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Impact of an insidious virus disease in the legume component on the species balance within self-regenerating annual pasture

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SUMMARY

The consequences of sowing seed of burr medic (*Medicago polymorpha*) cultivars Circle Valley and Serena that was either free of or infected to different extents with alfalfa mosaic virus (AMV) was studied in mixed species pasture swards that regenerated annually. The swards were grazed by sheep and the predominant plant species in them were burr medic and capeweed (*Arctotheca calendula*). Serena matured earlier and was more tolerant of AMV infection than Circle Valley. Each year, seed-infected medic plants were the source for virus spread by aphids to healthy medic plants. When the extent of infection was determined in the fourth to sixth growing seasons, the amount of virus spread in the medic component of the swards varied between seasons. Final infection in plots originally sown with infected seed ranged from 47 to 93% for Serena and from 25 to 79% for Circle Valley. Viral seed transmission rates in medic seed produced each year by these plots ranged from 19 to 31% with Serena and from 3 to 7% with Circle Valley. Final percentage infection within swards originally sown with healthy seed (control plots) was smaller regardless of cultivar (0·1–7%) as were transmission rates in their harvested seed (0–0·6%).

AMV infection of the burr medic in regenerated plots originally sown with Circle Valley seed diminished medic seed yields, thereby decreasing the proportion of medic in the seed bank. This decreased germination of medic but increased germination of capeweed. In control plots, plant densities were up to 36% greater in the medic and 52% smaller in the capeweed components than in plots originally sown with infected Circle Valley seed. In plots containing Serena there was a smaller decrease in medic seed yields due to AMV infection, so the impact on germination was less. In the fourth to sixth years from sowing, when yields were determined at different times after grazing ceased, there was either a small decrease (up to 8%) or no significant decrease in overall herbage yields due to infection with AMV. However, in plots originally sown with infected Circle Valley seed, the balance of medic to capeweed was altered in favour of capeweed, sometimes dramatically so (e.g. capeweed content increased from 19 to 45% in the fourth year from sowing). In contrast, by the end of the growing season the balance of medic to capeweed was little altered by the presence of the virus in plots containing Serena. Thus, infection with this insidious virus disease substantially diminished the ability of Circle Valley but not Serena medic to compete with other species such as capeweed in self-regenerated, mixed species pasture swards. It did this both directly by decreasing the competitive ability of the medic plants that became infected during the growing season, and indirectly *via* seed production and the seed bank, by altering the proportions in which the species germinated.

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INTRODUCTION

In regions of the world with Mediterranean climates, there is normally insufficient rainfall over summer to support pasture growth. This is the case in parts of southern Europe and North Africa, California, Chile, South Africa and southern Australia. The pastures that predominate in these regions are those containing annual species that persist over the summer as dormant seed and regenerate every year in autumn (Donald 1963; Rossiter 1966). They consist of a mixture of annual species, including clovers (*Trifolium* spp.), medics (*Medicago* spp.), grasses and broad-leaved weeds. The relative balance between beneficial and less desirable species in an annual pasture determines both the amount of feed available to livestock and feed quality, and is influenced by grazing and trampling pressure by herbivores and other biotic influences, and by geographic, climatic and edaphic factors (Rossiter 1966). However, although many aspects of the impact of these factors on interspecies competition have been investigated, there have been no published studies on the consequences of disease in one component species in annual pasture on the resulting balance between species. In mixed species, perennial pasture swards, decreased competitive ability of one species due to disease can result in increased proportions of other species (Lancashire & Latch 1970; Latch & Lancashire 1970; Eagling *et al*. 1992).

In agricultural areas of southern Australia, annual medics are widely used as the legume component of pastures in cereal/pasture rotations. Medic-based pastures provide nutritious feed, improve soil structure, benefit subsequent cereal crops by adding nitrogen to the soil and, if the grass component is removed, allow a break from cereal fungal root diseases (Cocks *et al*. 1980; Puckeridge & French 1983; Crawford *et al*. 1989; McDonald 1989). Several species of annual medic are sown in low to medium rainfall cropping zones (300–450 mm rainfall per year). The most widespread are barrel medic (*M. truncatula*) and, more recently, burr medic (*M. polymorpha*), which are grown on neutral to alkaline heavy textured soils or moderately acidic sandy soils respectively (Robson 1969; Thorn *et al*. 1988; Crawford *et al*. 1989; Ewing & Howieson 1989; Howieson & Ewing 1989). These two species are also important or potentially important in other parts of the world, e.g. barrel medic in South Africa and burr medic in West Asia (Lamprecht & Knox-Davies 1984; Cocks 1995). As with lucerne (*M. sativa*) in perennial pastures, medics growing in annual pastures suffer from a range of pests and diseases (Lamprecht & Knox-Davies 1984; Johnstone & Barbetti 1987; Panetta *et al*. 1993; Barbetti *et al*. 1996; Jones 1996). Worldwide, the commonest virus pathogen of lucerne and annual medics is alfalfa mosaic virus (AMV) which causes an insidious disease in *Medicago* spp. (Edwardson & Christie 1991). The virus is transmitted by aphids in a non-persistent manner (Garran & Gibbs 1982), and is readily seed-borne in annual medics. It survives the dry summer period in annual pastures inside dormant, infected medic seeds which, when they germinate in autumn, provide a primary infection source for further virus spread through the activity of aphid vectors. In cropping regions of southern Australia, AMV is most widespread in medic pastures in zones with longer growing seasons and greater rainfall (Jones 1988, 1996; Jones & Pathipanawat 1989; Jones & Nicholas 1992; Pathipanawat 1993; McKirdy & Jones 1994, 1995; Pathipanawat *et al*. 1995, 1997).

In experiments with sap-inoculated plants growing in pots, as spaced plants or in simulated swards, AMV caused substantial losses in herbage and seed production in barrel and burr medics (Dall *et al*. 1989; Jones & Pathipanawat 1989). In mown, first year mini-swards and mown or grazed swards sown with infected seed of burr medic, AMV spread by naturally occurring aphids caused herbage and seed yield losses of up to 32%. Losses of up to 29% occurred in grazed, monoculture swards of burr medic that regenerated after cereals (Jones & Nicholas 1992; Jones 1993). Moreover, AMV decreases nitrogen fixation in burr medic by impairing nodule function and in barrel medic by decreasing nodule numbers (Dall *et al*. 1989; Wroth *et al*. 1993). In burr medic, it also increases the content of the oestrogenic compound coumestrol (Jones & Ferris 1995).

