leaves of the harvested seedlings with seedborne infection were used to manually inoculate 48 of the healthy seedlings 2 days post-emergence and for a second time one day later. Healthy seedlings were mock inoculated. The plants were transferred to an insect proof screenhouse 3 days post-emergence. A randomised complete block design was used, with the two treatments of infected seedlings and a healthy control randomly arranged in two blocks. Plants were fertilised weekly with the complete liquid fertiliser Aquasol (Hortico), starting 40 days post-emergence.

For each measurement of growth, 8 plants were randomly selected from each treatment replicate and clipped at soil level and height and fresh weight measured.

6.2.10 Survival of CMV through seed transmission

To test whether CMV persisted naturally from one generation to the next via seed transmission, infection levels at the seedling stage of two successive generations of lupins were compared. Seeds were mechanically harvested from the *L. angustifolius* 'Warrah' plants in the 1989 field trial (see section 3.2.3) and a seed sample tested for CMV infection by ELISA. Samples of the seed used to sow experimental plots of 'Gungurru' and 'Danja' lupins at Walpeup, Victoria, and harvest samples of seed produced by these plants, were also tested for CMV transmission.

6.3 Results

6.3.1 Seed transmission in commercial seedlots

Table 6.2 shows the rates of transmission of CMV in commercially traded seedlots from south-eastern Australia. For the three years studied, CMV transmission was detected in seedlots from 11/26 South Australian crops, 12/25 Victorian crops and 0/1 crops from NSW. For the 19 seed samples from the 1988 harvest, there was no significant difference (P=0.395) between estimated rates of seed transmission by either the seedling assay or direct seed testing methods.

6.3.2 CMV transmission in seed from plants infected during the 1988 field trial

Table 6.3 lists the rates of seed transmission recorded for plants infected during the epidemic established in the 1988 field trial. The highest rate of CMV transmission (23.1 %) was found in seeds from plants that became systemically infected in the two week period ending on August 24, which was about one week before the commencement of flowering (*ca.* August 31). The rate of seed transmission declined thereafter.

Table 6.3: CMV transmission in lupin seed harvested from the 1988 field trial.

Period when CMV first detected	Germination rate (*a* proportion of total, *b* percent)		Seed transmission rate (*a* proportion of total, *b* percent)	
	a	*b*	*a*	*b*
[1] Jun. 3-Jun 17	13/30	43.3	2/13	15.4
Aug.10-Aug.24	134/145	92.4	31/134	23.1
Aug. 24-Sep. 7	379/400	94.8	67/379	17.7
Sep. 7-Sep. 21	380/400	95.0	63/380	16.6
After Sep. 21	364/400	91.0	29/364	8.0

[1] Plants which were inoculated at the cotyledon stage on June 3 and introduced to the field as sources of inoculum.

Table 6.2: Incidence and rates of CMV transmission in commercially traded seedlots in south-eastern Australia.

Seedlots from the 1986-1987 and 1987-1988 seasons were from South Australia and the seedlots from the 1988-89 season were from South Australia, Victoria and New South Wales. Rate of CMV transmission in each seedlot was determined by testing 200 seeds in 1987, 500 seeds in 1988, and 1000 seeds in 1989.

Cultivar	[1] 1986/87 harvest		[2] 1987/1988 harvest		[2] 1988/89 harvest	
	Proportion of seedlots infected (no. infected/total)	Rate of seed transmission	Proportion of seedlots infected (no. infected/total)	Rate of seed transmission	Proportion of seedlots infected (no. infected/total)	Rate of seed transmission
Danja	1/1	0.75	0/2	0	10/16	0 - 4.6
Geebung	1/1	1.3	1/1	11.5	-	-
Gungurru	-	-	2/6	0 - 0.6	3/8	0 - 0.1
Illyarrie	1/1	4.5	-	-	-	-
Wandoo	1/1	5.3	-	-	-	-
Warrah	-	-	2/6	0 - 1.4	0/1	0
Yandee	1/2	0 - 1.2	-	-	1/2	0 - 0.3
Yorrell	-	-	-	-	0/1	0

[1] Geering, A.D.W and Alberts, E., unpublished results.

[2] Ingham, B., Francki, R.I.B. and Geering, A.D.W., unpublished results.

6.3.3 Relationship between plant age at the time of inoculation, symptom severity and seed and dry matter yields

Fig 6.2 illustrates the symptoms of plants inoculated at different ages. Plant stunting was more severe at the earlier times of inoculation. Other symptoms of infection included leaf epinasty and distortion and yellowing of the margins of the leaflet. Symptoms of systemic infection started to appear at *ca.* 20 days following treatment 2 and at *ca.* 14 days following treatment 3. Plants in treatment 5, when tested prior to inoculation, were all uninfected, indicating that no natural spread had occurred and that plants only became infected as a result of the experimental inoculation.

The mortality rate of infected plants in treatment 1, as recorded on October 30, was 44.7 %, compared with a mortality rate of less than 1 % for plants in the other treatments. Dying plants in treatment 1 had symptoms of root rot. Only 39.2 % of the survivors in treatment 1 produced seed.

Fig. 6.3 illustrates the relationship between age of the plant at the time of inoculation and seed and dry matter production. Four plants became infected in treatment 5, and the sample number was considered too low to provide a good estimate of yield. Potential seed and dry matter production of healthy plants was obtained from uninfected plants in this treatment. Dry matter productivity was reduced only when the plant became infected during its vegetative stage, as dry matter yields from infected plants in treatments 3 and 4 and from healthy plants were not significantly different ($P=0.224$), however, yields from these plants significantly differed from that found for infected plants from treatments 1 and 2 ($P<0.001$). Infection resulting from inoculation during the reproductive stage of growth still reduced seed yields, as yields of plants in treatments 3 and 4 and healthy plants were significantly different ($P<0.001$) from each other.

Fig. 6.2: Symptoms of infection with CMV-B_{SA} (as seen on October 25).

(A) Infected plants in treatment 1.

(B) Infected plant in treatment 2.

(C) Infected plant in treatment 3.

(D) Growing point of an infected plant in treatment 4, showing pods of a L2 inflorescence and the flowers of L3 inflorescences.

Refer to Table 6.1 for descriptions of the treatments.


A


B
50
40
30
20
10

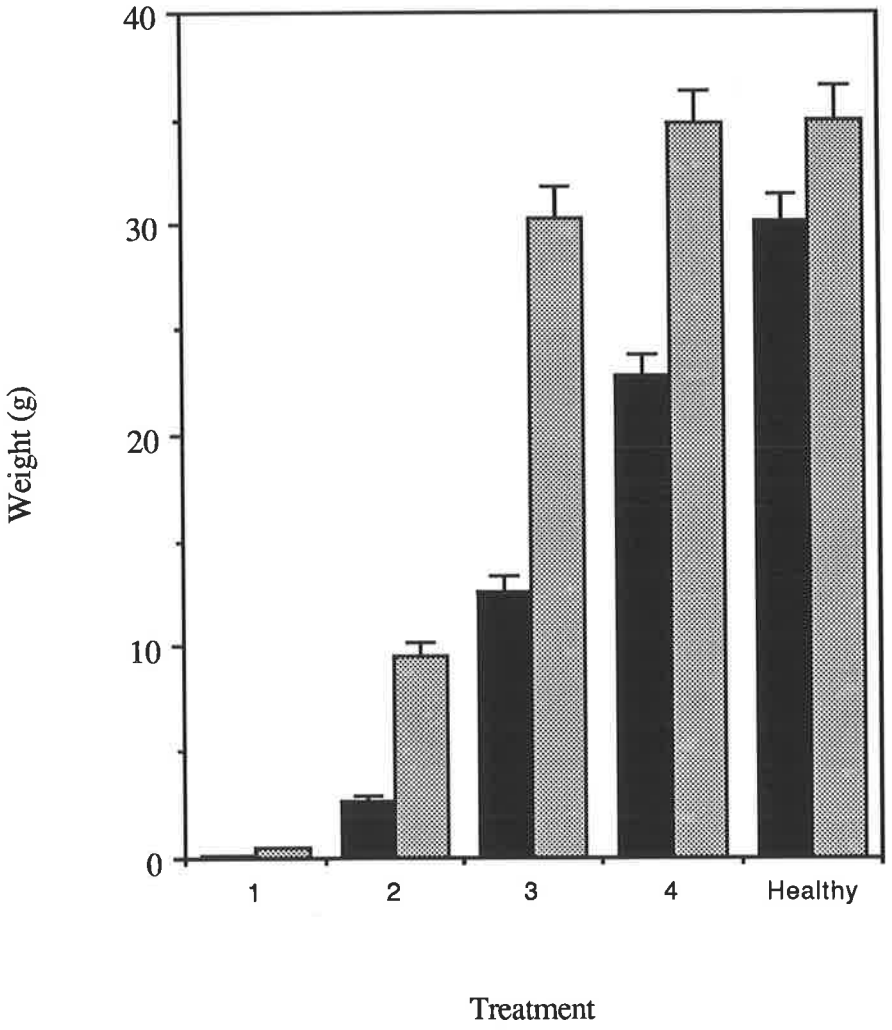

C
60
50
40


D

Fig. 6.3: Relationship between plant age at the time of inoculation with CMV and seed and dry matter productivity.

Seed yield (■) is the mean weight per plant of seed produced and dry matter yield (▩) is the mean weight per plant of stem and leaf from the dessicated plant, measured after the pods had been harvested. Bars represent the standard errors of the mean.

Refer to Table 6.1 for descriptions of the treatments.


40
30
20
10
0
Weight (g)
1
2
3
4
Healthy
Treatment

Seed filling was affected by the severity of disease, as individual seed weights were lower at the earlier times of inoculation (see Table 6.4). Germination rates did not differ significantly (P=0.224) between treatments 2, 3 and 4, which indicates that for these treatments, differences in seed size and levels of infection (see section 6.3.4), did not affect viability. The germination rate of seeds from treatment 1 was 59.7 ± 9.7 %, compared with an average of 86.8 ± 1.3 % for treatments 2, 3 and 4. The low rate of germination for seed from treatment 1 appears to be due to a decrease in the size of seed to a level at which viability was reduced (see Table 6.4).

Table 6.4: Relationship between time of inoculation, weights of individual seeds, and number of viable seeds produced per plant.

Treatment	1	2	3	4	Healthy
Seed yield (g)	0.080 ± 0.02	2.6 ± 0.3	12.5 ± 0.8	22.8 ± 1.1	30.2 ± 1.2
[1] Single seed	67.0 ± 3.1	101.5 ± 2.4	109.4 ± 3.4	117.4 ± 2.3	147.9 ± 3.1
[2] No. of viable seeds per plant	0.71	22.2	99.2	168.6	177.2

[1] Single seed weights are the average for 25 replicate plants from treatment 1 and 30 replicate plants from treatments 2, 3 and 4 and healthy plants.

[2] Number of viable seeds = [Yield (g) x germ. rate (%) x 10]/[single seed weight (mg)]. Rates of germination were 86.8 % for treatments 2-4 and healthy, and 59.7 % for treatment 1.

6.3.4 Effect of plant age at the time of inoculation on rate of seed transmission

Fig. 6.4 shows the relationship between plant age at the time of inoculation and the rate of CMV transmission in the seeds. The rates of transmission for seeds from treatments 2, 3 and 4 differed significantly ($P<0.001$). The highest rates of seed transmission occurred when plants became infected during the vegetative stage, with a maximum rate of 24.5 % for seeds from treatment 2. Later inoculations after the commencement of flowering gave progressively lower rates of seed transmission.

The largest absolute amounts of infected seedlings were produced by plants in treatment 3, as is illustrated in Fig. 6.5.

6.3.5 Distribution of infected seeds on the plant

Tables 6.5 and 6.6 show that the more developed the seed at the time of inoculation, the less probable it was that CMV was transmitted in that seed. For plants from treatment 3, higher rates of CMV transmission were found in seeds from later emerging inflorescences (see Table 6.5). This pattern was not found for plants from treatment 4.

Table 6.5: Rates of CMV transmission in seed from pods of the primary, first order lateral (L1) or second order lateral (L2) inflorescences.

Treatment	Rate of seed transmission (%)		
	Primary	L1	L2
3	6.5 ± 1.4	11.2 ± 1.4	17.5 ± 3.6
4	4.9 ± 1.2	2.0 ± 0.6	5.5 ± 1.3

Fig. 6.4: Relationship between plant age at the time of inoculation and rate of seed transmission of CMV.

Mean rates of transmission for seeds from 25 replicate plants in treatment 1, 30 replicate plants in each of treatments 2, 3 and 4 and 4 replicate plants in treatment 5, are shown. Bars represent the standard errors of the mean.

Refer to Table 6.1 for descriptions of the treatments.

