Table 1. Gel-diffusion precipitation bands formed by fungal antigenic preparations challenged with *E. eucalypti* antiserum

Fungal antigen	Number of precipitation bands
Phycomycetes	
Endogone eucalypti	6
Absidia spinosa	2
Allomyces macrogynus	—
Mortierella pusilla	2
Pythium ultimum	—
Thamnidium elegans	2
Ascomycetes	
Chaetomium globosum	—
Gaeumannomyces graminis	—
Leptosphaeria karrae	—
Rhodotorula sp.	—
Basidiomycetes	
Pholiota marginata	—
Polyporus vesicolor	—
Fungi Imperfecti	
Alternaria tenuis	—
Aspergillus niger	—
Curvularia sp.	—
Fusarium culmorum	—
Penicillium chrysogenum	—
Stemphylium sp.	—
Trichoderma lignorum	—

Table 2. Gel-diffusion precipitation bands formed by cross reacting various Phycomycete fungal antigenic preparations with *E. eucalypti* antiserum.

Fungal antigen	Number of precipitation bands
Mucoraceae	
Absidia coerulea	2
Actinomucor sp.	1
Phycomyces blakesleeanus	3
Phycomyces sp.	3
Mucor genevensis	2
M. ramannianus	2
M. hiemalis	1
Thamnidiaceae	
Thamnidium elegans	2
Choanephoraceae	
Cunninghamella echinulata	1
Mortierellaceae	
Mortierella sp.	2
M. pusilla	2
Endogonaceae	
Endogone eucalypti	6-7
Syncephalastraceae	
Syncephalastrum racemosum	1
Kickxellaceae	
Coemansia spiralis	1

effectiveness of FA staining as an ecological tool.

Vegetative mycelium was produced by growing *E. eucalypti* in static flask cultures containing a modified Whites tissue culture medium (3). Growth was slow but after 4 weeks sufficient mycelium was produced to form a thin mat. The mycelium was harvested by filtration, lyophilised and stored in a deep freeze. Antigenic suspensions and antisera were prepared (1, 2, 4). Gel-diffusion tests on slides (2) were carried out between antisera and antigenic suspensions of all test fungi. The direct FA staining technique was applied to the control and test fungi using the method outlined in Frankland *et al.* (2).

The serological data (Table 1) showed no cross-reactions with any member of the Ascomycetes, Basidiomycetes or Fungi Imperfecti but significant cross reactions with certain members of the Phycomycetes. These data indicate close serological relationship between *E. eucalypti* and this class of fungi. To further explore this relationship, antigenic material from a range of species from the main families of the Class Phycomycetes were cross reacted with *E. eucalypti* antiserum.

The data in Table 2 confirms the relationship of *E. eucalypti* with the Phycomycetes and further a particularly close relationship with the Mucoraceae. These data support the morphological taxonomic relationship of *Endogone*.

Positive F.A. staining of hyphae followed closely the cross reactions precipitation band data contained in Tables 1 and 2. *Endogone eucalypti* and members of the Mucoraceae, particularly *Phycomyces* spp., fluoresced brightly with an apple green fluorescence whereas species such as *Coemansia spiralis* showed only low fluorescence. Members of the Ascomycetes, Basidiomycetes and Fungi Imperfecti failed to react.

The wide cross reaction of *E. eucalypti* antigens would suggest that F.A. staining would not be applicable as a critical ecological tool in estimating penetration of hyphae in a substrate or hyphal biomass. However, in this exploratory study crude antigen preparations were used as taxonomic markers. The use of fractionated antigens, structural protein or enzymes and suitable cross absorption of the antiserum by cross reacting antigens could well result in a specific antiserum and consequently highly selective F.A. staining.

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Ecology and Control of Non-Persistent Viruses in Australia

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(This paper is based on a presentation to the 4th National Conference of the Australasian Plant Pathology Society held in Perth, Western Australia, May 1980.)