Capeweed (*Arctotheca calendula*) is a common broad-leaved weed of annual pastures in Australia and South Africa (Rossiter 1966; McIvor & Smith 1973*a*, *b*; Arnold *et al*. 1985). In AMV-infected annual medic-based, regenerated pasture swards, capeweed plants can become symptomlessly infected with the virus. However, capeweed is far less susceptible to infection than medic and AMV is not seed-borne in it (McKirdy & Jones 1994). This paper reports field experiments which investigated the impact of sowing AMV-infected seed of burr medic and the virus spread that resulted on the balance between medic and capeweed within grazed, self-regenerated, mixed species pasture swards. Experiments initiated by Jones & Nicholas (1992) which were sown with AMV-infected or healthy burr medic seed were used to provide the swards. Evidence is presented that an insidious virus disease in the legume component of annual pasture, that is often overlooked, can alter the species composition directly by decreasing the competitive ability of the legume plants that become infected, and indirectly by diminishing the proportion of legume to other species that germinate. A preliminary report was published (Jones & Nicholas 1997).

MATERIALS AND METHODS

Virus, isolate, virus cultures and antiserum used

Alfalfa mosaic virus (AMV) isolate EW (El Waset) from annual medic was obtained from previous work (Jones & Pathipanawat 1989). To inoculate plants, infected leaves were ground in 0·02 M phosphate buffer, pH 7·2, the infective sap mixed with 'celite' and rubbed onto leaves. AMV–EW was cultured in subterranean clover (*Trifolium subterraneum*) cvs Daliak or Woogenellup, and infected sap from this culture was used as a positive control in enzyme-linked immunosorbent assay (ELISA). The culture plants were grown in an insect-proofed, air-conditioned glasshouse in pots containing steam-sterilized potting mix inoculated with root nodule bacterium (*Rhizobium meliloti*). Antiserum to AMV was supplied by the late Dr R. I. B. Francki, Waite Campus, University of Adelaide, South Australia.

Field experiments

Experiments 1–3 were sown in 1988–90 at Avondale Research Station (average annual rainfall 415 mm) located *c*. 95 km east of Perth in Western Australia. They were previously used to study the effect of sowing AMV-infected seed and subsequent virus spread by naturally occurring aphids on the productivity of pure medic swards. Individual plots contained one medic cultivar only but up to three different medic cultivars (Circle Valley, Santiago and Serena) were sown per experiment. For each cultivar used, plots were sown with either healthy seed (control plots) or with seed with different amounts of AMV infection. Details of site preparation, fertilizers, sowing procedures, experimental design, mowing and/or grazing histories in 1988–90 were described by Jones & Nicholas (1992). Table 1 summarizes this information and shows the years in which the regenerated experiments were grazed, cropped with cereal and terminated. In Expts 2 and 3, in the year of sowing only, a portion of each plot was fenced off to prevent grazing, giving grazed and ungrazed subplots.

The corners of the plots were restaked each year at the start of the growing season. Buffers 5 m wide of annual ryegrass (*Lolium rigidum*), a non-host of AMV, surrounded each plot (Fig. 1*a*). These buffers were resown annually in Expts 1 and 2, except in years when they were sown to cereals or in 1992 and 1993 in Expt 1 when grass was left to regenerate naturally in the buffer zones. In Expt 1, in 1991 and 1992, but not in 1993, grass was removed from within the plots in July using the selective herbicide Kerb (propyzamine) at 1 litre/ha. During the summer in 1991 (Expts 1–3), and in 1992 and 1993 (Expt 1), the fences around Expts 1–3 were left open for grazing of the dead foliage by sheep from surrounding fields. To obtain an adequate grazing pressure during the

Table 1. *Details of field experiments*

Expt number	Previous designation in Jones & Nicholas (1992)	Year sown	Medic cultivars sown	Original experimental design	Defoliation treatments in first growing season	Annual ryegrass buffers resown	Grazing by sheep on regenerated experiments	Under cereal	Final year
1	Main mowing trial	1988	Circle Valley & Serena	Randomized block	Mowing/undefoliated	1990 & 1991	1990, 1991, 1992 & 1993	1989	1993
2	1989 grazing trial	1989	Circle Valley & Serena	Split plot	Grazing/undefoliated	1991	1991	1990*	1991
3	1990 grazing trial	1990	Circle Valley, Santiago & Serena	Split-split plot	Grazing/undefoliated	—	—	—	1991†

* Expt 2 sown with cereal in 1990 after medic germination assessments were completed.
† Expt 3 finished in 1991 following medic germination assessments.