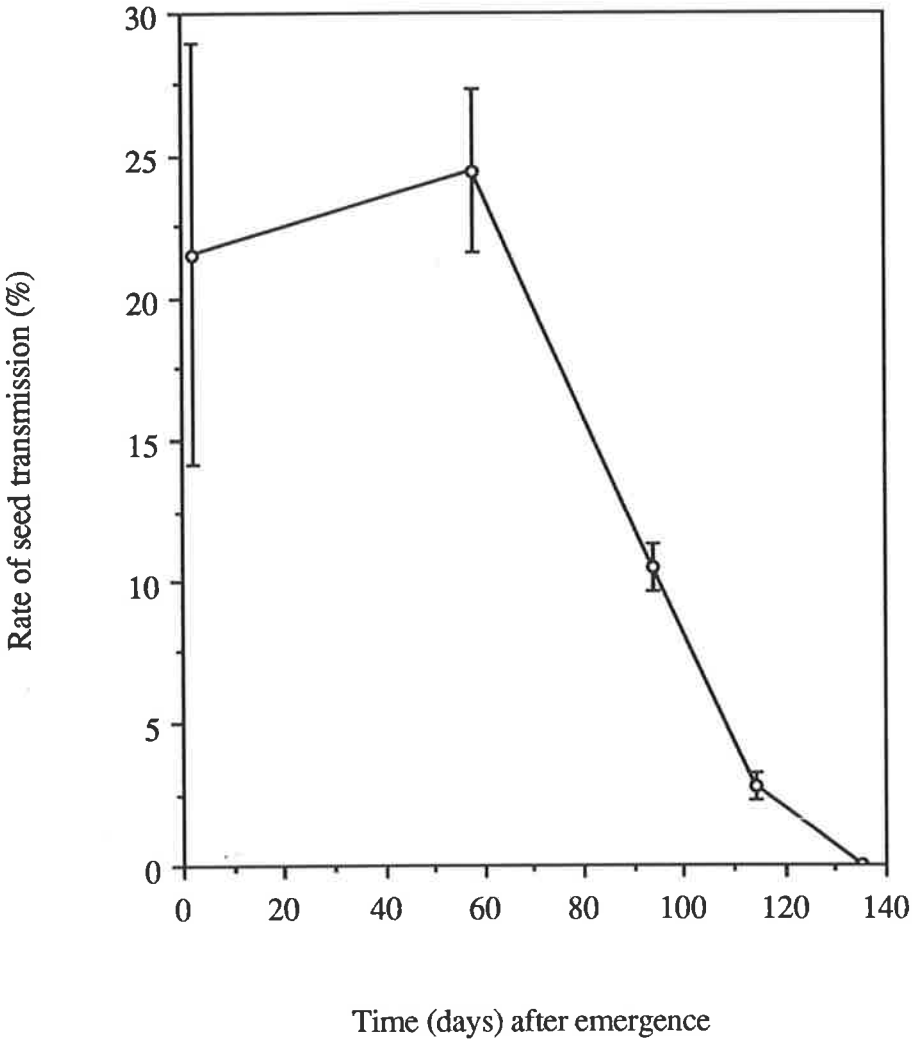
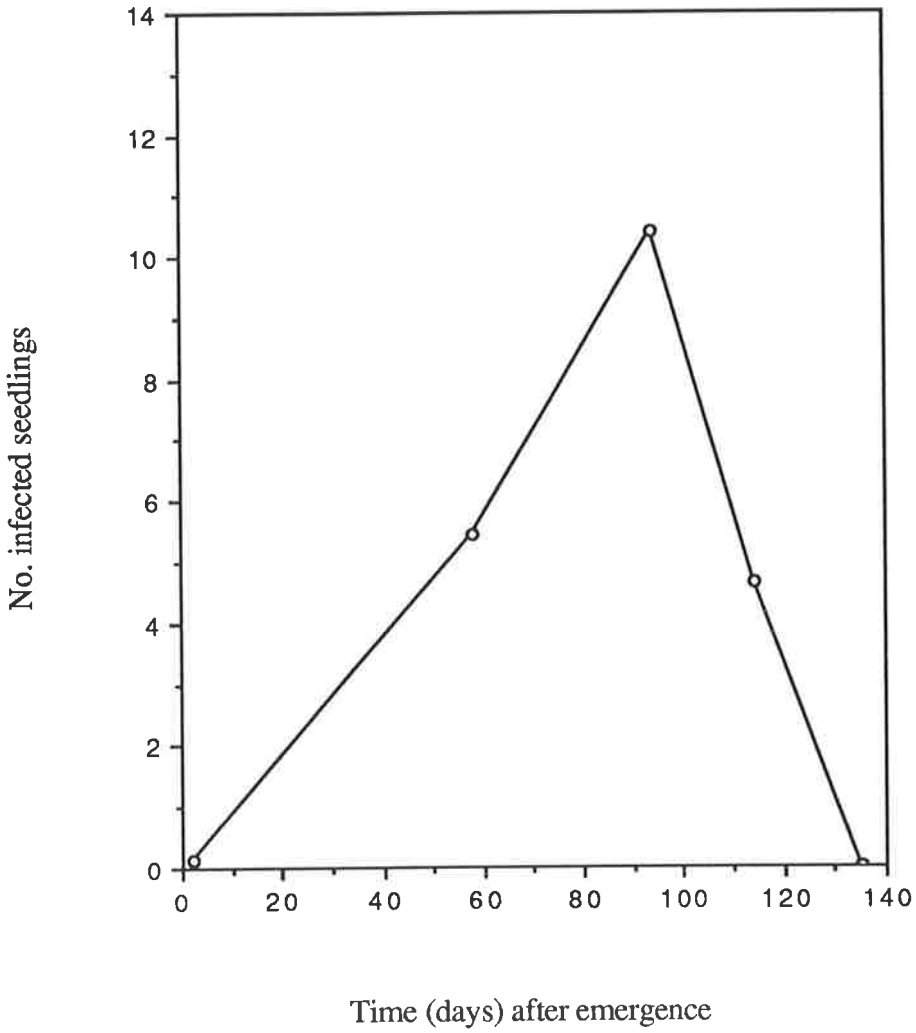

Rate of seed transmission (%)
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Time (days) after emergence

Fig 6.5: Relationship between age at the time of inoculation and the number of infected seedlings produced by each plant.

The number of infected seedlings was calculated by multiplying the number of viable seed produced per plant (see Table 6.4) by the rate of seed transmission (expressed as a proportion).


No. infected seedlings
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
Time (days) after emergence

Incidence and rates of seed transmission were greater in seeds from the later emerging flowers (pods 5-8) on the primary infloresence of plants from treatments 3 and 4 (see Table 6.6). Comparison of seeds from the primary inflorescence of plants in treatments 3 and 4, which matured simultaneously but differed in developmental stage at the time of inoculation, also showed that incidence and rates of CMV transmission were greater in seeds which were less developed at the time of inoculation (see Table 6.6).

Table 6.6: Rates of CMV transmission in seed from standard positions on the primary inflorescence.

Pod position	Pods 1-4		Pods 5-8	
Treatment	[1] Incidence of seed transmission (%)	[2] Mean rate of seed transmission (%)	[1] Incidence of seed transmision (%)	[2] Mean rate of seed transmission (%)
3	39.1	4.6 ± 1.4	54.6	6.7 ± 1.9
4	14.3	2.5 ± 1.5	27.3	3.5 ± 1.5

[1] Incidence of seed transmission refers to the percentage of plants with seed transmission of CMV, irrespective of rate, in the seed from these pods.

[2] Mean rate of seed transmission refers to the percentage of seeds transmitting CMV in all the pods.

6.3.6 Distribution of infectivity in seed parts

Infectious CMV was recovered from both the cotyledons and embryo of 2 of the 9 seeds which were individually tested. In a second experiment in which seedparts from

between 5 to 14 seeds were combined and tested, infectious CMV was recovered from all 5 pooled samples of cotyledons and all 5 pooled samples of embryos. CMV was not recovered from the testa in either experiment.

6.3.7 Effect of CMV infection on seed weight

Seeds from 8 infected plants were tested, of which 202/219 (92.2 %) germinated and 47/202 (23.3 %) transmitted CMV. There was no significant difference (P=0.187) between the mean individual weights of infected (97.6 $\pm$ 2.9 mg) and uninfected seeds (112.0 $\pm$ 5.2 mg). A large source of variation in seed weight was from differences between plants in the size of seed produced, as the mean individual weights of seeds from different plants were significantly different (P<0.001).

6.3.8 Rate of growth of seedlings relative to the time of infection

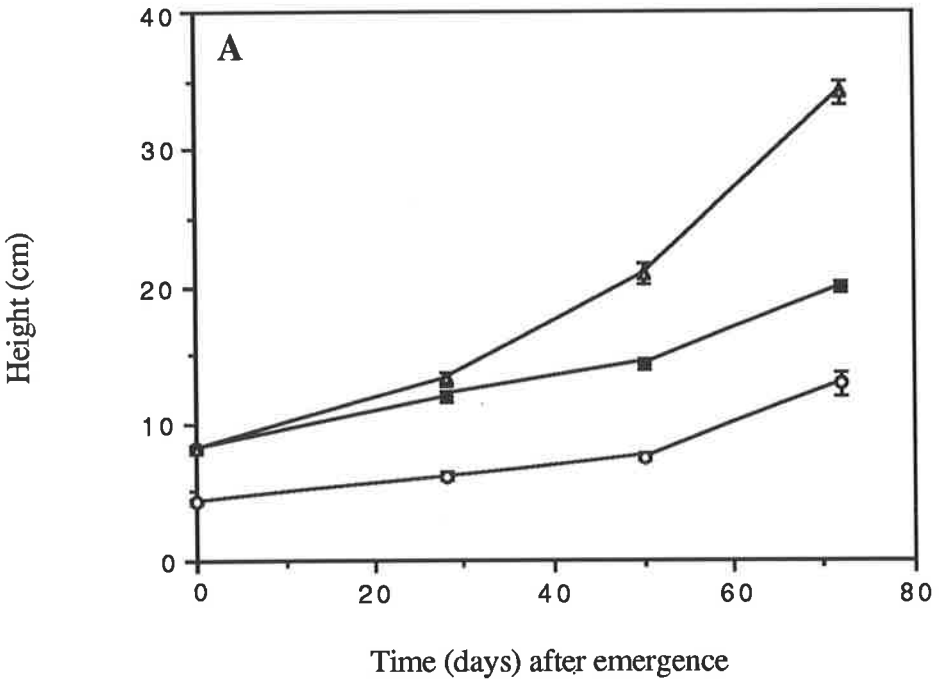
The growth rate of seedlings, relative to the time of infection, is shown in Fig 6.6. Stunting of lupins with seedborne infection was evident at emergence. Seedlings with seedborne infection showed no greater tolerance to infection than seedlings which were infected after emergence, as growth rates were similar.

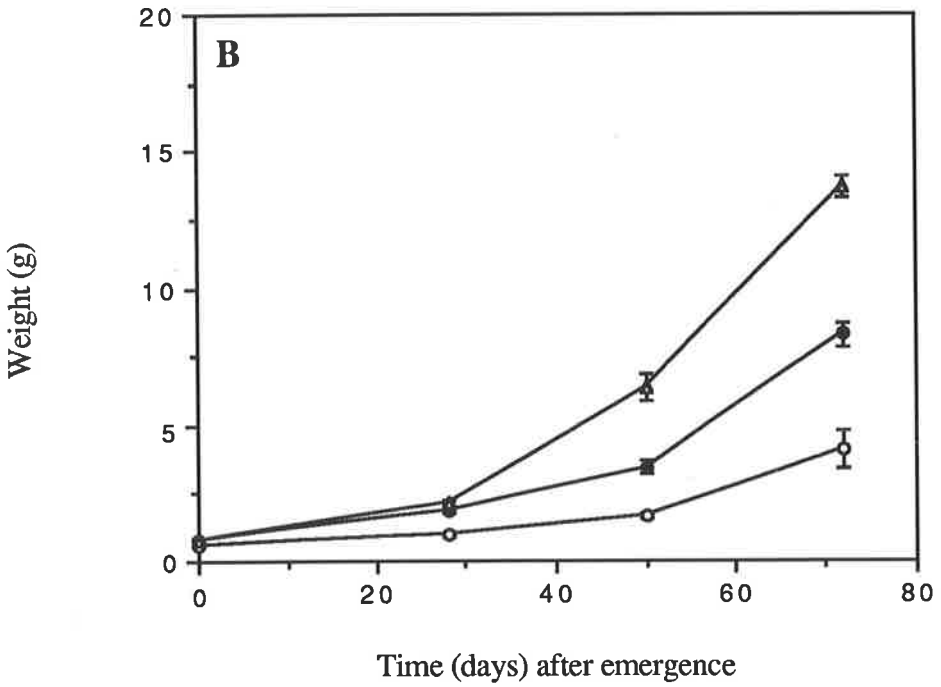
6.3.9 Increase in seedborne CMV through sequential generations

To assess the potential of CMV to persist and to increase in lupin crops, the levels of seedling infection in successive generations of lupin plants were measured. For the 1989 field trial, the incidence of infection at the seedling stage was 0.07%, if each cluster of 4 infected seedlings introduced as primary sources of inoculum is considered to be a single infection focus (see section 3.3.2.4). The final incidence of infection, recorded on October 31, was 4.7 %. Seed harvested from this plot transmitted CMV at a rate of 0.87 %. This

Fig. 6.6: Comparison of the growth rate of seedlings with seedborne infection (○) with that of seedlings that were inoculated at the cotyledon stage (●) and healthy plants (Δ).

Growth was measured by either (A) change in height or (B) change in fresh weight. Height was measured from the base of the stem to the apical bud. Growth measurements are the mean of 16 replicate plants. Bars represent the standard errors of the mean.


A
Height (cm)
0
10
20
30
40
0
20
40
60
80
Time (days) after emergence


B
Weight (g)
0
5
10
15
20
0
20
40
60
80
Time (days) after emergence

represented a thirteen fold increase in inoculum levels in seedlings in two successive generations.

Rates of CMV transmission in *L. angustifolius* seed used for sowing experimental plots at Walpeup, Victoria were 0.10 % for the cultivar Danja and 0.31 % for the cultivar Gungurru. Rates of CMV transmission in seed harvested from these plots were 8.22 % for the 'Danja' lupins and 11.0 % for the 'Gungurru' lupins.

6.4 Discussion

The following conclusions can be drawn from the experimental work described in this chapter.

(a) CMV transmission in commercially traded seedlots in south-eastern Australia in 1987-89 was common.

(b) Transmission of CMV in seeds can be tested either by assay of seedlings or by direct testing of seeds for infection.

(c) Infection of lupins with CMV-B_{SA} developing during vegetative growth, causes a reduction in both dry matter and seed productivity.

(d) Infection developing during reproductive growth only reduces seed productivity.

(f) Rate of seed transmission of CMV is dependent on the age of the plant at the time of inoculation.

(g) The greatest absolute amount of infected seedlings are produced by plants which are inoculated at the beginning of flowering of the primary inflorescence.

(h) Infection of the seed does not affect seed size.

(i) CMV can succesfully persist by transmission in lupin seed.

Seed of *L. angustifolius*, which was infected with CMV, was commercially traded in south-eastern Australia in the period from 1987-89, thus providing a means of long distance dispersal, and ensuring introduction of inoculum into farm crops.

Rates of seed transmission could be determined by either assay of seedlings or by testing seed extracts by ELISA. Infection of the seed did not affect its viability. Rate of seed infection is therefore the same as rate of transmission. For routine testing of seed samples for CMV transmission, batch testing of seed was most efficient. However, when testing individual seed for transmission of CMV-B_{SA}, the bioassay method was most rapid as an initial screening could be made based on symptoms, so that the number of samples for further serological testing was reduced.

Fig. 6.3 showed that dry matter yield was reduced when the plant was inoculated during its period of vegetative growth, but not at later times. In contrast, seed yield was reduced when the plant was inoculated after the commencement of flowering. These observations on the effect of CMV infection on lupin growth could be explained by changes in the pattern of distribution of carbon and nitrogen assimilate that occurred during plant maturation. During the vegetative stage of growth, assimilate is distributed to the roots and developing shoots. Following the onset of anthesis, the pod and developing seed becomes the overwhelming sink of assimilate (Thorne, 1985). Infection which developed during reproductive growth would therefore be expected to have its greatest effect on seed productivity. Infection which developed from inoculation at the beginning of flowering of the primary inflorescence, resulted in both a 44 % reduction in the number of seed produced as well as a reduction in individual seed weight. Later inoculation resulted in only a small

reduction in the number of seed produced and the reduction in seed yield was due mostly to lower individual seed weights.

The mortality rate of plants inoculated at the cotyledon stage was high. Plants in the experiment had symptoms of brown leaf spot, commonly associated with infection by *Pleiochaeta setosa* and the root rot in dying plants in treatment 1 resembled that caused by the same fungus (Woodcock, 1982; Sweetingham, 1986). The severe stress on the plant caused by CMV infection may have predisposed the plant to fungal root rot. Jones (1988) observed lower establishment rates for seedlings with seedborne infection during drought conditions. Rainfall at the site of the field trial, during the winter of 1989, was not considered to be limiting to plant growth. It is therefore unlikely that moisture stress alone caused the death of the infected lupins which were inoculated at the cotyledon stage.

Seedlings infected through seed showed symptoms at emergence and these plants were more stunted than plants inoculated at the time of emergence. A characteristic of plants infected by way of seed is that they are often symptomless, or show less severe symptoms than plants inoculated after germination (Stace-Smith and Hamilton, 1987). This phenomenon has been associated with viruses which infect plants, initially producing a shock reaction, which is followed by some form of recovery. The developing seeds presumably become infected during the recovery stage and the seedborne infected seedlings do not suffer the shock reaction. No such pattern of symptoms of shock reaction followed by recovery was observed for 'Illyarrie' lupins infected with CMV-B_{SA}.