Most aphid transmitted plant viruses may be placed in one or other of two categories dependent upon whether they persist or not in their vectors. The evolution of these two types of virus has undoubtedly resulted from selection

pressures acting upon opposing sets of properties which ensure the continued survival of the viruses concerned. There is generally a very intimate relationship between the persistent viruses, their host plants and their vectors with the result that vector species numbers are few and host ranges are narrow. On the other hand, the relationship between the non-persistent viruses and their vectors is less exacting but this is compensated for by these viruses generally having extensive host ranges and large numbers of vector species, e.g. cucumber mosaic virus has a host range of hundreds of species among more than 40 families of flowering plants and is transmitted by more than sixty species of aphid. The transmission of non-persistent viruses is also enhanced by conditions, such as starving the vectors, which stimulate them to probe briefly and move frequently around or between food plants.

Several non-persistent viruses presently cause important disease problems in crops in various regions of Australia. These include bean common mosaic in *Phaseolus vulgaris*, bean yellow mosaic in broad bean and lupin, cucumber mosaic in lupins and a range of ornamentals, passionfruit woodiness in passionfruit, potato Y in solanaceous field crops, sugar cane mosaic in maize, sorghum and sugar cane, and the watermelon mosaics in cucurbits.

Widespread infection with various non-persistent viruses also occurs in many ornamental species, and their importance is increasing with changing attitudes in Australia to amenity horticulture. They include bean yellow mosaic in gladiolus, carnation vein mottle in carnation, chrysanthemum B in chrysanthemum, cucumber mosaic in gladiolus, daphne S in daphne, the iris mosaics in iris, narcissus yellow stripe in daffodil and related species, tomato aspermy in chrysanthemum, tulip breaking in tulip and lily, and turnip mosaic in wallflower.

Some non-persistent viruses which formerly caused disease problems in particular crops are now under control. Two factors, either separately or in combination, have been particularly important in those instances where control has been achieved. Firstly, most non-persistent viruses spread over very short distances and, secondly, virtually only adult alatae appear to spread the viruses in the field. Non-persistent virus diseases are therefore controlled by eliminating sources of infection within or nearby susceptible crop plants and/or by restricting the activity of vector alatae within the crops.

1. Elimination of infection sources

(i) *Elimination of carriers of infection in crops.* Potato viruses S and Y commonly infected many potato cultivars until about ten years ago when the States started pathogen tested stock schemes by using heat treatment and meristem tip culture to produce virus-free plants. This strategy virtually eliminated viruses S and Y from ware crops.

In Victoria, it was shown that flower-breaking virus in tulips could be controlled when symptomless yellow and white flowered cultivars as well as coloured varieties were freed of virus (21). Similarly, narcissus yellow stripe was successfully controlled by a daffodil grower in Tasmania who planted virus-tested stock about 1.5 km from other infection sources in home gardens.

(ii) *Production of disease-free seed.* Transmission of plant viruses through seed was considered a rare event until recently. However, the number of non-persistent viruses known to be transmitted through seed has increased from 5 to 14 to 34 during the past two decades (2, 15). Seed transmission has a vital role in the ecology of many non-persistent viruses.

Production of clean seed stock has resulted in the extraordinarily efficient control of some non-persistent viruses. The classic example in Australia was the control of lettuce mosaic (20). Control of bean common mosaic in *Phaseolus* and of bean yellow mosaic in *Vicia* was also achieved through production of clean seed stocks.

(iii) *Elimination of carriers near crop plants.* Weeds within crops may also act as sources of infection, particularly if the virus is seed-borne in weed species, as is cucumber mosaic virus (24). Weeds are generally not a problem in broad-scale production where efficient control is achieved using herbicides and other cultural practices. However serious problems often arise in market gardens where weed control is far more difficult. Additional problems arise in urban areas from reservoirs of infection in a wide range of ornamentals in home gardens.

(iv) *Isolation of crops from infection sources.* Spread of non-persistent viruses generally occurs over relatively short distances and gradients of infection around infection sources therefore are steep. Plots of tulips in Victoria therefore remained free from infection with tulip breaking virus for two years by planting them at least 300 m distant from plots of infected bulbs.