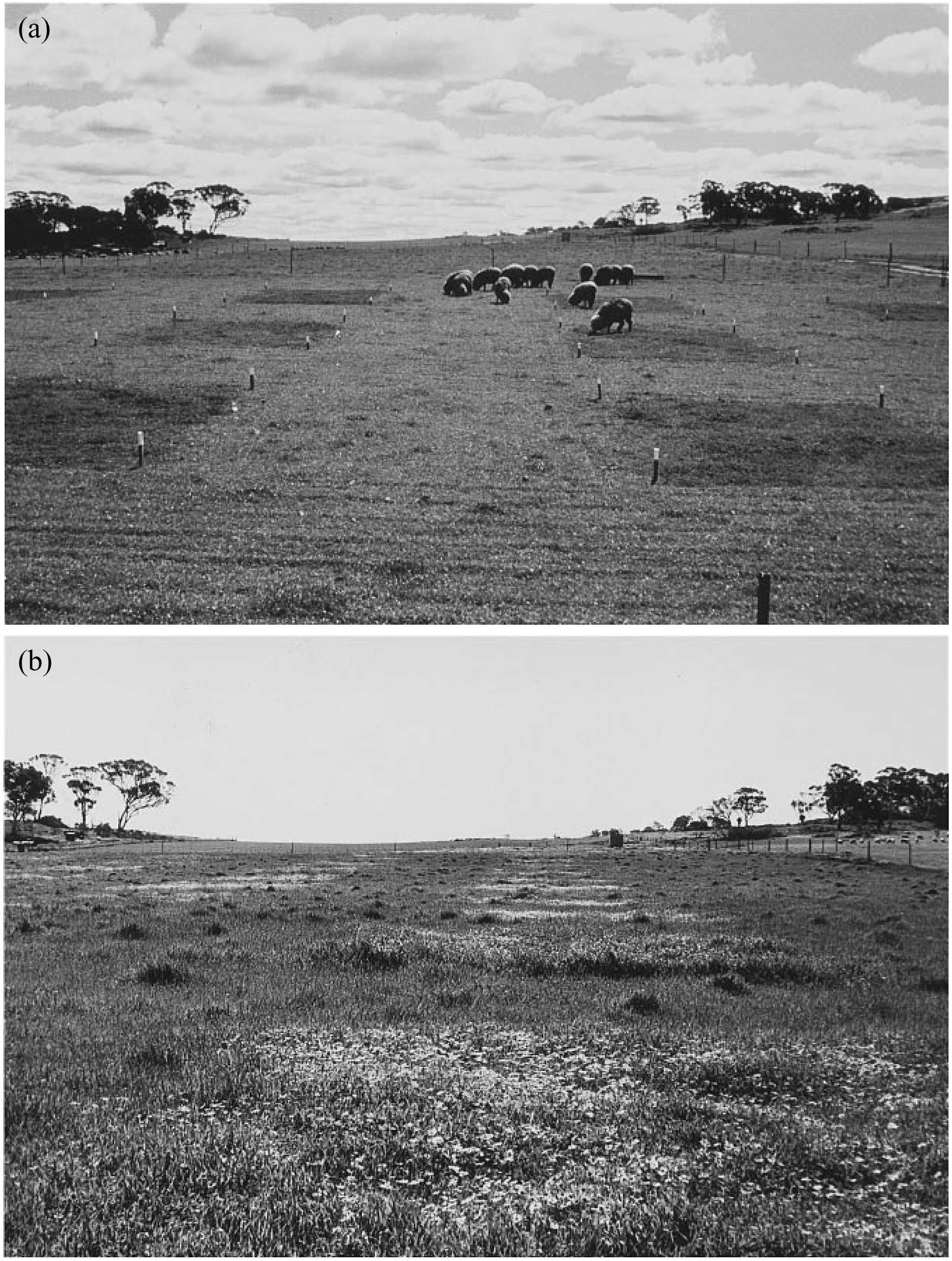

Fig. 1. (*a*) Grazing of Expt 1 by sheep in 1991. Note 5 m wide grass buffers around the plots and the extent of defoliation. (*b*) Experiment 1 at the end of the growing season in 1993. Note growth of plots and buffers following removal of grazing, and capeweed in flower within the mixed species plots.

growing season, the number of sheep fenced inside the experimental areas was raised or lowered as required. Experiment 1 was heavily grazed by sheep for the first part of the growing season, from 25 May to 5 August (1991), 5 July to 5 August (1992) and 2 July to 6 August (1993). It was then allowed to grow up without grazing (Fig. 1*b*). Experiment 2 was heavily grazed by sheep from 14 June to 31 July in 1991.

Sampling plots and testing samples by ELISA

In Expt 1, single shoot samples were taken at random from each plot at different times in the growing season, the numbers taken per plot being decreased gradually as virus spread took place (Jones & Nicholas 1992). The shoots were placed in sealed polythene bags to prevent wilting and stored at 4 °C. Then a single leaflet from each shoot was either combined with others (2–10 per grouped sample) for testing or tested singly for AMV by ELISA. To determine transmission rates of AMV in medic seed, samples of 200 seeds per plot were combined within individual treatments, scarified and then sown in rows in trays in an insect-proofed, air conditioned glasshouse. About 3–4 weeks after sowing, leaf samples from the seedlings or whole seedlings were grouped together (2–10 per grouped sample) for testing or were tested singly.

Grouped or single samples of leaflets, leaves or whole seedlings were extracted in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7·4, containing 0·5 ml/l Tween 20 and 20 g/l polyvinylpyrrolidone using a leaf press (Pollahne, Germany). The extracts were collected in labelled plastic blood tubes and tested for AMV by ELISA as described by Jones & Pathipanawat (1989) using duplicate wells in microtitre plates. The substrate was 0·6 mg/ml *p*-nitrophenyl phosphate in 100 ml/l diethanolamine, pH 9.8. Absorbance (A_{405}) values were measured with a Titertek Multiscan plate reader (Flow Laboratories, Finland). They were considered positive (virus present) when more than twice those recorded for healthy leaf sap controls. When grouped samples of leaves were tested, percentage infection was estimated using the formula of Gibbs & Gower (1960).

Plant establishment, sward composition and yield measurements

In Expt 1, establishment of medic plants was measured following germination in autumn by counting numbers of seedlings inside cores taken at random within each plot; 15 cores (89 mm diameter) per plot in 1991 and 1992, or 10 cores (83 mm diameter) per plot in 1993. In 1993, capeweed, grass and subterranean clover seedlings were also counted inside the same cores used to count the medics. In 1991 and 1992, capeweed seedlings were counted, inside eight or 12 quadrats (0·35 m^2) per plot respectively. In Expt 2 in 1990, before cereal was sown, establishment of medic plants was counted inside eight quadrats (400 cm^2) per plot placed at random, four each per grazed and ungrazed subplot from the previous year. In 1991, there were nine quadrats (400 cm^2) per plot all placed in the original ungrazed subplot areas. Numbers of capeweed plants were counted inside 16 quadrats (0·35 m^2) per plot of cv. Serena (eight inside each of the previously grazed and ungrazed subplot areas). In Expt 3 in 1991, medic seedlings were counted inside 20 cores (89 mm diameter) per plot (10 inside each of the previously grazed and ungrazed subplot areas).