Rate of seed transmission was dependent on the age of the plant at inoculation. Highest levels of seed transmission, of between 23-25%, were found in the seed of plants inoculated before emergence of the primary floral bud. Jones (1988) reported that the highest rates of CMV transmission in *L. angustifolius* 'Illyarrie' seed was 18 % for a crop which was 100 % infected early the growing season. Considering that there were probably

environmental differences and different virus isolates were investigated, these estimates of maximum seed transmission are very similar.

The later the time of inoculation of the parent plant after initiation of flowering, the lower the level of CMV transmission in the progeny seeds. The distribution of infected seeds on plants inoculated during reproductive growth showed that the more developed the seed at the time of inoculation, the less probable it was that CMV was transmitted by that seed.

Most infected seeds were produced by plants inoculated at the commencement of flowering of the primary inflorescence. At this time of inoculation, disease resulting from the infection was moderate, potential seed production was reduced by 44 % (see Table 6.4), and seed transmission was 10.5 %.

Infectious CMV was recovered from embryo and cotyledons, but not the testa. This suggests that CMV transmission results from infection of the embryonic tissues. This is to be expected as CMV is labile outside host tissue. A characteristic of viruses that are seed transmitted by infection of the testa, for example, transmission of TMV in tomato seed (Taylor *et al.*, 1961), is that they are very stable in an exposed environment and remain infective for long periods (Bennett, 1969).

The theory that developing seeds can be directly infected is supported by observations of seed development at the time of inoculation and subsequent occurrence of CMV transmission by that seed. At the time of application of treatment 4 in the 1989 experiment, pods were partially formed in the lowest positions on the primary inflorescence (see Table 6.1). Seeds from these pods transmitted CMV (see Table 6.6). Flowering commenced 3 weeks prior to the time of inoculation, and it is considered that variation in floral development was not great enough so that some flowers were not fertilised. Pollen is

released before the flower opens, and self-fertilisation occurs automatically (Crane and Walker, 1984).

There was no relationship between seed weight and CMV transmission. This is in contrast to the report of Jones (1988), who found higher levels of seed transmission in lighter weight categories of seed. The earlier the plant becomes infected, the more severe the disease and the higher the rate of seed transmission. With late plant infections, highest rates of seed transmission are found in the later produced pods, and filling of this seed may be affected by plant senescence. Both these phenomena would cause a bias towards CMV infection more frequently occurring in the lighter seed categories of the total crop seed.

For the three crops investigated, seed transmission levels increased over one generation. The results of the 1989 field trial showed that CMV could persist by seed transmission, even when secondary spread by aphids was small.

Chapter 7

General Discussion

The aim of this chapter is to discuss interactions between the components of the epidemic, to propose future directions for research and to make recommendations for control.

7.1 Sources of inoculum

In chapter 3, lupins which were derived from infected seed were shown to be important as primary sources of inoculum. The role of alternative hosts in the epidemiology of CMV was not investigated. It is possible that weeds are unimportant as sources of inoculum as -

(a) seed transmission of CMV is commonly found in commercially traded seedlots (see chapter 6; Jones, 1988).

(b) lupins with seedborne infection are efficient as sources of inoculum, and low levels of seedborne infection (≤ 5 %) can cause large epidemics (see chapter 3; Jones, 1988).

Only perennials, or annual species in which transmission of CMV occurs through seed, are likely to be important as primary sources of inoculum, as the summer period of drought restricts most hebaceous growth in the dryland agricultural areas of South Australia. Pasture legumes such as subterranean clover (*Trifolium subterraneum*), in which CMV is seed transmitted (Jones and McKirdy, 1990), could be important as alternate hosts.

7.2 Secondary spread by aphids

Results described in chapter 4 suggested that migratory alate aphids were the most important field vectors. In Western Australia, most severe disease epidemics have been observed in crops which were heavily colonised with aphids (Sandow, 1987; Jones, 1988; Jones and Proudlove, 1991). Colonising species such as *A. craccivora*, *A. kondoi* and *M. persicae* were therefore considered to be the most important vector species. The relationship between aphid flights, colony development and virus spread has not been investigated in Western Australia and the evidence to suggest that the colonising aphids are important vectors is very circumstantial. In the field trials described in this thesis, little aphid colonisation occurred, even when colonising aphids were artificially introduced into the plots, as in the 1987 field trial, and therefore the effect of aphid colonisation on epidemic development is unknown.

In 1987, the first period of rapid increase in incidence of CMV correlated with flights of *R. padi*. Further evidence obtained from using suction traps that collected from the boundary layer of the lupin canopy showed that *R. padi* were at a level where they could alight on the lupins and transmission studies showed that this species was capable of transmitting CMV. *R. padi* are therefore considered to be important vectors of CMV in lupin crops.

In 1988, the effect on epidemic progress of establishing colonies of *R. padi* next to the sources of inoculum was investigated. This experiment failed to provide further evidence on the importance of *R. padi* as a vector. An alternative to the experimental design used in the 1988 field trial would be to release alatae that were either reared in the glasshouse or collected from the field onto the sources of inoculum. Optimally, the aphids would be released after a period of flight or containment away from the host so that they were exhibiting settling behaviour when they were released.

R. padi and R. maidis have been shown to be important vectors of non-persistently transmitted viruses in soybean crops in the USA and potato crops in the Netherlands (van

Hoof, 1977, 1980; Halbert, Irwin and Goodman, 1981). Though *R. padi* colonises graminaceous plants (Eastop, 1983), it will alight on a wide range of plant species. Wiktelius (1982) studied the flight and settling behaviour of *R. padi* on wheat and potato and found that the aphid was slow at discriminating between a host and non-host species. *R. padi* invariably probed following alighting, irrespective of how short the previous period of flight and there was no significant difference in number of probes and probing time on wheat or potato. From this research it was concluded that the behaviour of *R. padi* on a non-host did not preclude it as a vector of a non-persistently transmitted virus.

Evidence was provided to suggest that *L. erysimi* was not a vector of CMV in lupins. *L. erysimi* was incapable of vectoring CMV in the glasshouse transmission tests. This evidence alone is not sufficient to conclude that the aphid is not a vector as only one aphid clone and one vector was tested. The results obtained from the suction traps in the 1989 field trial also suggest that *L. erysimi* is not a vector of CMV in lupins. Though large flights of *L. ersimi* did occur in Spring, 1989, as recorded using the yellow pan traps, this species was rarely collected in the suction traps and were therefore not flying in the boundary layer of the crop where they would be subjected to the airflow into the traps. Presumably, *L. erysimi* were not alighting on the lupins in large numbers and could therefore be considered to be unimportant as a vector.

It is possible that alates of *L. erysimi* can appraise host suitability at a distance, perhaps by using olfactory signals, and do not enter the boundary layer of the lupin crop. *L. erysimi* only colonises cruciferous plants with high levels of sinigrin (Dixon, 1987). Petterson (1973) showed that alate viviparous females of *Brevicoryne brassicae*, which have a similar host range to *L. erysimi*, are attracted to sources of sinigrin.

Further research is required to establish the importance of common aphid species as vectors of CMV in lupin crops. The most direct means of demonstrating whether an aphid species is a vector is to trap live aphids over the crop using suction traps (Raccah *et al.*,

1985) or nets (Halbert *et al.*, 1981) and testing the aphids for infectivity by placing them on healthy test seedlings.

From observations of the spatial pattern of infected plants in the field trials, two types of aphid spread were inferred -

(1) Short distance spread resulting in the formation of steep infection gradients.

(2) Longer distance spread resulting in the formation of secondary infection foci around which new clumps of infected plants formed.

When finding a host to colonise, flying alates enter an 'attack mood', in which they descend, alight on a plant and probe, then usually make a succession of smaller flights ('trivial flight') before entering the 'settling mood' (Robert, 1987). Short distance spread, resulting in the formation of steep infection gradients, may have resulted from this 'trivial flight'. The 'settling mood' is quickly inhibited if an aphid alights on an unfavourable host, and the aphid will recommence flight. The distance covered in the next period of flight is dependent on the urge to settle, which increases with repeated flight (Dixon, 1985). Aphids, to which the lupins were poor quality or non-hosts, may have taken several large 'hops' through the crop before leaving, and thereby initiated the secondary infection foci that were observed.

7.3 Patterns of aphid flights

Programmes of aphid trapping, like those described in chapter 4, and also conducted by others, have shown that in southern Australia, most abundant flights occur in spring. Aphid populations reach their maximum size in spring, as plant growth is rapid, senescence of new plant growth is slow, and predators of the aphids are frequently rare (Maelzer,

1981). Factors such as colony overcrowding trigger aphid morphogenesis and migratory alatae are produced (Maelzer, 1981; Dixon, 1985; Robert, 1987).

Daily patterns of flight are influenced by weather conditions. Low temperatures and high winds suppress flight. Adverse weather conditions modify a daily flight pattern that is primarily determined by the availability of flight mature aphids. For many aphid species, a model of bimodal flight periodicity has been proposed, which predicts that large flights will occur in the morning and late in the afternoon (Dixon, 1985). The first peak in aphid flights is one of alates that mature overnight and are prevented from flying by low temperatures and light intensity, and the second flight in the afternoon is due to a peak in the number of newly moulted alates in the morning that complete their teneral period by the afternoon (Dixon, 1985).

The daily flight patterns on 6 days in September are described in chapter 4. No consistent pattern was observed. Longer periods of observation may reveal that abundant aphid flights more frequently occur in the morning and late afternoon, as would be predicted by the model of bimodal flight periodicity.

Flight of *M. persicae* and *R. padi* was observed at temperatures which were below reported minimum thresholds for flight initiation. Previous studies on the flight behaviour of *R. padi* and *M.persicae* were done in Europe. In South Australia, the secondary hosts of the aphids are most abundant during winter and spring, which is in contrast to Europe, where the secondary hosts are most abundant during summer. It would therefore be advantageous for alates to have minimum temperature thresholds for flight initiation which were lower than those reported in Europe, so as to facilitate dispersal in the cooler months. Studies of different alate morphs of *R. padi* which are produced at different parts of the year, have shown that they have different minimum temperature thresholds for flight initiation (Wiktelius, 1981; Walters and Dixon, 1984). Gynoparae of *R. padi*, returning to the primary host *Prunus padus* in Autumn, have been observed to fly at temperatures as low as 9 to

10 C (Wiktelius, 1981). The different alate morphs of *R. padi* appear to adapt to the temperature regime of the season in which they develop.

7.4 Modelling disease progress as a function of vector numbers

In chapter 5, increase in incidence of infection in 1987 was described as a function of cumulative vector numbers. A problem existed in that more than one aphid species could have been a vector and no information was available on the vector propensity nor the relative landing rates of the different aphid species. During the early part of the epidemic, *R. padi* was overwhelmingly the most abundant aphid and for the purpose of modelling, was considered the only vector species.

Two important objectives of future vector work to allow more accurate modelling of epidemic progress are -

(a) To develop a method of measuring the landing rates of alate aphids on the lupin crop. There has been no method suggested to directly measure aphid landing rates, other than observation. The green tile trap has been designed to mimic a leaf in colour, size and texture and is therefore thought to provide an accurate estimate of landing rates (Irwin and Ruesink, 1986). The green tile traps used in the 1989 field trial trapped inefficiently and the numbers were too small to provide a good indication of the relative landing rates of aphids through the season. The numbers of aphids trapped in the green tile traps in the 1989 field trial were too small to provide a good estimate of the relative landing rates of the different aphid species. A larger number of tile traps would need to be exposed to provide a representative sample of the aphids landing on the lupin crop. The suction traps are considered to have provided a good estimate of the relative aerial densities of aphids above the crop, but aerial densities may not neccesarily correlate with landing rates.

(b) To determine the vector propensity of common aphid species. Tests to determine the vector propensity of aphids transmitting non-persistently transmitted viruses have been developed in the laboratories of Sigvald and Irwin (Sigvald, 1984; Irwin and Ruesink, 1986). The essential characteristics of these tests are that alate aphids are released into an aphid cage which contains a mixture of healthy and infected plants and the aphids are allowed to move at will between the plants in a set period of time; the number of new infections that arise is then recorded. These tests are considered to give a better estimate of vector propensity compared with the transmission tests described in chapter 4 as they allow greater behavioral freedom to the aphid. Behavioral freedom is still restricted due to the spatial limitation of the cage. Halbert, Irwin and Goodman (1981) have used a different approach to determining vector propensity whereby they trap live aphids flying over a field plot and test them for infectivity. The field plot is totally infected so that virus acquisition is independent of the amount of inoculum and other experiments have shown that for soybeans infected with soybean mosaic virus, virus acquisition is independent of plant age and the length of time the plant has been infected.

Once an aphid's landing rate and vector propensity is determined, then vector intensity (the product of these two parameters) can be calculated (Irwin and Ruesink, 1986). By incorporating a parameter for vector intensity, instead of vector numbers, in models to predict epidemic progress, the need to distinguish between vector species is avoided (Ruesink and Irwin, 1986).

7.5 Modelling spatial progression

In chapter 5, four models were fitted to gradient data obtained from the 1988 field trial. The interpretations of the better fitting models were that all or most inoculum originated from the infection focus and that inoculum was diluted with increasing distance from the source.

Description of infection gradients in the 1988 field trial was limited to one observation. Repeated observations of the spatial progression of the epidemic were limited because of the small size of the plots and the convergence of gradients arising from the linear sources of inoculum arranged on opposite sides of the plot. With time, not only can the height and steepness of the gradient change, but also the functional relationship between incidence of infection and distance from the infection focus (Campbell and Madden, 1990). Results from modelling the progress of the 1987 epidemic as a function of vector numbers suggests that the epidemic is polycyclic. It is therefore possible that at a late stage of the epidemic, the plants at the infection focus may become insignificant as sources of inoculum compared with the newly infected plants and that rate of spread is no longer dependent on the distance from the infection focus.

Epidemics which initiate from infection foci in the crop can be quantified in both dimensions of time and space by describing the rate of isopath movement. An isopath is defined as a line of equal incidence of infection (Berger and Luke, 1979). Rate of isopath movement can be determined by modelling gradients at successive times and determining the change in distance of the isopath over the time period (Campbell and Madden, 1990). A modification to this concept for epidemics of non-persistently transmitted aphid-borne viruses could be to relate movement of the isopath to vector numbers instead of time.

Both the vector and gradient models used to describe epidemic progress in this thesis are simplistic. To produce more sophisticated models, the interactions between the plant, virus, vector and environment need to be more intensively studied. For example, what is the effect of plant age on suceptibility to infection? How effective are plants inoculated at different ages or which have been infected for different lengths of time as sources of inoculum?

7.6 Persistence of CMV between lupin generations through seed transmission

Without aphid spread, CMV cannot persist indefinitely in *L. angustifolius* by seed transmission. This point can be demonstrated by considering a theoretical situation in which a crop is sown with seed that has the maximum level of CMV transmission of 25 %. If no secondary spread occurs, the rate of seed transmission in the next generation can be calculated using the following equation -

$$Q = N_{(j)}Y_{(j)}R_{(j)} / [N_{(j)}Y_{(j)} + (100 - N_{(j)})H] \qquad \text{Equation 1}$$

where Q is the rate (%) of CMV transmission in seed produced by the crop, N is the number of plants inoculated on day j (% of total plant population), H is the number of viable seed produced by healthy plants and $Y(j)$ and $R(j)$ is the number of viable seed and the rate (%) of CMV transmission in seed produced by the infected plants respectively.

