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Losses causes by and control of important seed-borne virus diseases affecting productivity of annual pasture legumes.

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1. 90V1 and 90W1 - Assessment of Pasture Productivity in Subterranean Clover Pastures Infected with SCMV

BACKGROUND:

SCMV was first identified in pastures in the Spring of 1980 and a pasture survey of 80 subterranean clover pastures carried out in the spring of 1989 found the virus to be widespread, extending from Harvey to east of Mt Barker. The virus was detected in 51% of those pastures tested and the incidence of infection ranged from 1-50%. In previous years, individual pastures have recorded incidence levels as high as 97%. Spaced plant trials showed the virus depressed herbage by 78-93% in cultivars Woogenellup and Daliak and seed production by 90% for Woogenellup when tested with three different field isolates of the virus.

The virus mechanism for spread is by contact transmission which is significantly increased by animals grazing and treading the pasture. Pasture trials to determine productivity loss in 1989 were unsuccessful because measures to spread the virus using mowing were ineffective and resulted in low levels of infection late in the season. The pattern of virus spread results in an initial localized infection which expands out into large patches and can affect all susceptible clover plants in the pasture sward.

AIM:

These trials measure SCMV disease progress, herbage and seed production losses in Woogenellup pasture swards which are:

(i) initially artificially infected by using infected transplants in the first year; and

(ii) naturally infected via seed transmission of the virus in year two. Trials are grazed by sheep throughout the season, maintaining the pasture height at less than 20 cm.

METHOD:

Trial Locations

The same trial was set up at two locations:

(i) Vasse, where during spring high levels of SCMV infection were found in the district, 50% in 1989 but even higher in earlier years; and

(ii) Wokalup where only low (< 10%) infection levels were measured.

The soil type at Vasse was a light sandy loam while at Wokalup the soil was a light textured loam.

Experimental Design

Nine plots each measuring 50 m^2 and arranged in a three by three block design, to even out soil variation within trial sites were prepared. Swards of healthy cv. Woogenellup were planted at a machine seeding rate of 90 kg/ha using certified seed with a germination rate of 92% on April 27 at Vasse and on April 30 at Wokalup. Each plot was separated by a 2 m buffer of Italian ryegrass cv. Tama sown at the rate of 30 kg/ha, and then plots were fully fenced down the middle of the ryegrass buffers. The average emergence rate for clover swards at Vasse was 760 plants per m^2 and at Wokalup was 735 plants per m^2. Plots were grazed down for seven days, prior to planting infected transplants, to assist establishment of diseased plants and then grazing recommenced two weeks later. Each plot contained five sheep but during September sheep numbers were increased to eight to maintain the height of the pasture at less than 20 cm. Grazing ceased at Vasse on October 1 and at Wokalup, one week later, on October 7.

Treatments

Three levels of virus infection using the SCMV isolate P23 were established in plots:

(i) nil;
(ii) low;
(iii) high (4 x low rate).

Each plot was sectioned every 10 m, along both plot axis, to create 25 discrete subplots. The internal nine subplots were then chosen as sites for introducing virus infection into plots. For low infection levels, a single site at the centre of a subplot was planted with five SCMV infected Woogenellup transplants. For the higher infection rate, four equally spaced sites within the subplot were planted with the same number of transplants (i.e. 45 plants for low infection rates/plot and 180 plants for high infections rates/plot).

Infected transplants were produced in the glasshouse by firstly germinating healthy Woogenellup seed and then sap-inoculating the youngest leaves with SCMV six weeks after germination. The young plants were then tested for virus infection by ELISA, three weeks later. Following a short acclimatization period outside the glasshouse, plants were transported and planted out at

Wokalup on July 4 and at Vasse on July 10. Plants were inspected again four to six weeks after transplanting to note the success of establishment of virus infected transplants and to observe any early spread of infection into healthy plants.

Sampling Procedures

At the time of recording emergence rates for the Woogenellup swards, 1000 leaflet samples from the swards for each trial were tested for AMV SCMV and CMV to ensure no seed-borne sources of virus infection were present in swards at the commencement of the trial.

Disease progress was monitored at Vasse on August 31, September 18, October 10 and October 22, and for Wokalup on August 30, September 17, October 10 and October 30. One hundred leaf samples were taken from each plot, grouped in tens and tested by ELISA for SCMV. Percentage infection was calculated using the formula of Gibbs and Gower (1980).