In Expt 1 in 1991, 1992 and 1993, species composition of herbage and overall herbage yield were assessed: (i) at the time grazing ceased in early August, (ii) during mid-September and (iii) just before the end of the growing season in early October. Four 0·2 m^2 quadrats (1991 and 1993) or five 0·1 m^2 quadrats (1992) were placed at random within each plot and and a visual estimate of percentage composition made within each. The herbage weight inside each quadrat was estimated by reference to calibration curves based on 0·1 m^2 calibration quadrats in which herbage was cut down to the soil surface using a power driven shearing handpiece (Alder & Richards 1962; Jones & Hargreaves 1979; Barbetti *et al*. 1996). The cut herbage from these was dried at 60 °C for at least 3 days and then weighed to obtain dry weight values. At two assessment times in 1991 and with the final assessment in 1992, before drying, each component species was separated from the others and the individual components dried and weighed separately.

In Expt 1, in 1991, 1992 and 1993, yields of newly produced medic seeds were determined after the end of the growing season by removing all pods down to the ground surface inside four randomly placed 0·2 m^2 quadrats per plot. Pods were threshed and seed cleaned before weighing.

Statistical analysis

Data on plant numbers, percentage species composition, herbage yields and seed yields were subjected to analysis of variance. Percentage estimates of species composition were transformed to angles before analysis. Although the original experimental designs from the year of sowing were retained for analysis of variance, for simplicity, the historical mowing/non-defoliation or grazing/non-defoliation treatment terms are omitted from the results shown.

RESULTS

Virus spread

In 1991, 1992 and 1993 in Expt 1, before spread of AMV each year by aphids, the medic components of plots originally sown with infected medic seed were 26–30% (Serena) and 5–7% (Circle Valley) infected (Table 2). In 1991, current season spread of AMV in the medic was first detected on 13 September and rose rapidly thereafter with both cultivars (Fig. 2). In 1992, spread started earlier and by 18 September had

Table 2. *Effect of sowing AMV-infected medic seed on annual infection of medic in swards and harvested seed over six growing seasons (Expt 1)**

Infection (%) in seed sown in 1988	Sward infection at end of growing season (%)						Infection in harvested seed (%)					
	1988	1989	1990	1991	1992	1993	1988	1989	1990	1991	1992	1993
cv. Serena												
20	98 (13)†	—	99 (22)	93 (30)	50 (28)	47 (26)	18	—	59	31	28	19
0	3 (0)	—	9 (0)	5 (—)	0·6 (0·2)	0·1 (0·1)	0·1	—	0·2	0·3	0·1	0
cv. Circle Valley												
4	98 (4)	—	99 (4)	79 (5)	32 (7)	25 (5)	3	—	26	7	6	3
0	2 (0)	—	11 (0·6)	7 (—)	0·6 (0·8)	0·3 (0)	0	—	1	0·2	0·1	0·6

* The plots were cropped with cereals in 1989 and then allowed to regenerate each year. Sward infection was determined by sampling shoots from each plot and testing leaflets for AMV by ELISA. Seed infection was determined by sowing seed samples and testing seedlings by ELISA. Data for 1988 and 1990 are from Jones & Nicholas (1992).

† Figures in parentheses are for percentage sward infection detected each year before spread by aphids.

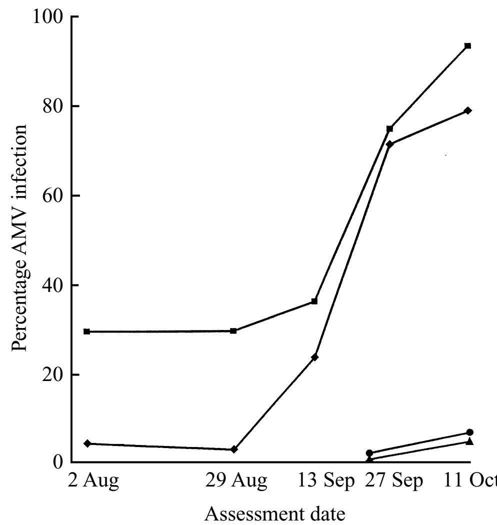

Fig. 2. Disease progress curves for AMV in 1991 (Expt 1). Symbols indicate plots sown in 1988 with infected seed of Serena (■) or Circle Valley (◆), or with healthy seed of Serena (▲) or Circle Valley (●).

risen to 40% in the medic within plots containing Serena and to 14% within those containing Circle Valley. In 1993, spread was not detected until 16 September. Final extents of infection in the medic component were high in 1991 (79–93%), as previously found in pure swards in 1988 and 1990 (> 97%), but lower in 1992 and 1993, especially in Circle Valley, only reaching 25% in this cultivar in 1993. In control plots, final infection amounts in medic were up to 7% in Circle Valley in 1991 but subsequently declined to < 1%.

In Expt 2 in 1991, amounts of AMV infection in July in the medic component of plots originally sown with infected medic seed were 10% (Serena) and 1% (Circle Valley). They rose to 47% (Serena) and 14% (Circle Valley) by early October. In the corresponding control plots, the amounts detected in early October were 0·7% (Serena) and 0·5% (Circle Valley). In Expt 3 in 1991, the corresponding figures for July were 23% (Serena), 2% (Santiago) and 3% (Circle Valley) in the plots originally sown with infected seed, and 0·2% (Serena), 0% (Santiago) and 0·3% (Circle Valley) in the control plots.

By September, AMV symptoms were visible in the medic component of the swards. These were faint mosaic in Serena. In Circle Valley and Santiago, they were mosaic plus leaf deformation. These symptoms were most obvious in plants infected *via* seed, which were also stunted. Circular symptom-affected patches centred on seed-infected plants were sometimes observed. No symptoms of AMV were seen in capeweed, but when capeweed plants growing in plots originally sown with infected medic seed were sampled

Table 3. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on medic seed yields in annually regenerating swards* (*Expt 1*)*

Infection (%) in seed sown in 1988	Seed yields (kg/ha)					
	1988	1989	1990	1991	1992	1993
			cv. Serena			
20	372	—	445	257	387	257
0	373	—	485	289	407	284
			cv. Circle Valley			
4	264	—	509	311	330	258
0	301	—	665	424	373	266
			S.E.			
Cultivar/infection	14·8	—	19·8	20·2	18·7	13·6
For interaction	21·0	—	28·2	28·6	26·5	19·2

* The plots were cropped with cereal in 1989 and then allowed to regenerate each year. Yields of newly produced medic seeds were determined each year inside quadrats placed at random within each plot. Data for 1988 and 1990 are from Jones & Nicholas (1992). D.F. = 28.