If it is assumed that seed yield and rate of seed transmission is the same for plants inoculated at the seedling stage as it is for plants infected through seed, then $Y_{(j)}$ is 0.71, $R_{(j)}$ is 25.0 and H is 177.2 (data were obtained from the 1989 field experiment on seed transmission which is described in chapter 6). Q is therefore calculated to be 0.033 %. In one generation, seed transmission levels will be reduced by 758 fold. This is a conservative estimate of the reduction in seed transmission levels, as neither the high mortality rate of seedlings with seedborne infection, nor the effects of competition from neighbouring healthy plants, were considered. From these simple calculations it can be seen that without secondary spread by aphids, either CMV will fail to persist through seed transmission or more mild variants of CMV that may occur will become the dominant genotype being seed transmitted.

The optimal time of inoculation for seed transmission is that time at which the effect of infection on seed yield is moderate and seed transmission levels are high. To determine which inoculation time was optimal for seed transmission, rates of transmission were calculated for a theoretical situation in which plants were either healthy or all inoculated at

one of the growth stages that were examined in the 1989 field experiment on seed transmission (see chapter 6). Rate of seed transmission was calculated using equation 1. The relationship between incidence of infection and rate of seed transmission is shown in Fig. 7.1.

Two interpretations of the data presented in Fig. 7.1 are -

(1) For epidemics in which incidence of infection at crop maturity is between 0 and 74.1 %, maximum levels of seed transmission will occur when virus spread by aphids is at the beginning of flowering (treatment 3 in the 1989 seed transmission experiment).

(2) The incidence of infection required to give seed transmission rates of between 0 and 6.5 % is minimised when the lupins are inoculated at the beginning of flowering of the primary inflorescence.

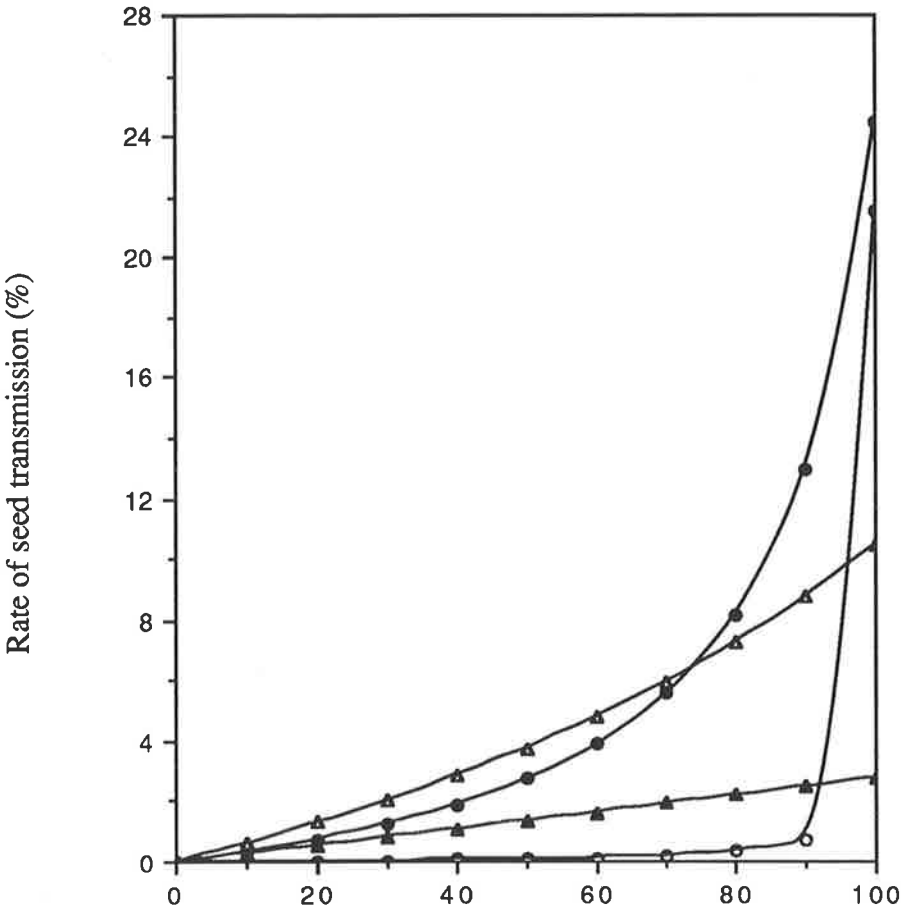
The first large aphid flights in 1987 and 1988 occurred in the period from mid-August to the beginning of September and this was the time at which flowering commenced. Aphid spread during flowering is optimal for survival of the virus by seed transmission. Maximum seed transmission rates of about 25 % would occur if the crop became completely infected during its vegetative stage, but the large reduction in seed production caused by virus infection would affect the survival chances of the plant population, which in turn would affect the survival chances of the virus.

7.7 The mechanism of seed transmission

Although the mechanism of seed transmission was not studied, observation of the development of the seed at the time of inoculation and the subsequent occurrence of CMV transmission in that seed, supported the theory that CMV can directly infect the developing seed. This is in contrast to the report from Davis and Hampton (1986), who speculated that

Fig. 7.1: Relationship between incidence of infection and rate of CMV transmission in the seeds produced by the crop.

To calculate rates, it was assumed that the crop consisted of either healthy plants or plants uniformly inoculated at one of the four growth stages examined in the 1989 seed transmission experiment (see Table 6.1). (○) is treatment 1; (●) is treatment 2; (△) is treatment 3; (▲) is treatment 4.


Rate of seed transmission (%)
28
24
20
16
12
8
4
0
0
20
40
60
80
100
Incidence of infection (%)

infection of the bean (*Phaseolus vulgaris*) seed resulted from infection of the megagametophyte and that direct infection of the developing seed did not occur, as seeds produced by flowers fertilised before infection of the plant, escaped infection.

The pattern of higher rates of CMV transmission in seed produced by the later emerging inflorescences, described in chapter 6 and also by Jones (1988), may be a consequence of -

(a) specific photosynthate source-sink relationships within the plant

For flowering soybeans, the major sink of photosynthate from a leaf is the pod in the axil of that leaf; most photosynthate is transported to pods no more than 2 nodes distance from the source leaf (Stephenson and Wilson, 1977). Only lupin leaves produced at least one or two weeks after inoculation become infected with CMV. Symptoms of systemic infection for plants inoculated at the beginning of flowering only showed in leaves of the first and second order laterals (see Fig. 6.2). It is possible that photosynthate movement from these infected leaves, which correlates with virus movement (Matthews, 1991), was predominantly to the later emerging inflorescences. The low rate of CMV transmission in seed from the primary inflorescence may be because this seed was primarily nourished by healthy leaves produced before the inoculation.

The pattern of higher rates of CMV transmission in seeds from the later emerging inflorescences was not observed for plants from treatment 4 of the 1989 seed transmission experiment. The high rate of transmission in seeds from the primary inflorescence may have resulted from the relatively crude method of patch grafting of infected strips of infected epidermis onto the main stem and branches of the lupin, which caused infection of the vascular bundles leading to these pods.

(b) the existence of a cut-off point in the development of the seed after which it avoided infection if the plant became infected.

Developing seeds are thought to avoid infection when the plant becomes infected because of the absence of a vascular connection between the embryo and the maternal tissues (Carroll, 1981). Compounds such as C and N assimilates, oxygen, water and some phytohormones are unloaded from the phloem in the legume seed coat and pass via an apoplastic pathway to the surface of the cotyledons (Thorne, 1985). The normal route for cell to cell movement of plant viruses is considered to be through the symplastic pathway (Matthews, 1991) .

Schoelz and Zaitlin (1989) have shown that TMV RNA can enter the chloroplast, a process that presumably requires active transport across the outer and inner membranes of the chloroplast. The theory that a symplastic connection is required for virus movement is questioned by this observation. It is possible that lupin seed can become infected by active transport of CMV RNA into the seed embryo.

Some fundamental questions exist as to how some seed manages to avoid infection when the plant is inoculated at the seedling stage. Plants inoculated at the seedling stage showed no signs of recovery from infection and therefore the failure of seed to become infected cannot be explained by a decrease in virus concentration.

A field trial was chosen to investigate the factors affecting rate of seed transmission as glasshouse grown lupins do not develop a normal phenotype. Experience with plants grown in the glasshouse showed that they did not develop a normal branching pattern, with most pods being produced on the primary inflorescence and only small L1 inflorescences being produced. Further experiments investigating the mechanism of seed transmission should be conducted in a glasshouse in which more uniform development of the lupin could

be achieved, plant inoculations better controlled and plant development and virus movement more closely monitored.

7.8 Recommendations for control

(a) Eradication of sources of inoculum

Seedlings that were infected through seed were shown to be important as primary sources of inoculum. Using seed which was free of CMV infection would therefore provide a method of control. To provide information to the farmers on the health status of seed, a seed testing service must be available.

In a review of literature, Stace-Smith and Hamilton (1987) found that for viruses which are efficiently spread by aphids, such as cucumoviruses and potyviruses, the inoculum threshold for seed transmission is close to zero. Farmers in Western Australia are recommended not to sow seed with CMV transmission levels greater than 0.5 %. Jones and Proudlove (1991) found in the two years studied, grain yield loss from CMV infection was not significant in plots sown with seed transmitting CMV at a rate of 0.5 %, whereas in plots sown with seed transmitting CMV at a rate of 5 %, grain yield loss was between 34 and 53 %. Further quantitative data on what is an appropriate seed transmission threshold value needs to be provided.

Results described in chapter 6 showed that rapid multiplication of seed transmission rates could occur in successive generations. Jones and Proudlove (1991) also found that seed harvested from plots sown with 0.5 % infected seed, transmitted CMV at a rates greater than 1.0 %. This presents a problem to farmers who buy seed of a new cultivar once and save a portion of each years harvest to sow next years crop. In one year, seed transmission rates may increase to levels which may cause severe disease epidemics. It is therefore

recommended that the threshold value for seed certification be 0.05 % seed transmission or lower.

Results described in chapter 6 showed that aphid spread during flowering may produce mild or symptomless infection which is difficult to detect, but CMV transmission rates in the seed may easily exceed a threshold value of 0.5 %. Crop inspection may therefore not be a satisfactory method to determine the health status of the seed.

To provide a routine seed testing service, a method by which dry seed is batch tested needs to be devised. The diagnostic assay used, the batch size and the total size of the seed sample tested is dependent on the objectives of the testing program. The size of the sample to be tested is dependent on the threshold value chosen. If the probability that a seed transmits CMV is p, the probability that it is healthy is q (where $q = 1 - p$) and a seed sample of size N is tested, then using the binomial equation (Lentner, 1976) it follows that -

$P\ (x \geq 1) = 1 - q^N$, where $P\ (x \geq 1)$ is the probability that at least 1 infected seed is detected in the seed sample.

To be able to detect one infected seed in 200 in 95 % of the tests applied, then the size of the seed sample needed is -

$N = \log 0.05 / \log 0.995 = 598$ seed.

Seed containing 1 infected seed in a sample of 598 should be rejected if the inoculum threshold is 0.5 % seed transmission. The probability of detecting that infected seed does not change whether the whole seed sample is pooled and batch tested or each seed is individually tested. To be able to confidently detect 1 infected seed in a pooled sample of 598, there is a need to develop a more sensitive diagnostic assay than DAS ELISA such as one based on nucleic acid hybridisation or the polymerase chain reaction (PCR).

If the objectives of the seed testing program are also to estimate the rate of seed transmission, then it is necessary to divide the seed sample into smaller groups to batch test (size dependent on the rate but between about 0 and 100). In this case, the sensitivity obtained by DAS ELISA is adequate.

(b) Sowing date

Surveys conducted in 1983 showed that severe disease epidemics occured more frequently in crops that were sown before April 26 (Alberts *et al.*, 1985). In southern Australia, aphid flights peak in abundance in autumn and spring (O'Loughlin 1962; Hughes *et al.*, 1965; Jayasena and Randles, 1984). In 1983, the earlier sown crops were probably more severely diseased because of the occurrence of aphid flights soon after emergence. Some control could be achieved by sowing in May or early June so as to avoid the autumn flights of aphids.

If the oversummering reservoirs of the aphid vectors could be identified and aphid numbers in autumn monitored, then the size and timing of vector flights could be better predicted and the risk to farmers of sowing early crops assessed.

(c) Mineral oils

Mineral oils applied to the lupins soon after emergence may be effective in protecting the crop from autumn vector flights. One or two applications may be sufficient to protect the crop in the short period of risk when autumn vector flights may occur. At the seedling stage, foliage area is small and good coverage of the leaves with the mineral oils may be achieved. The phytotoxity and the effect of oil on aphid transmission would need to be tested and the application technology developed.

(d) Sow at higher densities

Jones (1988) has suggested that a possible control method is to sow the seed at higher densities so that plant competition is greater and the severely stunted lupins which were infected through seed are shaded out. The effect of higher plant densities on epidemic development has yet to be tested. A higher plant density may have no effect on the number of infection foci if aphid spread occurs during the seedling stage, resulting in the formation of clumps of infected plants and the competitive advantage of the neighbouring plants is reduced. A more significant effect of higher plant densities may be to reduce the number of alate aphids landing on the crop. Halbert and Irwin (1981) found that the landing rates of some species were reduced by increased canopy closure, while for other species, landing rates were not affected or sometimes increased with increased canopy closure. The effectiveness of manipulating ground cover to reduce vector numbers would need to be tested.

(e) Breeding for resistance

Ideally, immunity or resistance to systemic infection should be sought, though it is unlikely that durable resistance of these types will be naturally found, due to the potential for rapid evolution of new CMV variants that overcome the selection pressures. Genetically engineered resistance, such as that produced when plants are transformed with viral coat protein (Cuozzo *et al.*, 1988), may offer better prospects for long term protection to CMV infection. For any type of plant virus resistance, the durability of resistance will be increased by implementation of other control methods, such as those mentioned above, which will reduce the infection pressure.

There are types of partial resistance which are useful in control by reducing the rate of progress of the epidemic. Aphid acquisition of SMV was shown to decrease with increasing leaf pubescence and when compared in the field, virus spread was greater in the sparsely pubescent cultivar (Gunasinghe *et al.*, 1988). The effiency of acquisition of CMV by an aphid is dependent on the concentration of the virus in the leaf (Normand and Pirone,

1968; Banik and Zitter, 1990; Zitter and Gonsalves, 1990). Field spread of CMV could be reduced by using cultivars in which virus replication was reduced. Any trait of the plant that reduces the number of cycles of infection that could occur in a season could be useful for disease control. what about resistance to seed transmission ?

7.8 Conclusions

This thesis makes the following original contributions to the knowledge of the epidemiology of CMV in *L. angustifolius* -

(a) Provides a quantitative description of the development of the epidemic.

(b) Shows that lupins with seedborne infection are important primary sources of inoculum. more already

(c) Provides descriptions of the seasonal and daily patterns of aphid flights. already done

(d) Shows that migratory alates, such as *R. padi*, which do not colonise, are important vectors.

(e) Provides simple models to describe both the spatial and temporal progress of the epidemic. based on these models

(f) Provides data on yield loss caused by CMV infection.

(g) Shows that the rate of seed transmission is dependent on the age of the plant at the time of inoculation.