Herbage production was measured on October 2-4 and again on October 22-24 at Vasse and on October 8-10 and October 29-31 at Wokalup. Fifty pasture plate metre counts were taken across each plot and 18 x 0.1 m^2 quadrat cuts of herbage taken. Each quadrat was assessed visually for the number of SCMV infected plants and checked by ELISA to confirm SCMV and not BYMV was causing the typical symptoms of vein clearing in infected plants. All plants in each quadrat were then counted and a percentage of infected plants for each quadrat calculated. Stakes were hammered into each site so that a second quadrat cut for herbage from the same site would measure the growth of pasture for the period between cuts for areas of pasture with known levels of virus infection. Later, after the pasture had dried, another quadrat from the same locations was taken for seed yield so that seed production could also be related to the level of SCMV infection. A final assessment of germination rate and virus transmission rate in newly germinated seedlings was carried out at the break of the winter season for each quadrat site.

RESULTS:

Disease Progress

The pasture swards established well and almost 100% of infected transplants survived at Vasse. At Wokalup where the pasture grew vigorously towards the end of August some of the infected transplants became shaded out and died. Spread of SCMV was very slow during July and August and only the odd plant was found with obvious symptoms of vein clearing indicating a newly infected plant. During September the incidence of SCMV infection increased slowly and then in October spread became very rapid (Table 1). Disease progress at Wokalup was much slower than at Vasse. This could partly be attributed to the rapid clover growth in the heavier soils at Wokalup and as a consequence grazing pressure was less. Three plots at Wokalup into which SCMV was introduced at the beginning of July had no visual symptoms of SCMV in late October and no leaflet samples tested by ELIZA were positive for SCMV. Diseased plants introduced into these plots had died away with the competition from healthy plants before any spread into surrounding healthy plants had occurred to set up the disease cycle. Inspection in late November, however, showed some infection had established in one plot (4) just before the pasture died.

Table 1. Subterranean clover mottle virus disease# progress in swards* of subterranean clover at two sites, Wokalup and Vasse

Date	Incidence of SCMV (Percentage)**	
	Vasse	Wokalup
August 30		0
August 31	1	
September 17		2
September 18	4	
October 2	9	
October 9		2
October 22	93	
October 30		8

* Swards of cv. Woogenellup (90 kg/ha) were established at the beginning of the 1990 season.

\# Infection with SCMV was introduced at the beginnning of July by transplanting five systemically infected transplants at each of 36 specific locations within the sward.

** 100 leaf samples, taken at random, were grouped in tens and then tested by ELISA to SCMV. The percentage infection was estimated using the formula of Gibbs and Gower (1980).

Pasture Productivity

Data from herbage cuts for year one is at the analysis stage and seed data is still being cleaned and assessed.

2. 90PE5 - Assessment of Sensitivity/Resistance to Subterranean Clover Mottle Virus in Selected Subterranean Clover Cultivars

BACKGROUND:

Cultivars of subterranean clover display different susceptibility to systemic infection with SCMV and have been catorgorized into groups known as non infected, limited infection and complete infection. Limited infection is defined here as a delay in the onset of systemic infection and not all the plants become infected following sap inoculation with virus. Disparity between glasshouse trials in the summer and field trials during the 1989 season demonstrated two systems of resistance to infection may be operating in some cultivars of sub. clover.

Firstly, cv. Karridale, which was fully susceptible to SCMV in tests in the glasshouse over summer, failed to develop disease in 98% of plants sap-inoculated during winter and as the season progressed a proportion of these plants grown as spaced plants in the field developed symptoms during spring, indicating a delay in the onset of systemic disease. This reduced susceptibility of Karridale to SCMV was also manifest in field sward trial in which cv. Woogenellup was significantly more susceptible to SCMV infection than Karridale.

Secondly, during the spring, 12.5% of cv. Woogenellup plants, which were systemically infected with SCMV and grown as spaced plants, were able to grow at a sufficient rate to escape the virus in shoots. These plants grew to almost similar size and produced similar levels of seed as uninfected plants. The seed (3,000 seedlings) when tested for virus transmission in seedlings was clean. These plants were called "growouts".

AIM:

Subterranean clover cultivars, recommended for growing in areas where SCMV has been found, and known to be susceptible to the SCMV following sap-inoculation in the glasshouse were assessed for susceptibility to SCMV. Included are measurements of herbage and seed productivity loss due to the disease. Seed transmission of SCMV in newly germinated progeny seedling from infected plants is also examined.

METHOD:

Treatments

(A) Virus Isolate P23.