(100 plants per plot) in October 1993 and the samples tested by ELISA, 0·4% infection was found.

Aphids

In 1991 in Expts 1 and 2, the blue-green aphid (*Acythrosiphon kondoi*) colonized the medic in large numbers during September. Some cowpea aphid (*Aphis craccivora*) also colonized the medic in Expt 1 and green peach aphid (*Myzus persicae*) colonized some capeweed plants. In 1992 and 1993, fewer blue-green aphids were observed on the medic and the other two aphid species were not found.

Seed infection

In Expt 1 in 1991–93, extents of AMV infection in medic seed harvested from the regenerated mixed species plots originally sown with infected seed were 19–31% for Serena and 3–7% for Circle Valley (Table 2). These infection percentages were considerably smaller than those for each cultivar in seed harvested from the pure regenerated swards in 1990 but comparable to those obtained in the year of sowing. Although only 5 m wide, grass buffers proved effective each year in preventing AMV spread to control plots in sufficient amounts to cause large-scale infection of seed. Infection of seed produced in 1991, 1992 and 1993 never exceeded 0·6%.

Medic seed yields

Because seed production each year had a cumulative impact on the medic seed bank within each plot and therefore on annual regeneration, the medic seed yields in Table 3 include those from the start of Expt 1. Overall medic seed yields were greatest in the pure regenerated swards (in 1990) and least in the mixed species swards in 1993. With both cultivars, seed yields in regenerated swards were always greater for control plots than for those originally sown with infected seed, although sometimes only marginally so (e.g. only a 3% increase in control plots with Circle Valley in 1993). However, in 1991, 1992 and 1993, these differences in seed yields due to the original sowing treatments were significantly different at $P < 0{\cdot}05$ only in 1991, when they were substantially greater with Circle Valley (36% increase in control plots) than with Serena (12% increase).

Relative plant densities

In Expt 1 in 1991, 1992 and 1993, when newly emerged medic seedlings were counted in regenerating, mixed species swards, the numbers per m^2 increased each year in plots originally sown with Circle Valley, as also occurred in 1992 and 1993 in plots originally sown with Serena (Table 4). In 1992, control plots had significantly more medic seedlings ($P < 0{\cdot}05$) than plots originally sown with infected seed (12% increase). In 1991 and 1993, when the data for Circle Valley were analysed alone, there were significantly more medic seedlings ($P < 0{\cdot}05$) in the control plots (31–32% increase). However, with Serena in 1993, there were no significant differences in plant density due to the original sowing treatments. In Expt 2, control plots had significantly greater numbers of medic seedlings ($P < 0{\cdot}05$ in 1990 and $P < 0{\cdot}01$ in 1991), a 23% increase in 1990 and a 36% increase in 1991 (Table 5). In Expt 3, again there were significantly more medic seedlings ($P < 0{\cdot}01$) in the control plots; overall there was a 16% increase for control plots, with the greatest increase (24%) in cv. Santiago.

Table 4. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on plant densities (plants/m²) of medic and capeweed in annually regenerating swards, Expt 1**

	1991		1992		1993	
Infection (%) in seed sown in 1988	Medic (19 June)	Capeweed (23 August)	Medic (23 June)	Capeweed (6 July)	Medic (6 June)	Capeweed (6 June)
			cv. Serena			
20	—	—	1341	21	1848	2541
0	—	—	1560	17	1889	1948
			cv. Circle Valley			
4	1040	29	1328	14	1752	1937
0	1364	23	1429	7	2304	924
			S.E.			
Cultivar/infection	80·9	2·6	54·2	1·2	143·6 [17·4]†	231·0
For interaction	—	—	76·7	1·7	203·1	326·7

* Plant densities were counted inside cores or quadrats placed at random within each plot. D.F. = 28 except where data for plots originally sown with cv. Circle Valley were analysed alone, when D.F. = 12.
† Figures in square brackets are from an analysis restricted only to data from plots originally sown with cv. Circle Valley.

Table 5. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on plant densities (plants/m²) of medic and capeweed in annually regenerating swards, Expts 2 and 3**

	Expt 2			Expt 3
	1990	1991		1991
Infection (%) in seed sown	Medic (3 July)	Medic (19 June)	Capeweed (25 August)	Medic (20 June)
		cv. Serena		
20	351	420	15	1147
0	443	548	10	1358
		cv. Circle Valley		
9	239	229	—	728
0	283	336	—	762
		cv. Santiago		
9	—	—	—	684
0	—	—	—	846
		S.E.		
For cultivar	20·5	23·3	—	49·8
For infection	20·5	23·3	1·5	35·2
For interaction	28·9	33·0	—	65·3
D.F. (infection)	42	21	21	18

* The plots were sown in 1989 (Expt 2) or 1990 (Expt 3) and allowed to regenerate each year. Plant densities were counted inside cores or quadrats placed at random within each original subplot. In Expt 2 in 1990 the plots were sown with cereal after the counts were done. D.F. (cultivar) was the same as D.F. (infection) except in Expt 3, in which it was 12.

In Expt 1, densities of capeweed plants were small in 1991 and 1992 but increased greatly in 1993 (Table 4). In 1992 and 1993, there were significantly more capeweed plants ($P < 0{\cdot}001$ in 1992 and $P < 0{\cdot}05$ in 1993) in regenerating plots originally sown with infected medic seed than in control plots (46 and 56% more in 1992 and 1993 respectively). Also, there were significantly more capeweed plants ($P < 0{\cdot}001$ in 1992 and $P < 0{\cdot}05$ in 1993) in plots originally sown with Serena than in those sown with Circle Valley. In 1993, there was an average across all original sowing treatments of 164 grass and 98 subterranean clover plants per m². There were no significant differences in grass plant numbers due to any of the original sowing

Table 6. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread in medic on overall herbage yields (kg/ha) in the mixed-species swards that regenerated in the subsequent fourth to sixth growing seasons (Expt 1)**

Infection (%) in seed sown in 1988	1991		1992			1993		
	5 August	8 October	5 August	16 September	6 October	6 August	14 September	6 October
				cv. Serena				
20	204	3562	1422	3452	—	227	2744	3857
0	224	3664	1474	3692	—	284	2874	4032
				cv. Circle Valley				
4	191	3600	1346	3676	5560	250	2920	4241
0	261	3687	1082	4028	5812	205	3022	4416
				S.E.				
Cultivar/infection	21·8	100·0	80·6	72·1	169·3	18·9	45·6	55·1
For interaction	30·8	141·4	114·0	101·9	—	26·9	64·5	77·9