(h) Shows that aphid spread during flowering is optimal for suvival of CMV. and summary

Appendices

Appendix 1: Description of the CMV epidemic in *L. angustifolius*.

Appendix 1.1: Temporal progress of the epidemic in the 1987 field trial (diagnosis by symptoms).

	Incidence of infection (%)								
Date	Treatment VV			Treatment V			Treatment C		
	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
10/6	4.1	1.3	3.8	5.0	3.7	2.2	3.2	1.9	1.9
2/7	5.5	1.3	4.6	5.5	4.2	2.5	3.2	2.5	2.9
23/7	8.0	2.4	5.7	7.6	5.7	2.8	4.1	2.5	2.9
6/8	8.6	2.9	6.8	8.2	6.5	3.0	4.4	2.7	3.5
20/8	9.1	3.7	7.0	9.2	7.1	3.0	4.4	2.7	3.0
3/9	9.9	4.5	7.3	12.4	9.3	3.9	4.4	3.8	6.7
17/9	17.1	14.1	13.0	27.6	23.2	12.9	13.7	10.9	12.3
1/10	30.4	30.9	26.8	38.4	37.1	18.7	19.7	15.6	27.5
15/10	48.9	41.2	34.3	62.4	51.8	32.5	29.5	34.4	32.9
30/10	62.2	56.4	44.3	79.5	55.2	51.5	47.3	44.5	50.3

Appendix 1.2: Analysis of variance to test for differences in incidence of infection between treatments VV, V and C of the 1987 field trial.

Variate: incidence of infection (%)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	453.07	226.54		
block.treatment stratum					
treatment	2	621.48	310.74	2.55	0.193
residual	4	488.05	122.01		
block.treatment.time stratum					
time	9	26881.21	2986.80	176.16	<0.001
treatment.time	18	492.82	27.38	1.61	0.089
residual	54	915.58	16.96		
total	89	29852.22			

Appendix 1.3: Temporal progress of the epidemic in treatment C of the 1987 field trial (diagnosis by ELISA).

Date	Incidence of infection (%)																	
	Replicate plot 1						Replicate plot 2						Replicate plot 3					
	Row					Mean	Row					Mean	Row					Mean
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
10/6	0	0	0	0	6.7	1.3	5.6	6.3	0	0	5.0	3.4	5.0	5.0	0	0	0	2.0
2/7	6.7	0	0	0	6.7	2.7	5.6	6.3	0	5.6	5.0	4.5	5.0	5.0	0	0	0	2.0
23/7	7.1	0	0	0	6.7	2.8	5.6	6.3	0	5.6	5.0	4.5	5.0	5.0	0	0	0	2.0
6/8	7.7	0	0	0	6.7	2.9	5.6	6.3	0	5.6	10.0	5.5	5.0	5.0	0	0	0	2.0
20/8	15.4	6.7	0	0	7.1	5.8	5.6	6.3	0	5.6	10.0	5.5	10.0	10.0	0	0	0	4.0
3/9	15.4	6.7	0	5.9	14.3	8.4	11.1	12.5	10.0	5.6	25.0	12.8	25.0	30.0	10.5	0	0	13.1
17/9	30.8	26.7	18.8	35.3	35.7	29.4	16.7	43.8	35.0	33.3	65.0	38.8	35.0	40.0	42.1	22.2	36.8	35.2
1/10	46.2	40.0	62.5	52.9	71.4	54.6	27.8	56.3	55.0	50.0	75.0	52.8	45.0	45.0	42.1	61.1	68.4	52.3
15/10	76.9	64.3	81.3	94.1	84.6	80.2	50.0	75.0	75.0	55.6	90.0	69.1	75.0	60.0	52.6	88.9	84.2	72.2
30/10	92.3	85.7	87.5	100.0	100.0	93.1	50.0	81.3	95.0	83.3	95.0	80.9	90.0	65.0	73.7	94.4	94.7	83.6

Appendix 1.4: Temporal progress of the epidemic in the 1988 field trial (diagnosis by ELISA).

Date	Incidence of infection (%)															
	Treatment VOA				Treatment VO				Treatment V				Treatment C			
	Block				Block				Block				Block			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
14/7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28/7	3.4	0	0	1.1	0	0	0	0	0	0	0	0	0	0	0	0
10/8	3.4	1.8	0	1.7	0	2.0	0	0.7	2	1.9	2.3	2.1	0	0	0	0
24/8	3.4	5.5	2.1	3.7	0	4.0	2.1	2.0	4.1	11.3	5.1	6.8	1.8	1.8	2.2	1.9
7/9	45.8	42.6	54.2	47.5	47.4	24.0	43.8	38.4	34.7	49.1	46.3	43.3	12.5	21.4	22.7	18.9
21/9	88.1	96.4	97.9	94.1	94.7	82.0	100	92.2	95.8	96.2	100	97.3	91.1	94.5	81.8	89.1
5/10	96.6	96.4	100	97.7	98.2	90.0	100	96.1	97.9	98.1	100	98.7	96.4	100	97.7	98.0

Appendix 1.5: Spatial pattern of infected plants in the 1988 field trial on September 7.

Appendix 1.5.1: Incidence of infection in rows at varying distance from the linear source of inoculum.

	Incidence of infection (%)							
Treatment	VOA		VO		V		C	
Distance (cm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
50	85.5	4.7	67.4	14.3	70.9	10.2	30.6	7.4
100	62.4	6.3	45.4	10.0	57.1	10.2	16.2	6.7
150	52.9	10.1	44.6	8.0	51.5	14.1	20.4	6.8
200	39.5	8.2	28.1	8.8	35.6	8.0	18.8	5.1
250	19.5	7.0	24.2	6.0	27.1	7.5	12.8	4.2
300	18.7	5.8	36.4	9.6	29.1	7.4	20.1	4.5

Appendix 1.5.2: Incidence of infection in columns at varying distance from the edge of the plot.

	Incidence of infection (%)							
Treatment	VOA		VO		V		C	
Distance (cm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
50	42.2	4.1	36.2	7.3	37.2	10.7	21.3	7.9
100	58.1	4.1	47.7	10.9	36.8	10.6	17.3	7.8
150	45.6	4.0	37.7	9.3	43.2	8.1	18.0	4.9
200	48.7	4.3	40.1	8.9	52.3	3.6	15.3	7.1
250	44.9	6.3	44.6	4.4	45.2	5.4	26.1	6.1

Appendix 1.6: Analyses of variance to test for differences in incidence of infection between treatments, between rows at varying distance from the linear source of inoculum, and between halves of the plot.

Appendix 1.6.1: Analysis using data from treatments VOA, VO, V and C.

Variate: Incidence of infection (proportion)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.14239	0.07120		
block.plot stratum					
distance	5	2.93083	0.58617	16.30	<0.001
half	1	0.35980	0.35980	10.01	0.002
treatment	3	1.66211	0.55404	15.41	<0.001
distance.half	5	0.06951	0.01390	0.39	0.857
distance.treatment	15	0.84202	0.05613	1.56	0.100
half.treatment	3	0.40451	0.13484	3.75	0.014
distance.half.treatment	15	0.69329	0.04622	1.29	0.227
residual	94	3.37965	0.03595		
total	143	10.48410			

Appendix 1.6.2: Analysis using data from treatments VOA, VO and V.

Variate: Incidence of infection (proportion)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.18912	0.09456		
block.plot stratum					
distance	5	3.34203	0.66841	16.64	<0.001
half	1	0.55210	0.55210	13.74	<0.001
treatment	2	0.05706	0.02853	0.71	0.495
distance.half	5	0.16531	0.03306	0.82	0.538
distance.treatment	10	0.32283	0.03228	0.80	0.626
half.treatment	2	0.20459	0.10230	2.55	0.086
distance.half.treatment	10	0.49244	0.04924	1.23	0.290
residual	70	2.81227	0.04018		
total	107	8.13776			

Appendix 1.6.3: Analysis using data from treatment C.

Variate: Incidence of infection (proportion)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.01180	0.00590		
block.plot stratum					
distance	5	0.10798	0.02160	0.93	0.478
half	1	0.00762	0.00762	0.33	0.572
distance.half	5	0.10505	0.02101	0.91	0.494
residual	22	0.50884	0.02313		
total	35	0.74129			

Appendix 1.7: Analyses of variance to test for differences in incidence of infection between treatments and between columns at varying distance from the edge of the plot.

Appendix 1.7.1: Analysis using data from treatments VOA, VO, V and C.

Variate: Incidence of infection (proportion)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.05233	0.02617		
block.treatment stratum					
treatment	3	1.42505	0.47502	5.09	0.044
residual	6	0.56045	0.09341		
block.treatment.plot stratum					
distance	4	0.08130	0.02032	0.79	0.535
treatment.distance	12	0.16929	0.01411	0.55	0.877
residual	92	2.36663	0.02572		
total	119	4.65504			

Appendix 1.7.2: Analysis using data from treatment C

Variate: Incidence of infection (proportion)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	0.01014	0.00507		
block.plot stratum					
distance	4	0.04141	0.01035	0.34	0.846
residual	23	0.69309	0.03013		
total	29	0.74464			

Appendix 2: Vector studies.

Appendix 2.1: Numbers of aphids trapped in the yellow pans in 1987.

Date	Aphid species											
	Aphis craccivora		*Brachycaudus rumexicolens*		*Lipaphis erysimi*		*Myzus persicae*		*Rhopalosiphum padi*		Other species	
	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2
Jun. 10	2	1	8	10	6	1	6	1	7	1	11	6
Jun. 17	0	0	12	8	0	0	8	3	3	1	9	2
Jun. 24	0	0	15	2	0	0	5	0	0	0	3	0
Jul. 1	0	0	6	3	0	0	2	0	3	0	4	1
Jul. 8	0	0	4	1	0	0	5	1	6	2	5	1
Jul. 15	0	0	0	0	1	0	4	0	2	0	1	0
Jul. 22	0	0	0	0	0	0	0	0	1	2	0	0
Jul. 29	0	0	0	0	0	0	0	0	2	0	0	0
Aug. 5	0	0	0	0	0	0	0	1	5	2	1	0
Aug. 12	0	0	0	0	0	0	0	0	10	4	0	0
Aug. 19	0	0	0	1	16	2	11	2	7	10	2	1
Aug. 26	0	0	0	0	0	0	1	0	59	34	0	1
Sep. 2	0	0	1	3	38	3	15	4	32	23	4	0
Sep. 9	0	0	3	1	0	0	4	1	19	24	2	0
Sep.16	3	1	11	8	0	0	13	1	11	14	3	0
Sep. 23	24	3	17	19	9	0	88	16	13	18	10	6
Sep. 30	0	39	6	6	59	16	125	32	3	6	20	8
Oct. 7	15	14	60	67	1	0	45	15	2	8	15	6
Oct. 14	46	23	126	181	1	0	15	6	0	0	7	9
Oct. 21	52	30	71	96	15	11	17	11	2	2	18	17
Oct. 28	13	13	99	123	6	0	9	1	0	0	18	13
Total	155	114	439	529	152	33	373	104	187	151	133	71

Appendix 2.2: Numbers of aphids trapped in the yellow pans in 1988.

	Aphid species					
Date	*Aphis craccivora*	*Brachycaudus rumexicolens*	*Lipaphis erysimi*	*Myzus persicae*	*Rhopalosiphum padi*	Other species
	Trap 1	Trap 1	Trap 1	Trap 1	Trap 1	Trap 1
22/6	0	0	0	4	2	0
29/6	1	0	0	4	0	4
6/7	0	0	0	3	1	0
13/7	0	3	0	7	5	9
20/7	0	1	0	9	7	10
27/7	0	0	0	6	2	2
3/8	0	0	0	10	4	1
10/8	2	0	6	6	0	9
17/8	0	0	130	65	11	1
24/8	0	0	0	24	0	0
31/8	17	0	85	243	22	19
7/9	29	16	212	566	43	36
14/9	28	3	50	645	20	28
21/9	6	0	1	199	8	1
Total	83	23	484	1791	125	120

Appendix 2.3: Numbers of aphids trapped in the yellow pans in 1989.

Date	Aphid species											
	Aphis craccivora		*Brachycaudus rumexicolens*		*Lipaphis erysimi*		*Myzus persicae*		*Rhopalosiphum padi*		Other species	
	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2	Trap 1	Trap 2
Jun. 13	0	0	1	0	0	0	21	5	2	3	0	4
Jun. 20	0	0	0	0	0	0	9	5	5	4	0	3
Jun. 27	0	1	1	1	0	0	37	25	7	4	14	2
Jul. 4	0	0	2	0	0	0	35	4	0	1	11	0
Jul. 11	0	0	0	0	0	0	0	0	1	0	0	0
Jul. 18	0	0	0	0	0	0	9	15	2	1	1	2
Jul. 25	0	0	0	0	0	0	15	24	6	5	6	2
Aug. 1	0	0	0	0	0	0	2	3	21	5	1	0
Aug. 8	0	0	0	0	0	0	0	1	2	1	0	0
Aug. 15	0	0	0	0	0	0	0	0	5	6	0	1
Aug. 22	0	0	0	0	0	0	4	5	8	11	3	2
Aug. 29	0	0	0	0	0	0	0	1	3	0	2	0
Sept. 5	0	0	0	1	3	1	64	45	49	9	6	4
Sept. 12	1	0	0	0	27	8	49	23	35	8	5	0
Sept. 19	1	0	2	0	37	2	116	49	89	16	9	2
Sept. 26	1	0	5	0	159	50	305	78	118	9	18	10
Oct. 3	1	0	2	0	71	23	96	47	7	3	4	0
Oct. 10	1	1	2	0	1	1	14	2	1	1	3	0
Oct. 17	0	0	0	0	0	0	10	2	1	0	9	0
Oct. 24	0	0	1	0	0	0	1	0	0	0	2	1
Oct. 31	1	0	0	0	0	1	0	0	4	0	0	0
Total	6	2	16	2	298	86	787	334	366	87	94	33

Appendix 2.4: Aphid species trapped in the yellow pans in 1987.

Aphid species	Abundance (% of total trapped)
Acyrthosiphon kondoi	1.1
Acyrthosiphon malvae	0.41
Acyrthosiphon pisum	0.69
Aphis craccivora	11.3
Aulacorthum solani	0.16
Brachycaudus helichrysi	0.081
Brachycaudus rumexicolens	39.4
Brevicoryne brassicae	0.081
Calaphis flava	0.041
Capitophorus eleagni	0.24
Cavariella aegopodii	0.041
Dysaphis sp.	3.0
Hyperomyzus carduellinus	0.041
Hyperomyzus lactucae	0.28
Lipaphis erysimi	7.5
Macrosiphum euphorbiae	1.5
Metapolophium dirhod um	0.57
Myzus cerasi	0.041
Myzus persicae	19.0
Rhopalosiphum insertum	0.49
Rhopalosiphum padi	13.7
Rhopalosiphum rufiabdominalis	0.081
Sitobion miscanthi	0.081
Tetraneura nigriabdominalis	0.041

Appendix 2.5: Aphid species trapped in the yellow pans in 1988.

Aphid species	Abundance (% of total trapped)
Acyrthosiphon kondoi	0.53
Aphis craccivora	3.2
Aulacorthum solani	0.15
Brachycaudus rumexicolens	0.88
Capitophorus eleagni	0.076
Dysaphis sp.	1.26
Hyperomyzus lactucae	0.38
Lipaphis erysimi	18.4
Macrosiphum euphorbiae	0.88
Metapolophium dirhod um	1.26
Myzus persicae	68.2
Rhopalosiphum maidus	0.038
Rhopalosiphum padi	4.8

Appendix 2.6: Aphid species trapped in the yellow pans in 1989.