2. Control associated with vector activity

To control virus spread by controlling vectors, one needs to know what vectors are actively transmitting in the field and also how they behave. Most evidence suggests that non-persistent viruses are spread by adult alatae and not be apterae. For example, apterae of *Aulacorthum solani* move freely in Tasmanian broad bean crops spreading the persistent sub-clover red leaf virus (9); *Myzus persicae* moved similarly but there was little spread of bean yellow mosaic virus (BYMV) in the crops, a non-persistent virus that was present in the crops and is transmitted experimentally by both these species. The apterae probably fed for too long on infected BYMV plants before moving to adjacent healthy plants to permit efficient transmission of the non-persistent virus. Furthermore there are many reports that aphicides do not control the spread of non-persistent viruses and this suggests that apterae are not responsible for their spread.

Results from experiments using bait plants and from tests with trapped alatae demonstrated that alatae often carry persistent viruses but more rarely non-persistent (1, 27; I. D. Geard, personal communication). Alatae experience a feeding inhibition in warm and relatively calm conditions during the teneral period before flying (22); these alatae take off and may fly for at least 2-3 hours under such conditions, too long for efficient transmission of non-persistent viruses (5).

However, when conditions are cool and turbulent, commencement of flight is normally delayed, with consequent feeding before flight and the distances flown later are often short. Alatae may introduce non-persistent viruses into crops in these circumstances (4). In any event, the flying urge of alatae is rarely satisfied by a single flight from their source and a series of short flights and probes are normally made following the initial flight before finally settling (10, 11). This phenomenon is readily observed in crops on calm sunny days during the Tasmanian spring and it is under these conditions that non-persistent viruses are optimally transmitted, whether the source of virus is within the crop or introduced from outside it. The infection patterns produced indicate short distance spread with steep gradients.

The control of non-persistent viruses by attention to the activity of vector alatae has been achieved in several different ways —

(i) *Control through adjustment of cropping period.* Australian winters in the cropping regions are so mild that, unlike in Europe and North America, aphids survive throughout the year on their secondary hosts. However, with most species very large populations fly once or twice a year (6). There are therefore opportunities to grow annual crops during periods of the year when few vector alatae are active (8, 18, 20). Sometimes it is sufficient to merely ensure that the young stages of plant growth do not coincide with the peak flight times because older plants in crops often are not liable to virus infection (8).

(ii) *Control through inhibition or enhancement of aphid settling.* Alatae, after a period of flight, become attracted to long spectral wavelength light and fly away from sources of short blue and ultra-violet wavelength light (12). This feature is the basis for the success of reflective aluminium foil mulches to control non-persistent viruses, but is of course only possible for high-value annual crops such as capsicum, lettuce and tomato (16). Additional side-benefits of mulches include weed control and assistance in the control of some fungal diseases such as those due to *Botrytis*, *Bremia* and *Sclerotinia* by keeping leaves off moist soil.

Sticky yellow polythene sheets placed around small potato plots in Israel successfully attracted aphids away from the crop and trapped them (28). Losses due to infection with the non-persistent viruses alfalfa mosaic and potato Y were reduced as a result.

(iii) *Control through diverting aphids from the crop.* There have been reports that barrier crops such as maize, mustard and oats can reduce the incidence of non-persistent virus infection within small plots surrounded by them (3). It was thought that the barrier plants were effective because alatae probed them and lost infectivity before reaching the protected susceptible plants. However, barrier crops often are not effective (21), perhaps because such barriers and hedges set up persistent air turbulence patterns which consistently deposit alatae on the leeward side (13). Incidence of infection generally seems less in fields without hedges around them.

(iv) *Control through field size and plant density factors.* Incoming alatae congregate near the windward edges of fields (23). Infections with non-persistent viruses therefore generally tend to be concentrated close to paddock boundaries (17) irrespective of whether the sources of infection are within or outside the crop. It was therefore proposed that the incidence of infection in crops could be reduced by establishing large fields so that a relatively smaller proportion of plants were adjacent to the perimeter (25).

A related factor is that alatae are more attracted to isolated plants with contrasting bare earth about them (14) which results in a greater incidence of infection in plots sown at lower densities (3). This effect is not merely due to similar numbers of settling aphids being forced onto fewer plants. The absolute numbers of infections resulting from activity of vector alatae can be much larger in plots with fewer plants and settling of alatae in plots of susceptible plants with a high leaf area index may not occur (8). The incidence of infection at specific densities may be reduced by arranging the plants in rectangular arrays rather than establishing them on more square patterns (7).