* The plots were allowed to regenerate naturally each year and herbage yields were determined by cutting foliage inside quadrats placed at random within each, drying the herbage and obtaining dry weight values. D.F. = 28 on all occasions except with the 6 October 1992 assessment where D.F = 12.

treatments (range 150–182 per m^2) but numbers of clover plants were significantly greater ($P < 0·01$) in plots sown with Serena (154 per m^2) than in those sown with Circle Valley (44 per m^2). In Expt 2 in 1991, there were insufficient capeweed plants to count in plots originally sown with Circle Valley but, within plots sown with Serena, numbers of capeweed plants in plots originally sown with infected seed were significantly greater ($P < 0·05$) than in control plots (50 % increase) (Table 5).

These results show that previous sowing of AMV-infected medic seed can result in decreased medic but increased capeweed germination in subsequent years. The magnitude of these effects varied with year and medic cultivar. Differences in plant densities between sowing treatments tended to be greater with capeweed than with medic, e.g. in the sixth year of Expt 1 when, for swards containing Circle Valley, control plots had 32 % more medic but 52 % fewer capeweed seedlings than plots originally sown with infected seed.

Overall herbage yields

In Expt 1, when grazing ceased in early August, overall herbage yields in the mixed species swards were very low in 1991 and 1993 but substantially greater in 1992, reflecting the lighter grazing pressure in the intervening year (Table 6). At this time, there was no significant impact of previous sowing of infected seed on overall herbage yields. Overall yields increased markedly from then on until the end of the growing season in October, when those in 1991 and 1993 resembled those for mid-September in 1992. In the September and October assessments, within plots originally sown with the same cultivar, control plots always outyielded those sown with infected seed, though sometimes by only small amounts. However, control plots had significantly greater overall herbage yields than those originally sown with infected seed only in September in 1992 ($P < 0·01$; 8 % yield increase) and at the end of the growing season in 1993 ($P < 0·05$; 4 % yield increase).

Species composition of herbage

In Expt 1 in 1991, when grazing ceased in August, the plots consisted of medic, capeweed and grass, but a trace of subterranean clover (< 1 %) was also noted. In subsequent assessments in 1991, they were 97–98 % medic and capeweed, the grass having been removed. Both in August and at the end of the growing season in October, the proportion of medic present was significantly smaller ($P < 0·001$) in the plots that contained Serena than in those that contained Circle Valley (Table 7). The opposite was the case with capeweed and grass in August ($P < 0·01$), and with capeweed in October ($P < 0·001$). In both August ($P < 0·05$) and October ($P < 0·01$), the medic content was significantly greater in control plots than in plots

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Table 7. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on the species composition of swards that regenerated in the fourth growing season (1991)**

Infection (%) in seed sown	Visual estimates (%)					Herbage dry weight (kg/ha)				
	5 August			8 October		5 August			8 October	
	Medic	Capeweed	Grass	Medic	Capeweed	Medic	Capeweed	Grass	Medic	Capeweed
	cv. Serena					cv. Serena				
20	22 (22)	33 (29)	43 (47)	37 (36)	52 (62)	48	60	96	1280	2244
0	29 (25)	32 (28)	42 (45)	36 (35)	53 (63)	55	69	97	1273	2331
	cv. Circle Valley					cv. Circle Valley				
4	42 (44)	28 (21)	35 (33)	47 (53)	42 (45)	78	44	68	1868	1655
0	51 (60)	25 (18)	25 (18)	62 (78)	26 (19)	155	51	50	2786	821
	S.E.					S.E.				
Cultivar/infection	1·6	1·0	1·6	1·8	1·8	11·2	8·3	8·2	101·8	139·1
For interaction	2·3	1·4	2·2	2·5	2·6	15·8	11·8	11·6	143·9	196·7

* The plots were allowed to regenerate naturally in each year and herbage composition was determined by estimating the proportions of each species. Dry weight values were also estimated and these estimates converted to kg/ha based on yield curves for each species determined by calibration cuts. Percentage estimates were converted to angles before analysis. Figures in parentheses are de-transformed % estimates. D.F. = 28.

Table 8. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on the species composition of swards that regenerated in the fifth growing season (1992)**

Infection (%) in seed sown	Visual estimates (%)						Herbage dry weight (kg/ha)	
	5 August		16 September		6 October		6 October	
	Medic	Capeweed	Medic	Capeweed	Medic	Capeweed	Medic	Capeweed
	cv. Serena						cv. Serena	
20	36 (35)	54 (65)	42 (45)	48 (55)	—	—	—	—
0	32 (28)	57 (71)	44 (49)	46 (51)	—	—	—	—
	cv. Circle Valley						cv. Circle Valley	
4	39 (40)	51 (60)	45 (50)	45 (50)	42 (44)	49 (56)	2442	3137
0	52 (62)	38 (38)	58 (72)	32 (28)	53 (64)	37 (36)	3606	2211
	S.E.						S.E.	
Cultivar/infection	2·3	2·4	2·3	2·3	2·9	2·9	261·8	306·0
For interaction	3·3	3·3	3·3	3·3	—	—	—	—

* The plots were allowed to regenerate naturally in each year and herbage composition was determined by estimating the proportions of each species. On 6 October the dry weight values were also estimated and these estimates converted to kg/ha based on yield curves for each species determined by calibration cuts. Percentage estimates were converted to angles before analysis. Figures in parentheses are de-transformed % estimates. D.F. = 28, except where data from plots originally sown with cv. Circle Valley were analysed alone, when D.F. = 12.