(B) Susceptible subterranean clover cvs Green Range, Dalkeith, Woogenellup, Daliak, Seaton Park, Denmark, Karridale, Junee and Goulburn. Trikkala was included as a resistant control although it should be noted that in the 1989 season one plant of Trikkala was identified with SCMV.

Certified seed for each of the different cultivars was germinated in peat pots in the glasshouse on May 9, watered with "Nodulaid C" at the two leaf cotyledon stage and then half the plants sap-inoculated with SCMV six weeks later. The other uninoculated half set of plants were prepared as healthy controls. Sap-inoculation was repeated again three weeks later to all plants not showing symptoms of vein clearing which indicated the commencement of systemic infection with SCMV. A representative sample of infected plants from each cultivar was also tested by ELIZA to SCMV.

Experimental Design

One hundred and twenty plots, measuring 2.5 m x 3 m and surrounded by a dense buffer row of oats cv. Mortlock were prepared in early May. Plots were divided into twelve blocks of ten treatments and within each block treatments were fully randomized. Four blocks of treatments were used for assessing losses in herbage production and eight blocks were for assessing losses in seed production. Within a single plot five healthy transplants and five virus-infected transplants were planted in two 1 m corresponding rows with plants spaced equi-distance apart.

Establishment of Transplants

Glasshouse grown transplants were transplanted into the field 11 weeks after germination and watered in with half strength "Thrive" nutrient solution. Weeds were controlled and plants were fertilized, by broadcast application, at the rate of 100 kg superphosphate and 40 kg potash in late August. Redlegged earth mite were controlled with "Lorsban" and aphids were controlled with "Pirimor". Oat buffers developed stem rust and this was controlled with one application of "Impact" in late September.

Sampling

Herbage

On October 15, 16 weeks after plants were sap-inoculated with virus, plants were removed from soil and the shoot material cut immediately above the main tap root. The root material was washed and then both shoot and root material was separately oven dried at 60°C and then weighed to obtain herbage and root dry weights.

Seed Yield, Size, Germination Rate and Virus Seed Transmission Rate

Clover burrs were harvested manually, threshed and the seed cleaned before measuring the average seed weight from the five plants in each treatment row. One hundred seeds were then counted and an average seed size assessed. Seed was then pooled from the different replicates to give a single sample for assessing germination rate and virus transmission rate for cultivars which were fully infected and had produced sufficient seed. Because of the low transmission rate already detected in Woogenellup, 2,000 seedlings were germinated in trays in the glasshouse and the first trifoliate leaf from each seedling picked and grouped in 10's before testing by ELISA for SCMV. Percentages of seedlings infected with SCMV were than estimated using the formula of Gibbs and Gower (1960).

RESULTS:

Susceptibility to Infection

Systemic infection of sub. clover cultivars following sap-inoculation of virus to youngest leaves resulted in three distinct catorgories of susceptibility to infection with SCMV (Table 2).

1. Plants which became readily infected and had no resistance to infection, namely cvs Woogenellup, Daliak, Dalkieth and Junee.

2. Plants for which only a few could be infected systemically, namely Karridale, Seaton Park, Goulburn, Denmark and Green Range.

3. Plants which did not become systemically infected, namely Trikkala.

The second phenomenon of plants outgrowing the virus in shoots was observed in two plants of Woogenellup and one plant of Seaton Park.

Table 2. Development of systemic infection in a range of subterranean clover cultivars following sap-inoculation with SCMV

Cultivars	Numbers of plants* testing positive to SCMV by ELISA		
	1-8-90	23-8-90	29-9-90
Woogenellup	50	53	55
Daliak	60	60	60
Dalkeith	57	57	57
Junee	48	51	52
Karridale	3	5	5
Seaton Park	4	4	6
Goulburn	7	10	10
Denmark	1	1	2
Green Range	1	3	3
Trikkala	0	0	0

* Sixty, six week old plants of each cultivar were sap-inoculated with SCMV isolate P23 on June 20 and then transplanted into plots at South Perth five weeks later.

Herbage Yields

Decreases in herbage dry weight ranged from 63% for Daliak, an early maturing cultivar, to 73-80% for Woogenellup, Junee and Dalkeith. Decreases in root dry weight between healthy and diseased plants were not significant except for the cultivar Junee (60.47%).

Table 3. Comparison of the effect of SCMV on herbage for a range of fully susceptible cultivars of subterranean clover

Cultivars	Herbage yields (g/plant)		
	Healthy mean	Infected mean	% Reduction
Woogenellup	46.6	11.7	72.7
Daliak	28.8	6.7	62.7
Junee	51.0	9.1	79.8
Dalkeith	63.4	10.5	76.4

Significant disease ($P < 0.01$).