(v) *Control with oil sprays.* Oils sprayed on plants inhibit transmission of non-persistent, but not of persistent viruses by aphids. The oils do not directly affect viral infectivity or vector feeding behaviour but apparently act by modifying virus-vector relationships.

Conclusions

Non-persistent viruses are numerous and can be responsible for important crop losses. However some non-persistent virus diseases are extremely well controlled in Australia. A variety of different approaches have been successful but all are dependent for their success upon the facts that most spread of non-persistent viruses is over short distances and results from activity of alatae. These indications suggest ways to approach the control of other disease situations caused by non-persistent viruses that are currently causing crop losses. One can presume that the sources of infection are probably very near or within the crop, seed-borne or vegetatively propagated.

There is scope in the future for general control of aphid-borne viruses through biological control of the vectors to reduce the vector populations. Many of the aphid species important as virus vectors in Australia were apparently introduced with few or any of their parasites. The potential for biological control along such lines was indicated by Stubbs (19) who reported that the introduction of *Aphidius salicis* from California was a potent controlling influence on *Cavariella aegopodii*, vector of the carrot motley dwarf disease.

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A Quantitative Method of Inoculating Plants with Uniform Densities of Fungal Spores

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An inoculation chamber, adapted from those used by J. A. Browning at Iowa State University, by G. J. Green at the Canada Department of Agriculture's Research Station at Winnipeg, by J. S. Melching at the United States Department of Agriculture's laboratory at Frederick, Maryland, and the one reported by Brown & Kochman (1973) (1) was constructed so that uniform densities of dry spores (e.g. urediospores of rusts) or spores suspended in a liquid (e.g. pycnidiospores of *Septoria* spp.) could be inoculated onto plant surfaces. In principle, the inoculation chamber consisted of a large rotating turntable (about 1200 mm diam) which could be rotated at various speeds up to 50 r.p.m. in a clockwise direction. Mounted on this turntable were 16 smaller turntables (100 mm diam) which rotated in an anti-clockwise direction at the same number of revolutions per minute as the large turntable (Fig. 1). Pots containing plants to be inoculated were clamped (Fig. 2) onto the smaller turntables (to prevent movement due to centrifugal force) and the turntables were rotated using a sprocket and chain mechanism driven by an electric hydraulic motor. The inoculation chamber itself, into which wet or dry spores were discharged, was 1200 mm in diameter and 1200 mm high and was fitted above the turntables (Fig. 3).

Plants were inoculated with dry spores by placing a known quantity of spores into an explosive device located at the centre of the large turntable. Spore discharge was effected by an explosive press release system attached to a compressed air cylinder. Experiments using microscope slides (coated with silicone grease) that were located at various positions within the inoculation chamber showed that the most uniform deposition of spores onto the slides occurred when an ejection pressure of 414 kPa (60 p.s.i.) was used.

A series of experiments was made to investigate the factors (rotation speed of turntable, period of exposure to inoculum, quantity of inoculum used) that influenced the efficiency with which spores were deposited onto leaf surfaces. In these studies 16 pots of the wheat cultivar Federation were placed in the inoculation chamber and were inoculated with known quantities of urediospores of *Puccinia recondita* Rob. ex Desm. f.sp. *tritici* Erikss. & Henn. (strain 68 ANZ 1, 2, 3, 4). Ten wheat seedlings were planted in a single row across the diagonal (through the centre) of each pot. After inoculation, eight pots were placed in each of two dew chambers kept at 25°C for 16 hours. The dew chambers were identical to those described by Brown, Clark and Kochman (1974) (2). After incubation, the plants were placed in a glasshouse and the number of pustules that developed on leaves was determined at 10 to 14 days after inoculation.

To determine the effects of the rotation speed of the turntable on urediospore deposition the turntable was rotated at speeds of 20, 25, 30, 35, 40 and 45 r.p.m. for 5 min after 50 mg of urediospores had been discharged