Aphid species	Abundance (% of total trapped)
Acyrthosiphon kondoi	0.52
Acyrthosiphon pisum	0.095
Aphis craccivora	0.38
Aulacorthum solani	0.047
Brachycaudus rumexicolens	0.85
Brevicoryne brassicae	0.19
Capitophorus eleagni	0.095
Dysaphis sp.	2.5
Hyperomyzus lactucae	0.52
Lipaphis erysimi	18.2
Macrosiphum euphorbiae	0.14
Metapolophium dirhod um	0.47
Myzus cerasi	0.43
Myzus persicae	53.1
Rhopalosiphum maidis	0.43
Rhopalosiphum padi	21.5

Appendix 2.7: Numbers of aphids collected in the suction traps in 1989.

Date	*Aphis craccivora*				*Brachycaudus rumexicolens*				*Lipaphis erysimi*				*Myzus persicae*				*Rhopalosiphum padi*				Other species			
	Trap 1	Trap 2	Trap 3	Trap 4	Trap 1	Trap 2	Trap 3	Trap 4	Trap 1	Trap 2	Trap 3	Trap 4	Trap 1	Trap 2	Trap 3	Trap 4	Trap 1	Trap 2	Trap 3	Trap 4	Trap 1	Trap 2	Trap 3	Trap 4
Jun. 20	0	0	0	0	1	0	0	0	0	0	0	0	1	8	1	1	2	2	3	6	1	0	0	0
Jun. 27	0	0	0	0	0	0	0	0	0	0	0	0	17	21	6	15	12	15	20	16	4	3	0	1
Jul. 4	0	0	0	0	2	2	0	1	0	0	0	0	39	32	15	20	20	11	14	19	1	0	3	1
Jul. 11	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Jul. 18	0	0	0	0	0	0	0	0	0	0	0	0	7	7	3	9	16	9	14	7	1	1	0	0
Jul. 25	0	0	0	0	1	0	0	0	0	0	0	0	6	6	1	8	32	31	36	39	4	1	3	0
Aug. 1	0	0	0	0	0	0	1	0	0	0	0	0	2	2	3	3	10	10	11	12	2	0	2	0
Aug. 8	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	4	5	4	8	0	1	0	0
Aug. 15	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	3	17	12	34	19	0	1	0	0
Aug. 22	0	0	0	0	0	0	0	0	0	0	0	0	5	5	1	0	19	22	15	23	0	5	1	5
Aug. 29	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	6	6	8	9	4	0	3	0
Sep. 5	0	0	0	0	1	2	1	1	2	0	1	0	5	5	1	4	71	61	39	52	3	2	5	3
Sep. 12	0	0	0	0	0	1	1	0	2	5	1	1	3	12	1	3	38	37	33	27	3	4	2	0
Sep. 19	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0	0	14	14	9	12	1	0	0	3
Sep. 26	0	0	0	1	1	3	3	0	0	1	0	0	2	2	1	4	46	56	48	37	1	1	0	1
Oct. 3	0	0	0	0	1	1	0	2	0	1	0	2	1	2	1	3	49	51	111	129	2	3	2	5
Oct. 10	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3	3	0	0	2	0	1	3
Oct. 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	8	5	1	1	1	1
Oct. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Oct. 31	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	2	6	2	1	1	3	4	1
Total	0	2	0	3	7	14	7	4	4	7	2	3	93	109	39	76	390	386	434	460	31	26	27	24

Appendix 2.8: Hourly trap collections of aphids on September 5, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1	0	0	0	0	0	1	1	0	0	1	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	1	2	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	1	1	1	1
	Total	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**3**	**3**	**1**
	Yellow trap 1	-	0	0	3	6	1	6	9	1	10	4	1
	Yellow trap 2	-	0	2	0	3	2	2	5	3	7	0	0
	Total	**-**	**0**	**2**	**3**	**9**	**3**	**8**	**14**	**4**	**17**	**4**	**1**
R. padi	Suction trap 1	0	0	0	1	1	0	3	7	9	9	7	3
	Suction trap 2	0	0	0	0	4	1	2	5	3	7	6	3
	Suction trap 3	0	0	0	0	0	0	3	4	2	3	2	1
	Suction trap 4	0	0	0	0	0	0	4	4	1	4	8	2
	Total	**0**	**0**	**0**	**1**	**5**	**1**	**12**	**20**	**15**	**23**	**23**	**9**
	Yellow trap 1	-	0	0	1	1	1	2	1	0	3	0	0
	Yellow trap 2	-	0	0	0	0	0	1	0	0	3	0	0
	Total	**-**	**0**	**0**	**1**	**1**	**1**	**3**	**1**	**0**	**6**	**0**	**0**
L. erysimi	Suction trap 1	0	0	0	0	0	0	0	1	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	Yellow trap 1	-	0	0	0	0	0	0	2	0	0	0	0
	Yellow trap 2	-	0	0	0	0	0	0	0	0	0	0	0
	Total	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**
Other species	Suction trap 1	0	0	0	0	0	0	0	0	1	1	0	0
	Suction trap 2	0	0	0	0	0	0	0	1	0	1	0	0
	Suction trap 3	0	0	0	2	1	0	0	1	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	1	0	0	0	0
	Total	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**3**	**1**	**2**	**0**	**0**
	Yellow trap 1	-	0	0	2	0	0	1	0	0	0	0	0
	Yellow trap 2	-	0	0	0	0	1	0	1	0	2	0	0
	Total	**-**	**0**	**0**	**2**	**0**	**1**	**1**	**1**	**0**	**2**	**0**	**0**

Appendix 2.9: Hourly trap collections of aphids on September 7, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1	-	0	0	0	0	0	0	1	0	0	0	0
	Suction trap 2	-	0	0	0	0	0	0	0	0	0	0	2
	Suction trap 3	-	0	0	0	0	0	1	0	0	0	0	0
	Suction trap 4	-	0	1	1	0	0	0	0	0	0	0	0
	Total	**-**	**0**	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**2**
	Yellow trap 1	-	0	0	1	0	1	0	0	0	0	14	13
	Yellow trap 2	-	0	0	0	0	0	0	0	1	1	3	7
	Total		**0**	**0**	**1**	**0**	**1**	**0**	**0**	**1**	**1**	**17**	**20**
R. padi	Suction trap 1	-	0	0	0	0	0	1	1	1	1	2	2
	Suction trap 2	-	0	1	0	0	3	0	0	0	1	3	4
	Suction trap 3	-	0	1	1	1	0	1	0	2	1	2	4
	Suction trap 4	-	0	1	0	0	0	0	1	0	0	2	5
	Total	**-**	**0**	**3**	**1**	**1**	**3**	**2**	**2**	**3**	**3**	**9**	**15**
	Yellow trap 1	-	0	0	0	0	0	0	1	1	0	4	17
	Yellow trap 2	-	0	0	0	0	0	1	0	0	1	1	1
	Total	**-**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**5**	**18**
L. erysimi	Suction trap 1	-	0	0	0	0	0	0	0	0	1	0	0
	Suction trap 2	-	0	0	0	0	0	0	0	0	0	2	0
	Suction trap 3	-	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	-	0	0	0	0	0	0	0	0	0	0	0
	Total	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**	**0**
	Yellow trap 1	-	0	0	0	0	0	0	0	0	0	2	10
	Yellow trap 2	-	0	0	0	0	0	0	0	0	1	1	2
	Total	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**3**	**12**
Other species	Suction trap 1	-	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	-	0	0	0	0	0	0	0	0	0	0	2
	Suction trap 3	-	0	0	0	1	0	0	0	1	0	0	0
	Suction trap 4	-	0	0	0	0	0	0	0	0	0	0	0
	Total	**-**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**2**
	Yellow trap 1	-	0	1	0	0	0	0	0	0	0	1	3
	Yellow trap 2	-	0	0	0	0	0	0	0	0	0	0	0
	Total	**-**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**3**

Appendix 2.10: Hourly trap collections of aphids on September 8, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1									1	0	0	0
	Suction trap 2									0	0	4	2
	Suction trap 3									0	0	0	0
	Suction trap 4									0	0	1	0
	Total									**1**	**0**	**5**	**2**
	Yellow trap 1									0	5	6	4
	Yellow trap 2									0	1	5	1
	Total									**0**	**6**	**11**	**5**
R. padi	Suction trap 1									2	4	7	4
	Suction trap 2									2	1	4	6
	Suction trap 3									0	0	6	3
	Suction trap 4									0	1	3	3
	Total									**4**	**6**	**20**	**16**
	Yellow trap 1									0	8	1	1
	Yellow trap 2									0	1	0	0
	Total									**0**	**9**	**1**	**1**
L. erysimi	Suction trap 1									0	0	1	0
	Suction trap 2									0	0	2	0
	Suction trap 3									0	0	1	1
	Suction trap 4									0	0	1	0
	Total									**0**	**0**	**5**	**1**
	Yellow trap 1									1	1	8	4
	Yellow trap 2									0	1	3	0
	Total									**1**	**2**	**11**	**4**
Other species	Suction trap 1									0	0	0	0
	Suction trap 2									0	0	0	0
	Suction trap 3									0	0	0	0
	Suction trap 4									0	0	0	0
	Total									**0**	**0**	**0**	**0**
	Yellow trap 1									0	0	1	0
	Yellow trap 2									0	0	0	0
	Total									**0**	**0**	**1**	**0**

Appendix 2.11: Hourly trap collections of aphids on September 20, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	1	1	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
	Yellow trap 1	0	2	9	0	1	17	20	21	18	58	63	10
	Yellow trap 2	0	2	1	0	2	1	10	1	1	3	17	11
	Total	**0**	**4**	**10**	**0**	**3**	**18**	**30**	**22**	**19**	**61**	**80**	**21**
R. padi	Suction trap 1	0	0	1	1	1	0	1	1	2	1	2	3
	Suction trap 2	0	0	1	4	2	2	4	1	1	4	2	2
	Suction trap 3	0	0	4	6	3	3	1	1	1	1	2	1
	Suction trap 4	0	0	1	3	2	1	0	1	1	0	4	2
	Total	**0**	**0**	**7**	**14**	**8**	**6**	**6**	**4**	**5**	**6**	**10**	**8**
	Yellow trap 1	0	0	4	0	0	6	5	7	4	29	30	12
	Yellow trap 2	0	0	1	0	0	1	1	0	0	0	0	0
	Total	**0**	**0**	**5**	**0**	**0**	**7**	**6**	**7**	**4**	**29**	**30**	**12**
L. erysimi	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	1	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
	Yellow trap 1	0	0	0	0	1	7	4	3	0	4	93	6
	Yellow trap 2	0	0	0	0	0	0	0	0	0	0	37	6
	Total	**0**	**0**	**0**	**0**	**1**	**7**	**4**	**3**	**0**	**4**	**130**	**12**
Other species	Suction trap 1	0	0	0	0	0	2	0	0	0	1	0	0
	Suction trap 2	0	0	0	0	0	2	2	1	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**4**	**2**	**1**	**0**	**1**	**0**	**0**
	Yellow trap 1	0	0	0	0	0	4	0	1	1	4	1	0
	Yellow trap 2	0	0	0	0	0	0	0	0	0	0	0	6
	Total	**0**	**0**	**0**	**0**	**0**	**4**	**0**	**1**	**1**	**4**	**1**	**6**

Appendix 2.12: Hourly trap collections of aphids on September 21, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	1	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
	Yellow trap 1	0	7	22	3	0	0	0	0	1	1	2	12
	Yellow trap 2	0	0	1	0	0	0	0	0	1	1	2	4
	Total	**0**	**7**	**23**	**3**	**0**	**0**	**0**	**0**	**2**	**2**	**4**	**16**
R. padi	Suction trap 1	0	0	0	0	1	0	0	1	1	1	1	0
	Suction trap 2	0	1	2	0	0	1	0	2	1	0	1	0
	Suction trap 3	0	1	1	2	1	1	1	2	1	3	2	1
	Suction trap 4	0	0	0	1	0	0	1	3	1	2	3	1
	Total	**0**	**2**	**3**	**3**	**2**	**2**	**2**	**8**	**4**	**6**	**7**	**2**
	Yellow trap 1	1	8	6	0	0	0	0	1	0	0	1	0
	Yellow trap 2	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**1**	**8**	**6**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	**0**
L. erysimi	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Yellow trap 1	0	1	23	9	1	0	0	0	2	0	2	2
	Yellow trap 2	0	0	1	1	0	0	0	0	0	1	0	3
	Total	**0**	**1**	**24**	**10**	**1**	**0**	**0**	**0**	**2**	**1**	**2**	**5**
Other species	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	1
	Suction trap 2	0	0	0	0	0	1	0	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**1**
	Yellow trap 1	0	1	2	0	0	0	1	1	0	3	1	0
	Yellow trap 2	0	0	0	0	0	0	0	0	0	0	1	0
	Total	**0**	**1**	**2**	**0**	**0**	**0**	**1**	**1**	**0**	**3**	**2**	**0**

Appendix 2.13: Hourly trap collections of aphids on September 29, 1989.

Aphid species	Time (hours)	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
M. persicae	Suction trap 1	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	1	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	1	1	0	0	0	0	0	0	0	0	0
	Total	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
	Yellow trap 1	0	0	0	0	0	8	6	26	2	0	0	0
	Yellow trap 2	0	0	0	0	2	3	3	7	3	0	0	1
	Total	**0**	**0**	**0**	**0**	**2**	**11**	**9**	**33**	**5**	**0**	**0**	**1**
R. padi	Suction trap 1	0	0	0	0	1	4	11	9	3	2	0	0
	Suction trap 2	0	0	0	1	1	4	3	6	3	0	0	0
	Suction trap 3	0	0	0	0	3	16	26	13	10	1	0	0
	Suction trap 4	0	0	0	0	1	8	19	26	6	4	0	0
	Total	**0**	**0**	**0**	**1**	**6**	**32**	**59**	**54**	**22**	**7**	**0**	**0**
	Yellow trap 1	0	0	0	0	0	0	1	1	0	0	0	0
	Yellow trap 2	0	0	0	0	1	0	0	1	0	1	0	0
	Total	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**2**	**0**	**1**	**0**	**0**
L. erysimi	Suction trap 1	0	0	0	0	0	0	0	1	0	0	0	0
	Suction trap 2	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	0	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Yellow trap 1	0	0	0	0	2	6	25	23	1	1	0	0
	Yellow trap 2	0	0	0	0	0	4	6	8	1	0	0	0
	Total	**0**	**0**	**0**	**0**	**2**	**10**	**31**	**31**	**2**	**1**	**0**	**0**
Other species	Suction trap 1	0	0	0	0	0	0	0	0	0	1	0	0
	Suction trap 2	0	0	0	0	0	0	1	1	0	0	0	0
	Suction trap 3	0	0	0	0	0	0	1	0	0	0	0	0
	Suction trap 4	0	0	0	0	0	0	1	1	1	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**2**	**1**	**1**	**0**	**0**
	Yellow trap 1	0	0	0	0	0	1	3	2	0	0	0	0
	Yellow trap 2	0	0	0	0	0	0	2	0	0	0	0	0
	Total	**0**	**0**	**0**	**0**	**0**	**1**	**5**	**2**	**0**	**0**	**0**	**0**

Appendix 2.14: Temperature and wind conditions in September, 1989.