Table 4. Comparison of the effect of SCMV on seed yields for a range of fully susceptible cultivars of subterranean clover

Cultivars	Seed yields (g/plant) Healthy mean	Infected mean	% Reduction
Woogenellup	14.86	2.86	83.2
Daliak	6.99	0.83	88.3
Junee	9.05	1.03	86.9
Dalkeith	12.14	1.70	85.2

Significant disease ($P < 0.001$).

Table 5. Comparison of the effect of SCMV on seed size for a range of fully susceptible cultivars of subterranean clover

Cultivars	Seed size (mg/seed) Healthy mean	Infected mean	% Reduction
Woogenellup	9.20	6.66	27.97
Daliak	6.37	3.72	41.33
Junee	6.56	3.95	36.51
Dalkeith	9.02	6.75	24.79

Significant disease ($P < 0.001$).

Seed viability and virus transmission rates in seed from infected plants are still being assessed.

CONCLUSIONS:

Deducing resistance to SCMV for cultivars which were not readily systemically infected when inoculated during the winter may have very little correlation with what actually happens under natural conditions in the field during spring. Most spread of SCMV occurs in spring. Observations made on December 12 of a trial at Pemberton growing mid-late season subterranean clover varieties which had become heavily infected with SCMV found no evidence of resistance in the cultivars Goulburn, Denmark, Karridale, Woogenellup or Mt Barker. Based on the severity of stunting, SCMV affected Goulburn the most and Mt Barker the least. The Trikkala and Karridale mixture which surrounded the trial as a buffer showed Trikkala maintains its resistance when interspersed with SCMV infected Karridale plants.

3. SCMV Infection and Systemic Movement in Trifolium spp.

BACKGROUND:

The susceptibility of Trifolium spp. to infection with SCMV and the subsequent systemic movement of virus throughout the plant appears to influenced by the environmental conditions under which plants are grown. Karridale, when grown in an air conditioned glasshouse during the summer months was easily infected

but during the winter months the majority of plants failed to become systemically infected. Testing of inoculated leaves by ELISA indicated that primary infection had taken place but systemic movement throughout the plant was impeded. Clarification between plants which are non hosts to SCMV or plants which are resistant to SCMV depends therefore on their ability to establish a source of primary infection in inoculated leaves and not in the observation and subsequent testing of systemically infected leaves. Trifolium spp. shown to be resistant to systemic infection were therefore retested to determine if sap inoculated leaves were infected with the virus 15 days after sap inoculation. Whether Karridale and other sub. clover cultivars have useful field resistance to SCMV due to the delay in systemic infection during winter months is not tested here.

(i) In air conditioned-glasshouse environment

METHOD:

Sub. clover cultivars and other Trifolium spp. were germinated in UC soil mix, pH 6.0, in peat pots and transplanted into 12.5 cm diameter plastic pots at approximately five weeks after emergence. Rhizobium trifolii WU 95 ("Nodulaid C") was watered on as a peat-inoculum suspension at seedling emergence. Infection with SCMV P23 by sap inoculation was carried out when plants were six weeks old. All fully expanded leaves on each plant were sap inoculated, 10-12 plants per sub. clover cultivar or Trifolium spp. As the plants continued to grow, newly forming leaves were removed to prevent senescence of inoculated leaves and furthermore, easy identification of virus-inoculated leaves. Inoculated leaves from each pot were picked on day 15, grouped together and ground in phosphate buffered saline before testing by ELISA to SCMV.

RESULTS:

Testing of inoculated leaves confirmed that all subterranean clover cultivars are hosts of SCMV (Table 6) and that T. dubium is the only Trifolium spp. behaving as a non host. It is possible, however, that there is resistance to primary infection, evidenced by the group in which not all plants became infected in their inoculated leaves. True plant resistance to SCMV infection within the plant takes the form of resistance to virus systemic-movement and also possibly virus multiplication. Resistance genes are thought to operate in subterranean clover and T. repens, T. pratense and T. balansa. Plants which are readily infected in the inoculated leaves (not the partially infected group) have all produced a limited number of plants which became systemically infected. For example, systemic infection of balansa clover has only occurred in one plant despite numerous attempts to systemically infect plants. T. arvense was found to have no resistance to infection and all plants developed systemic infection.