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Table 9. *Effect of sowing AMV-infected medic seed and the resulting annual virus spread on the species composition of swards that regenerated in the sixth growing season (1993)**

Infection (%) in seed sown	Visual estimates (%)						Visual estimates (%)					
	6 August		14 September		6 October†		6 August		14 September		6 October†	
	Medic	Non-medic	Medic	Non-medic	Medic	Non-medic	Legume	Non-legume	Legume	Non-legume	Legume	Non-legume
	cv. Serena						cv. Serena					
20	22 (14)	68 (86)	34 (32)	56 (68)	38 (38)	52 (62)	29 (24)	61 (76)	39 (40)	51 (60)	39 (39)	51 (61)
0	24 (17)	66 (83)	35 (33)	55 (67)	36 (35)	54 (65)	32 (27)	58 (73)	37 (36)	53 (64)	37 (37)	53 (63)
	cv. Circle Valley						cv. Circle Valley					
20	28 (22)	62 (78)	40 (42)	50 (58)	42 (44)	48 (56)	32 (27)	58 (73)	41 (44)	49 (56)	42 (44)	48 (56)
0	34 (31)	56 (68)	42 (45)	48 (55)	44 (49)	46 (51)	36 (35)	54 (65)	42 (46)	47 (55)	44 (49)	46 (51)
	S.E.						S.E.					
Cultivar/infection	1·0	1·0	1·1	1·1	0·9	0·9	0·9	0·9	1·1	1·1	0·8	0·8
For interaction	1·5	1·5	1·5	1·5	1·3	1·3	1·3	1·3	1·5	1·5	1·1	1·1

* The plots were allowed to regenerate naturally in each year and herbage composition was determined by estimating the proportions of each species. Percentage estimates were converted to angles before analysis. Figures in parentheses are de-transformed % estimates. D.F. = 28, except where data from plots originally sown with cv. Circle Valley were analysed alone, when D.F. = 12.

† When data for 6 October 1993 from plots originally sown with Circle Valley were analysed alone, differences between healthy and infected seed treatments were significant ($P = 0{\cdot}03$, S.E. = 0·8).

originally sown with infected seed. Conversely, the grass content in August ($P < 0{\cdot}05$) and the capeweed content in October ($P < 0{\cdot}01$) were both significantly smaller in the control plots. Because these differences in species composition due to AMV infection of the medic component were large in plots containing Circle Valley but small (August) or absent (October) in those containing Serena, there were also significant interactions between infection and cultivar ($P < 0{\cdot}01$). Serena was senescing by the time the October assessment was done. In general, both types of herbage assessments used gave similar results showing that the use of percentage content estimates alone in 1992 and 1993 was valid.

In 1992, the capeweed content of the swards was greater than in 1991 and they were mixtures of medic and capeweed (Table 8) with just a trace of subterranean clover (< 1%) and no grass. The same general trends were evident with (i) capeweed growing significantly more profusely in the plots containing Serena ($P < 0{\cdot}01$, August assessment; $P < 0{\cdot}05$, September assessment), and (ii) AMV significantly diminishing the ability of medic to compete with capeweed ($P < 0{\cdot}05$ in both September and October assessments). On all three assessment dates, plots originally sown with infected Circle Valley seed had substantially smaller medic and greater capeweed contents than control plots containing this cultivar. In contrast, there was no (August) or little (September) impact on the balance between the two species in plots originally sown with Serena. In the August assessment, this difference between the two cultivars in impact of AMV resulted in significant interactions between cultivar and infection ($P < 0{\cdot}05$) for both medic and capeweed contents. When both herbage yield assessment methods were used in October in plots containing Circle Valley, they gave similar results.

In 1993, at the August assessment time the proportion of medic in the swards was smaller than in 1991 and 1992 (Table 9) and there were far more capeweed plants (Table 4). Also, the grass and subterranean clover contents were more substantial. In August, they ranged from 6% (grass) and 10% (clover) in plots containing Serena to 6% (grass) and 5% (clover) in plots containing Circle Valley. By October, there was an average of 11% (grass) but only 0·5% (clover) with no difference evident between plots containing different medic cultivars. The grass component was predominantly a mixture of annual ryegrass and barley grass (*Hordeum leporinum*) but also contained ratstail fescue (= silver grass, *Vulpia myuros* forma *megalura*), wild oats (*Avena fatua*) and three species of brome grass (*Bromus diandrus*, *B. mollis* and *B. rubens*). The species composition data for herbage were examined in two ways: (i) medic *v*. non-medic and (ii) legume *v*. non-legume (Table 9). Plots originally sown with Serena always had significantly less medic/legume and more non-medic/non-legume than those sown with Circle Valley ($P < 0{\cdot}05$–$0{\cdot}001$ depending on assessment date). In August, there were significantly greater medic and legume contents and significantly smaller non-medic and non-legume contents in control plots than in plots originally sown with infected seed ($P < 0{\cdot}05$). When the September and October data for both cultivars were analysed, there were no longer any significant differences due to infection. However, when the Circle Valley data from October 1993 were analysed alone, a significant ($P = 0{\cdot}03$) decrease in medic and legume contents was evident due to originally sowing infected seed (see footnote in Table 9).

These results show that species composition in mixed pasture swards can still be affected by AMV infection of the medic component several years after its introduction in sown medic seed. However, the magnitude of this effect varied with year. Thus in plots originally sown with Circle Valley, the capeweed:medic ratios in October in the fourth year were 45:53 (infected seed originally sown) and 19:78 (control plots), while in the fifth year the corresponding ratios were 56:44 and 36:64. In the sixth year, the impact of AMV on medic content was smaller than in the previous two years: non-medic:medic (or non-legume:legume) ratios in October in Circle Valley plots were 56:44 (infected seed sown originally) and 51:49 (control plots). Each year, AMV had a much smaller impact on the ability of Serena than Circle Valley to compete with capeweed, despite the generally greater numbers of seed-borne AMV sources and extents of virus spread in plots containing it. This presumably reflects the greater tolerance of Serena to infection with the virus. In the October assessments, it undoubtedly also reflected the influence of senescence on competitive ability in the earlier maturing Serena.

These results also showed that AMV can influence the ability of medic to compete with grass as well as capeweed. Thus when grass content was assessed in the fourth year before being killed by herbicide application, there was less in control plots, the difference being substantial with Circle Valley (18 *v*. 33% grass content) but minimal with Serena (45 *v*. 47%).