Date	Time (hours)	0630	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800
September 5	Wind distance (km)		1.68	1.01	4.80	7.28	8.16	6.16	5.71	5.45	6.39	7.36	8.45	6.89
	Temperature (C)	2.5	3.5	8.9	12.2	15.2	17.0	18.0	20.0	19.2	19.5	18.4	17.0	13.2
September 7	Wind distance (km)			5.91	8.08	12.07	12.96	11.98	11.65	10.37	12.05	10.33	7.33	3.23
	Temperature (C)		6.0	10.0	14.5	17.8	20.0	21.2	21.8	21.7	21.3	21.0	19.5	15.0
September 8	Wind distance (km)	Average wind speed between 0900 and 1400 = 27.73									14.67	9.14	7.46	5.85
	Temperature (C)				20.5					22.4	22.3	22.2	19.2	14.9
September 20	Wind distance (km)		2.76	4.49	4.67	5.46	4.95	8.70	7.48	7.40	6.97	7.10	2.08	3.43
	Temperature (C)	7.4	8.5	12.0	14.5	15.4	15.9	16.7	17.5	18.0	18.2	18.3	17.4	13.6
September 21	Wind distance (km)		3.60	6.59	7.34	11.50	13.47	16.17	12.38	9.98	8.38	7.72	9.13	2.68
	Temperature (C)	9.0	10.3	15.1	18.0	21.1	22.4	22.3	21.0	19.6	21.1	20.0	18.1	14.0
September 29	Wind distance (km)		6.38	13.43	11.04	11.26	8.38	4.48	3.63	3.39	10.37	12.00	12.63	9.85
	Temperature (C)	17.1	18.0	18.1	18.5	19.3	20.0	18.6	19.5	20.1	17.4	16.1	15.0	13.5

Appendix 3: Modelling of epidemic progress.

Appendix 3.1: Regression analyses to fit the linear forms of the vector models to the epidemic observed in treatment C of the 1987 field trial.

Appendix 3.1.1: Vector species - *A. craccivora*, *B. rumexicolens*, *D. aucupariae*, *M. persicae* and *R. padi*.

Appendix 3.1.1.1: Vector model 1.

Response variate: ln [1/(1-*y*)]
Fitted terms: Constant, *A*

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	12.3405	12.34048
residual	25	0.9503	0.03801
total	26	13.2908	0.51118

percentage of variance accounted for: 92.6

Appendix 3.1.1.2: Vector model 2.

Response variate: ln [1/(1-*y*)]
Fitted terms: Constant, ln*A*

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	10.652	10.65186
residual	22	1.764	0.08020
total	23	12.416	0.53984

percentage variance accounted for: 85.1

Appendix 3.1.1.3: Vector model 3.

Response variate: ln [*y*/(1-*y*)]
Fitted terms: Constant, *A*

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	94.47	94.471
residual	19	50.78	2.673
total	20	145.25	7.263

percentage variance accounted for: 63.2

Appendix 3.1.1.4: Vector model 4.

Response variate: ln [y/(1-y)]
Fitted terms: Constant, ln$A$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	130.97	130.9656
residual	19	14.29	0.7520
total	20	145.25	7.2627

percentage variance accounted for: 89.6

Appendix 3.1.2: Vector species - *R. padi*.

Appendix 3.1.2.1: Vector model 1.

Response variate: ln [1/(1-y)]
Fitted terms: Constant, A

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	1.38314	1.383135
residual	19	0.05722	0.003011
total	20	1.44035	0.072018

percentage variance accounted for: 95.8

Appendix 3.1.2.2: Vector model 2.

Response variate: ln [1/(1-y)]
Fitted terms: Constant, ln$A$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	1.0968	1.09682
residual	16	0.2337	0.01461
total	17	1.3306	0.07827

Percentage variance accounted for: 81.3

Appendix 3.1.2.3: Vector model 3.

Response variate: ln [y/(1-y)]
Fitted terms: Constant, A

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	58.35	58.348
residual	13	15.84	1.218
total	14	74.19	5.299

percentage variance accounted for: 77.0

Appendix 3.1.2.4: Vector model 4.

Response variate: ln [y/(1-y)]
Fitted terms: Constant, ln$A$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	69.123	69.1227
residual	13	5.064	0.3895
total	14	74.186	5.2990

percentage variance accounted for: 92.6

Appendix 3.2: Regression analyses to compare the epidemic observed in the 1987 field trial with that predicted by the vector models.

Appendix 3.2.1: Vector species - *A. craccivora*, *B. rumexicolens*, *D. aucupariae*, *M. persicae* and *R. padi*.

Appendix 3.2.1.1: Vector model 1.

Response variate: y
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	2.72441	2.724407
residual	25	0.07976	0.003190
total	26	2.80416	0.107852
change	-1	-2.72441	2.724407

percentage variance accounted for: 97.0

Appendix 3.2.1.2: Vector model 2.

Response variate: y
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	2.2642	2.26420
residual	22	0.2715	0.01234
total	23	2.5357	0.11025
change	-1	-2.2642	2.26420

percentage variance accounted for: 88.8

Appendix 3.2.1.3: Vector model 3.

Response variate: *y*
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	2.2194	2.21945
residual	25	0.5847	0.02339
total	26	2.8042	0.10785
change	-1	-2.2194	2.21945

percentage variance accounted for: 78.3

Appendix 3.2.1.4: Vector model 4.

Response variate: *y*
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	2.4340	2.434031
residual	22	0.1017	0.004623
total	23	2.5357	0.110250
change	-1	-2.4340	2.434031

percentage variance accounted for: 95.8

Appendix 3.2.2: Vector species - *R. padi*.

Appendix 3.2.2.1: Vector model 1.

Response variate: *y*
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	0.73463	0.734629
residual	19	0.02461	0.001295
total	20	0.75923	0.037962
change	-1	-0.73463	0.734629

percentage variance accounted for: 96.6

Appendix 3.2.2.2: Vector model 2.

Response variate: y
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	0.5729	0.572874
residual	16	0.1213	0.007581
total	17	0.6942	0.040834
change	-1	-0.5729	0.572874

percentage variance accounted for: 81.4

Appendix 3.2.2.3: Vector model 3.

Response variate: y
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	0.73107	0.731070
residual	19	0.02817	0.001482
total	20	0.75923	0.037962
Change	-1	-0.73107	0.731070

percentage variance accounted for: 96.1

Appendix 3.2.2.4: Vector model 4.

Response variate: y
Fitted terms: Constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	0.67700	0.676997
residual	16	0.01718	0.001074
total	17	0.69417	0.040834
change	-1	-0.67700	0.676997

percentage variance accounted for: 97.4

Appendix 3.3: Regression analyses to fit the linear forms of the gradient models to the infection gradients observed in the 1988 field trial on September 7.

Appendix 3.3.1: Gradient model 1.

Appendix 3.3.1.1: Distinct lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln 1/(1-*y*)
Fitted terms: Constant + distance + treatment + distance.treatment

Summary of analysis

	d.f.	s.s.	m.s.
regression	5	10.83	2.1664
residual	96	18.60	0.1938
total	101	29.44	0.2915
change	-5	-10.83	2.1664

percentage variance accounted for: 33.5

Appendix 3.3.1.2: Parallel lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln 1/(1-*y*)
Fitted terms: Constant + treatment + distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	3	9.99	3.3316
residual	98	19.44	0.1984
total	101	29.44	0.2915
change	-3	-9.99	3.3316

percentage of variance accounted for: 31.9

Appendix 3.3.1.3: One coincident line fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [1/(1-*y*)]
Fitted terms: Constant + distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	9.78	9.7775
residual	100	19.66	0.1966
total	101	29.44	0.2915
change	-1	-9.78	9.7775

percentage variance accounted for: 32.5

Appendix 3.3.2: Gradient model 2.

Appendix 3.3.2.1: Distinct lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [*y*/(1-*y*)]
Fitted terms: Constant + distance + treatment + distance.treatment

Summary of analysis

	d.f.	s.s.	m.s.
regression	5	41.94	8.3879
residual	89	73.09	0.8212
total	94	115.03	1.2237
change	-5	-41.94	8.3879

percentage variance accounted for: 32.9

Appendix 3.3.2.2: Parallel lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [*y*/(1-*y*)]
Fitted terms: Constant + treatment + distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	3	37.93	12.6429
residual	91	77.10	0.8472
total	94	115.03	1.2237
change	-3	-37.93	12.6429

Appendix 3.3.2.3: One coincident line fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [*y*/(1-*y*)]
Fitted terms: Constant + distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	37.52	37.5182
residual	93	77.51	0.8334
total	94	115.03	1.2237
change	-1	-37.52	37.5182

percentage variance accounted for: 31.9

Appendix 3.3.3: Gradient model 3.

Appendix 3.3.3.1: Distinct lines fitted to gradient data from treatments VOA, VO and V.

Response variate: [ln 1/(1-*y*)]
Fitted terms: Constant + ln distance + treatment + ln distance.treatment

Summary of analysis

	d.f.	s.s.	m.s.
regression	5	11.70	2.3406
residual	96	17.73	0.1847
total	101	29.44	0.2915
change	-5	-11.70	2.3406

percentage variance accounted for: 36.6

Appendix 3.3.3.2: Parallel lines fitted to gradient data from treatments VOA, VO and C.

Response variate: ln 1/(1-*y*)
Fitted terms: Constant + treatment + ln distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	3	10.97	3.6559
residual	98	18.47	0.1885
total	101	29.44	0.2915
change	-3	-10.97	3.6559

percentage variance accounted for: 35.3

Appendix 3.3.3.3: One coincident line fitted to gradient data from treatments VOA, VO and C.

Response variate: ln [1/(1-*y*)]
Fitted terms: Constant + ln distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	10.72	10.7245
residual	100	18.71	0.1871
total	101	29.44	0.2915
change	-1	-10.72	10.7245

percentage variance accounted for: 35.8

Appendix 3.3.4: Gradient model 4.

Appendix 3.3.4.1: Distinct lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [y/(1-y)]
Fitted terms: Constant + ln distance + treatment + ln distance.treatment

Summary of analysis

	d.f.	s.s.	m.s.
regression	5	45.17	9.0348
residual	89	69.85	0.7848
total	94	115.03	1.2237
change	-5	-45.17	9.0348

percentage variance accounted for: 35.9

Appendix 3.3.4.2: Parallel lines fitted to gradient data from treatments VOA, VO and V.

Response variate: ln [y/(1-y)]
Fitted terms: Constant + treatment + ln distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	3	42.36	14.1193
residual	91	72.67	0.7985
total	94	115.03	1.2237
change	-3	-42.36	14.1193

percentage variance accounted for: 34.7

Appendix 3.3.4.3: One coincident line fitted to gradient data from treatments VOA, VO and V.

Response variate: ln y/(1-y)
Fitted terms: Constant + ln distance

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	41.84	41.8416
residual	93	73.18	0.7869
total	94	115.03	1.2237
change	-1	-41.84	41.8416

percentage variance accounted for: 35.7

Appendix 3.4: Regression analyses to compare the infection gradients observed in the 1988 field trial with those predicted by the models.

Appendix 3.4.1: Gradient model 1.

Response variate: y
Fitted terms: constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	2.753	2.75271
residual	106	5.385	0.07605

percentage variance accounted for: 33.2

Appendix 3.4.2: Gradient model 2.

Response variate: y
Fitted terms: constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	3.078	3.07814
residual	106	5.060	0.04773
total	107	8.138	0.07605

percentage variance accounted for: 37.2

Appendix 3.4.3: Gradient model 3.

Response variate: y
Fitted terms: constant, $y_{pred.}$

Summary of analysis

	d.f.	s.s.	m.s.
regression	1	3.144	3.14384
residual	106	4.994	0.04711
total	107	8.138	0.07605

percentage variance accounted for: 38.1

Appendix 3.4.4: Gradient model 4.

Response variate: y
Fitted terms: constant, $y_{pred.}$

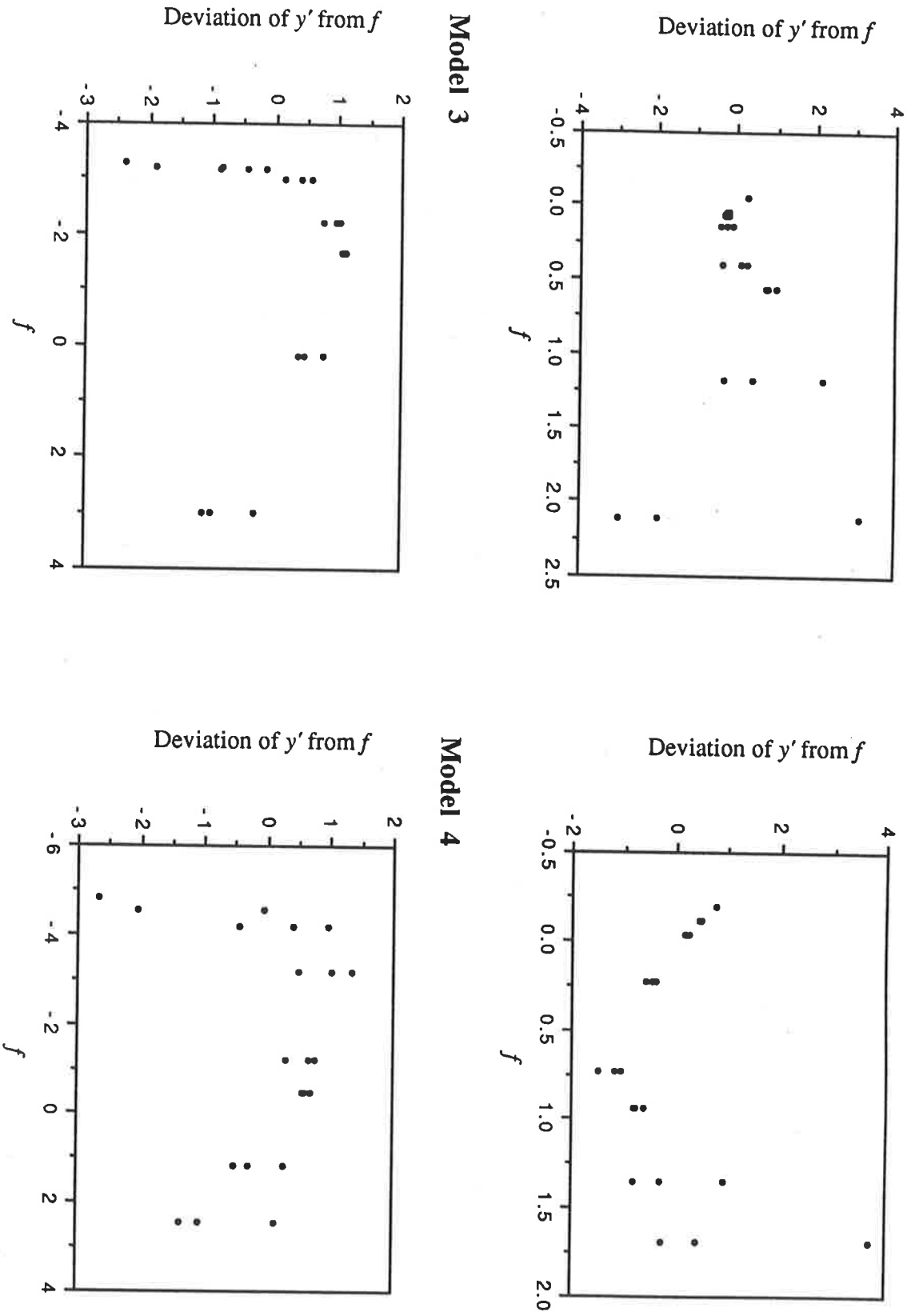
Summary of analysis

	d.f.	s.s.	m.s.
regression	1	3.193	3.19337
residual	106	4.944	0.04665
total	107	8.138	0.07605

percentage variance accounted for: 38.7

Appendix 3.5: Residual plots for the linear forms of the vector models fitted to data from the 1987 field trial (vector species - *A. craccivora*, *B. rumexicolens*, *D. aucupariae*, *M. persicae* and *R. padi*).

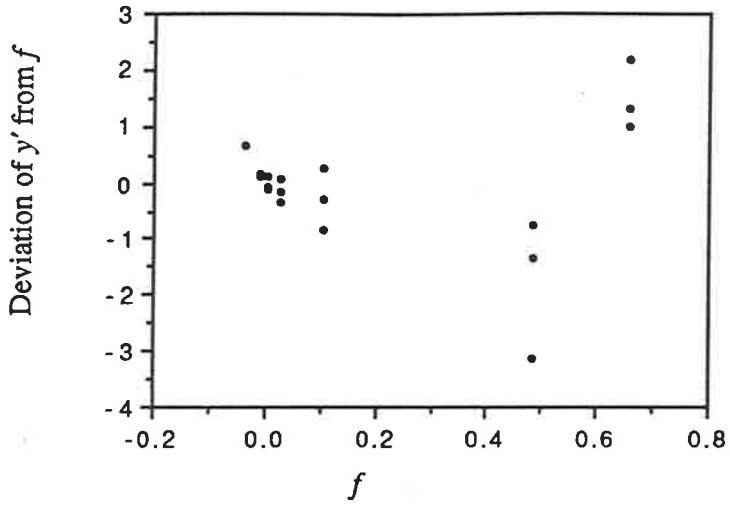
y' is the transformed value of y (either ln [1/(1-y)] for models 1 and 2 or ln [y/(1-y)] for models 3 and 4); f is the fitted value of y'.


Model 1
Deviation of y' from f
f
Model 2
Deviation of y' from f
f
Model 3
Deviation of y' from f
f
Model 4
Deviation of y' from f
f

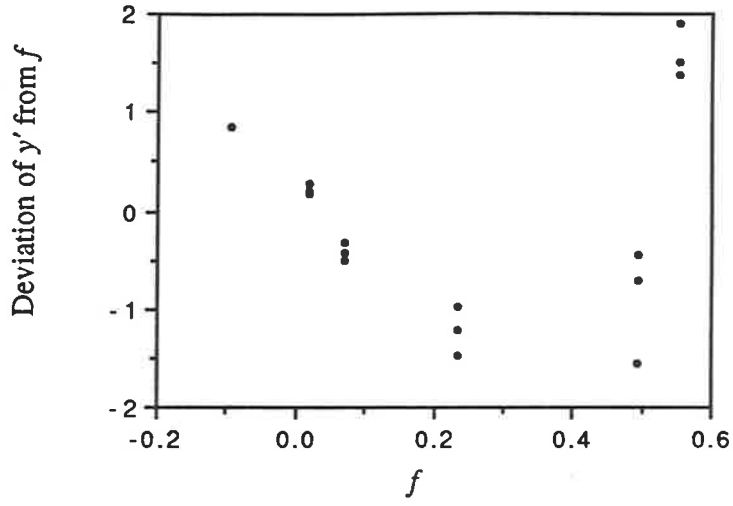
Appendix 3.6: Residual plots for the linear forms of the vector models fitted to data from the 1987 field trial (vector species - *R. padi*).

y' is the transformed value of y (either ln [1/(1-y)] for models 1 and 2 or ln [y/(1-y)] for models 3 and 4); f is the fitted value of y'.

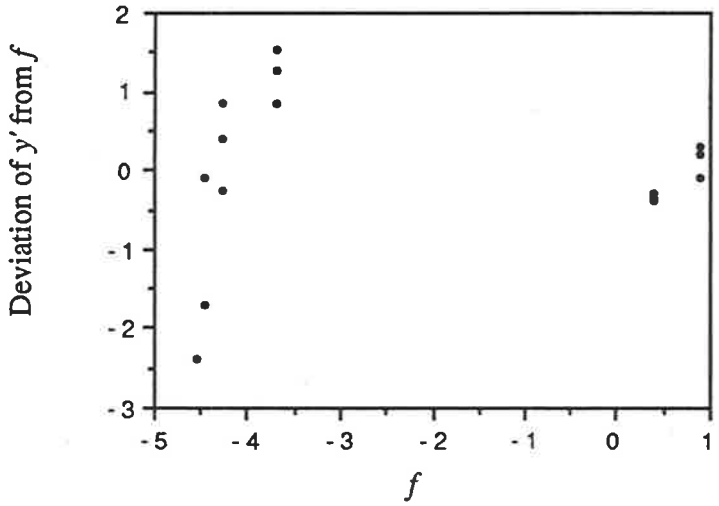
Model 1



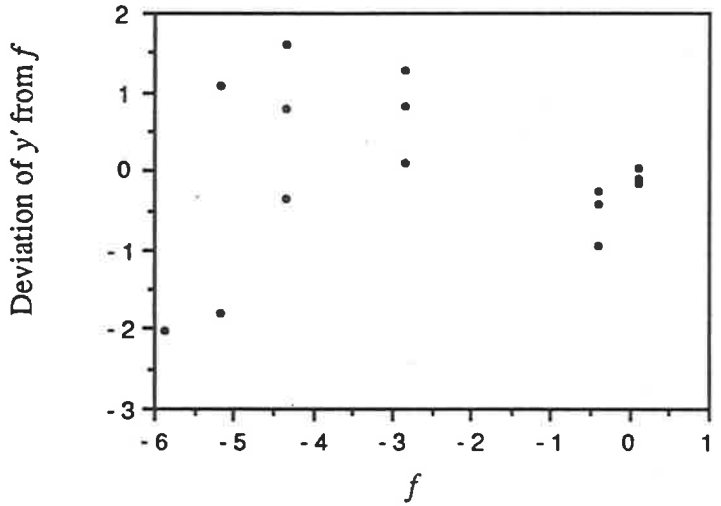
Model 2



Model 3

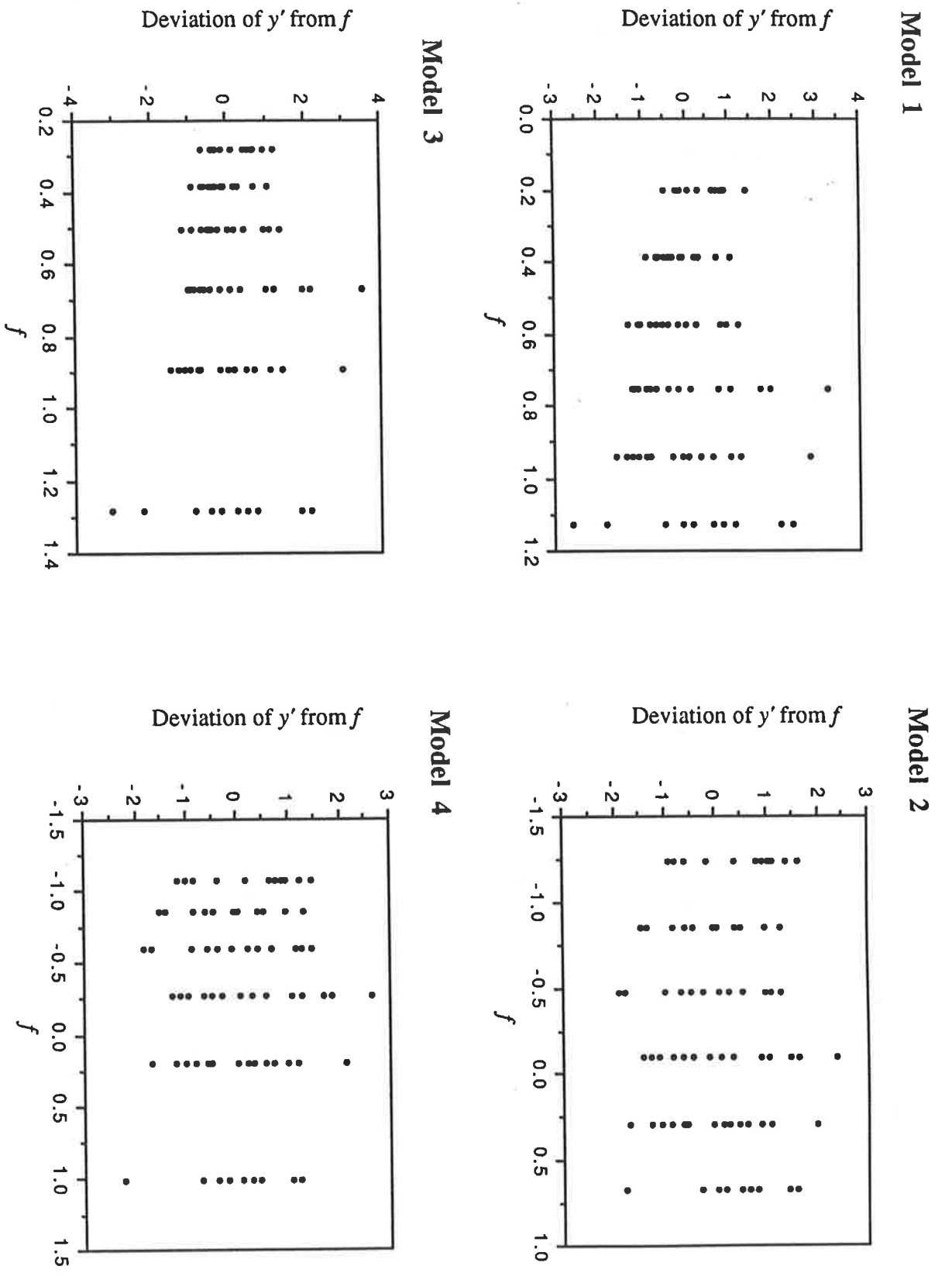


Model 4



Appendix 3.7: Residual plots for the linear forms of the gradient models fitted to data from the 1988 field trial.

y' is the transformed value of y (either ln [1/(1-y)] for models 1 and 3 or ln [y/(1-y)] for models 2 and 4); f is the fitted value of y'.


Model 1
Deviation of y′ from f
f
Model 2
Deviation of y′ from f
f
Model 3
Deviation of y′ from f
f
Model 4
Deviation of y′ from f
f

Appendix 4: Seed transmission of CMV and the effect of CMV infection on lupin productivity.

Appendix 4.1: Analysis of variance to test for differences between the seedling assay and testing of seed by ELISA to determine seed transmission rates.

Variate: rate of seed transmission (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivar	4	4.489	1.122	1.04	0.395
cultivar.testing method	5	0.304	0.061	0.06	0.998
residual	46	49.452	1.075		
total	55	54.245			

Appendix 4.2: Effect of age at the time of inoculation on seed and dry matter productivity (1989 field experiment).

Appendix 4.2.1: Seed and dry matter yields.

Treatment	Number of replicates	Seed yield (g) Mean	SE	Dry matter yield (g) Mean	SE
1	48	0.08	0.018	0.50	0.061
2	56	2.60	0.31	9.44	0.73
3	58	12.58	0.80	30.25	1.52
4	92	22.74	1.08	34.84	1.50
Healthy	110	30.16	1.17	34.90	1.71

Appendix 4.2.2: Analyses of variance to test for differences in seed yields between treatments.

Appendix 4.2.2.1: Analysis using data from treatments 1 - 5.

Variate: seed weight (g; log transformed)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	4	497.2491	124.3123	606.71	<0.001
residual	359	73.5575	0.2049		
total	363	570.8067			

Appendix 4.2.2.2: Analysis using data from treatments 3 - 5.

Variate: seed weight (g; log transformed)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	28.2869	14.1434	67.78	<0.001
residual	257	53.6277	0.2087		
total	259	81.9145			

Appendix 4.2.3: Analyses of variance to test for differences in dry matter yields between treatments.

Appendix 4.2.3.1: Analysis using data from treatments 1 - 5.

Variate: dry matter weight (g; log transformed)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	4	412.3444	103.0861	547.21	<0.001
residual	359	67.6305	0.1884		
total	363	479.9749			

Appendix 4.2.3.2: Analysis using data from treatments 3 - 5.

Variate: dry matter weight (g; log transformed)

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	2	0.5999	0.3000	1.51	0.224
residual	257	51.19181	0.1992		
total	259	51.7917			

Appendix 4.3: Effect of plant age at the time of inoculation on seed viability (1989 field experiment).

Appendix 4.3.1: Germination rates.

Treatment	Number of replicates	Germination rate (%)	
		Mean	SE
1	25	59.7	9.7
2	30	86.4	2.1
3	30	84.4	2.8
4	30	89.5	1.6
Healthy	10	77.7	4.0

Appendix 4.3.2: Analysis of variance to test for differences in germination rates between treatments 2, 3 and 4.

Variate: germination rate (%; arcsin transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	751.78	375.89		
block.plant stratum					
inoculation time	2	247.76	123.88	1.50	0.228
residual	85	7003.32	82.39		
total	89	8002.86			

Appendix 4.4: Effect of plant age at the time of inoculation on rate of seed transmission of CMV (1989 field experiment).

Appendix 4.4.1: Rates of seed transmission of CMV.

Treatment	Number of replicates	Rate of seed transmission (%) Mean	SE
1	25	21.55	7.42
2	30	24.48	2.88
3	30	10.49	0.875
4	30	2.77	0.464
5	4	0	0

Appendix 4.4.2: Analysis of variance to test for differences in rates of seed transmission between treatments 2, 3 and 4.

Variate: rate of seed transmission (%; arcsin transformed)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	45.23	22.62		
block.plant stratum					
inoculation time	2	6249.90	3124.95	64.58	<0.001
residual	85	4113.16	48.39		
total	89	10408.28			

Appendix 4.5: Relationship between seed weight and infection of that seed.

Appendix 4.5.1: Weights of infected and uninfected seeds.

	Individual seed weight (mg)					
Plant	Uninfected			Infected		
	Number	Mean	SE	Number	Mean	SE
1	12	107.5	8.6	4	91.9	9.6
2	18	129.0	9.3	4	116.8	24.8
3	4	143.3	13.9	5	124.4	13.7
4	17	91.2	9.1	4	79.8	16.1
5	51	118.1	3.2	13	110.9	5.8
6	22	139.7	7.1	5	102.9	10.7
7	12	100.5	8.3	7	95.6	2.7
8	19	66.8	4.3	5	58.2	3.2

Appendix 4.5.2: Analysis of variance to test for differences in weight between infected and uninfected seeds.

Variate: seed weight (mg)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
reps	7	88533.9	12647.7	15.68	<0.001
reps.infect	8	8903.8	1113.0	1.38	0.208
residual	186	150052.7	806.7		
total	201	247490.4			

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