DISCUSSION

The disease symptoms AMV causes in medics are often mild and therefore overlooked or considered unimportant in annual medic-based pastures. However, our findings with annually regenerating, mixed species pasture swards originally sown with infected medic seed show that, although overall herbage yield is normally little altered by the virus, AMV can still have a major impact on species composition several years after its introduction in sown seed. Its presence in the medic component allows the proportion of less desirable species such as capeweed to increase at the expense of the medic, sometimes dramatically so. Moreover, it is likely to continue increasing the content of less desirable species to varying extents in different years once infected medic seed becomes established in the pasture seed bank. The magnitude of its effect on competitive ability varied with medic cultivar and was much smaller in the more tolerant and early maturing Serena than in Circle Valley.

Because of prolonged seed dormancy, the extent of regeneration of different component species from the seed bank within annual pastures reflects seed production history over the years since sowing (Rossiter 1966; Taylor *et al*. 1991). Decreased medic but increased capeweed germination due to sowing of infected medic seed in earlier years was evident in Expts 1–3, especially in plots containing Circle Valley. Moreover, it still showed after an intervening cropping phase. Differences in plant densities between the original AMV-infected and healthy medic sowing treatments tended to be of greater magnitude with capeweed than with medic.

The impact of infection with AMV on the species balance visible at the end of the growing season within the regenerated, mixed species swards containing Circle Valley came from: (i) the smaller numbers of medic plants and corresponding greater numbers of capeweed plants germinating in plots originally sown with infected than healthy medic seed, and (ii) the diminished competitive ability of AMV-infected than healthy medic plants following virus spread during the growing season. Infection in the medic rose to the highest amounts in the fourth year, which resulted in the greatest divergence in capeweed content between the two types of plots containing Circle Valley. In the sixth year, grass was not removed, some subterranean clover was present and there was a vastly greater germination of capeweed: such big differences in germination from one year to the next are normal for capeweed and reflect its specific environmental requirements for breaking seed dormancy (Rossiter 1966). This provided much greater competition pressure for the Circle Valley plants to deal with, regardless of whether they were infected or not. Also, final AMV infection in Circle Valley did not exceed 25% in 1993. Whether this result would be more typical of the commercial pasture situation than those obtained in the fourth and fifth years is not clear because, although herbicide was not used to remove grass, capeweed germination was exceptionally high and AMV spread somewhat smaller.

In addition to competing with other species in mixed species pasture swards, AMV-infected Circle Valley medic plants have to contend with healthy medic plants. Healthy medic plants have greater vigour, which allows them not only to compete effectively with other aggressive species such as capeweed but also to compensate for the decreased competitive ability of neighbouring infected medic plants. The dynamic system that results in virus-infected, mixed species swards is also complicated by variations in the extent of defoliation due to differing grazing pressures and different rates of virus spread depending on (i) on the proportion of seed-infected virus source plants present initially and (ii) on the relative abundance and activity of aphid vectors in different years.

As in newly sown and regenerated pure medic swards (Jones & Nicholas 1992), AMV spread by aphids in the medic component of the mixed species swards took place towards the end of the growing season (especially in early spring), continuing until stand collapse due to lack of moisture in October. The greater virus spread in the fourth year reflected the greater aphid abundance in the swards in that year. Presence of non-hosts of AMV (e.g. grasses) or less susceptible hosts (e.g. capeweed) would be expected to slow its spread more in medic when the proportion of non-medic:medic is greater. That capeweed is far less susceptible to AMV than medic was confirmed here when only very low amounts of symptomless AMV infection were found in tests on capeweed plants from mixed species swards compared with much higher amounts in the medic. AMV infection both in the growing medic and in newly produced medic seed remained substantial for at least six growing seasons after sowing infected seed. Previously, Jones & Nicholas (1992) and McKirdy & Jones (1994) had found that AMV persisted through seed bank infection for at least 7 years in self-regenerating, grazed swards originally sown with infected burr medic seed, but the extent of infection decreased year by year as the medic eventually became only a very minor component. Percentage AMV infection of newly produced seed also decreased year by year but amounts in the seed bank did not decline nearly as much, due to the presence of older medic seed.

Virus spread by aphid vectors from plots containing AMV-infected medic plants over the 5 m wide grass buffers to the medic component of control plots remained surprisingly low even after six growing seasons. Moreover, there was no evidence of a build-up of infection over time in the seed they produced. This presumably reflected the spread of AMV predominantly by wingless blue-green aphids walking between plants within swards with infrequent spread by winged forms over distances of as little as 5 m (Jones & Nicholas 1992). As a result, infection in control plots was never sufficient to cause much seed infection by the time that plants died from lack of moisture in October. This contrasts with experiments with perennial pasture species where contamination of control plots has been a major obstacle to obtaining data on the impact of virus infection (Campbell 1986).

In annual pasture, diminished legume content, and a corresponding increase in undesirable species such as capeweed, results in poor feed quality at the critical late autumn/early winter 'feed gap' period and subsequently during the growing season. It also results in diminished medic seed yields which, when compounding year by year, gradually deplete the seed bank, eventually leading to deteriorated, weed-dominated pastures. To prevent AMV infection from contributing to declining legume contents, virus-tested seed should always be used when medic is first sown in pastures. The lack of substantial spread to control plots regenerating naturally over a 6-year period in the work reported here and the low overall incidence of AMV in medic pastures (Jones & Pathipanawat 1989; McKirdy & Jones 1995) suggest that sowing seed with very low amounts of infection will rarely result in substantial virus spread or a major impact on pasture composition. These observations support previous conclusions that a tolerance of $< 0{\cdot}1\%$ seed infection should be adequate for medic seed stocks used to sow new pastures (Jones & Pathipanawat 1989; Jones & Nicholas 1992; McKirdy & Jones 1994).

Our findings on the impact of sowing AMV-infected burr medic seed on the relative germination of medic and capeweed and on the species balance within regenerated, mixed species pasture have wide implications. Similar consequences arising from the presence of insidious diseases of annual pasture legume plants caused by other viruses, nematodes feeding on roots, or fungi infecting leaves or roots are likely to result in other types of legume-based pastures. We suggest that greater attention should be given to avoiding introduction of pathogens causing insidious diseases to new pastures, particularly through diseased seed. Should they become established, consideration should be given to minimizing both their spread during the growing season and their build-up in the seed bank, e.g. by sowing disease-resistant cultivars, using cultural methods designed to decrease the carry-over of infection between growing seasons and the application of appropriate pesticides or fungicides to slow the spread.

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