Table 6. SCMV infection of inoculated leaves of Trifolium spp. and subterranean clover cultivars following sap inoculation

	Inoculated Leaves		
	Non infection	Partial infection	Complete infection
Subterranean clover cultivars		Yarloop (3/9) Larisa (3/10) Trikkala (2/7) Meteora (5/9) Rosedale (9/12) Dwalganup (5/8)	Denmark (9/9) Mt Barker (12/12) Bacchus Marsh (12/12)
Trifolium spp.	T. dubium (0/10)	T. repens (5/10) T. pratense (2/9)	T. arvense (11/11) T. balansa (11/11)

Infection within inoculated leaves was confirmed by testing by ELISA, 15 days after sap inoculation.

The figures in parentheses show the number of plants testing positive to SCMV/total number of plants inoculated.

SCMV Stability in Sap

Relatively stable in plant extracts, infectivity surviving for one month when kept at room temperature (approximately 25°C). However, unable to withstand temperatures in excess of 70°C. for 10 minutes.

4. 89GL17 - The Effect of AMV on Nodulation of Burr Medic

BACKGROUND

Previous experiments demonstrated that AMV infection of burr medic (Medicago polymorpha cv. Circle Valley) interfered with nodule function and resulted in a 25% decrease in herbage dry weight and a 16% decline in the quantity of nitrogen fixed when compared to healthy plants. AMV infected plants were all grown from virus-infected seed and there was no evidence of a reduction in the number of nodules formed but there was a significant decrease (19%, $P < 0.05$) in nodule weight for infected plants. However, relative to plant weight, infected plants produced more nodules than uninfected plants and the nodulation index (nodule mass x shoot mass^{-1} x 100) which provides a useful paramenter for measuring nodulation by compensating for the effect of plant size indicated no significant difference between virus-infected and uninfected plants.

Loss in nodule function without a significant loss in nodulation was considered the major cause of decreased nitrogen fixation but the cause may have resulted from:

(i) depleted photosynthate supply to the nodules;
(ii) lower capacity within the nodule to metabolize current photosynthate;
(iii) a reduction in the assimilation of ammonia in the nodule cytoplasm;
(iv) reduced translocation of N products out of the nodule or;
(v) a lower concentration of bacteroids in nodules of virus-infected plants.

To date, there is no information comparing virus concentrations in the nodules and shoots to determine preferred sites for virus multiplication or whether virus concentration declines in shoots and nodules as plants age. The aims of this experiment were to examine which of these factors limited nitrogen fixation in burr medic nodules infected with AMV.

METHOD:

Experiment

A randomized block of 32 non-draining pots was set up in an air conditioned glasshouse with alternating 12 hour periods at 13°C/21°C. Each pot contained 6 kg of potting soil mixed with basal nutrients and was adjusted to field capacity with deionized water before planting. Twelve burr medic seedlings were planted in each pot, half the pots with virus-free seedlings and half with AMV infected-seedlings. Single leaflets from the first trifoliate leaf from each seedling were tested for AMV by ELISA before thinning plants to five well-spaced plants/pot. Plants were watered to field capacity daily and pot positions were randomized weekly to reduce environmental variations.

The experimental design included two treatments (infected plants raised from AMV infected-seed and healthy plants) and two harvest dates with eight replications of each treatment. Plants were harvested on days 53 and 75 following germination.

Data Collection

The following parameters were measured; nodule number, bacteroid concentration, dry weights of nodules, roots and shoots, shoot and nodule nitrogen content, shoot and nodule virus concentration, nodule amino acid content, nodule soluble sugar and carbohydrate content and the specific activity of nitrogenase.

RESULTS:

Virus Concentration

Absolute values for virus concentration were not estimated but comparisons of the absorbance values for shoots and nodules tested by ELISA were compared. There was a significant difference between the concentration in nodule tissue and the youngest trifoliate leaf in plants from both harvests (Table 7), with the nodules of virus-infected plants containing 42% more virus antigen than the corresponding leaf tissue on the same plant. The level of AMV detectable in nodules and shoots fell between harvests but was only significant at the 10% level.

Table 7. Detection of AMV in shoots and nodules of M. polymorpha cv. Circle Valley by means of the ELISA assay

Summary of means* - Absorbance (405 nm)**

1. Detection of changing virus concentration in plant parts at harvest 1 and 2

	Harvest 1		Harvest 2
Mean	464		413
Significance		P = 0.096	

2. Detection of changing virus concentration between plant parts for both harvests

	Nodules		Shoots
Harvest 1 means	571		358
Harvest 2 means	538		289
Combined means	554		323
Significance		P < 0.001	
LSD		56	
% Change		42	

* Sample means; nodule and shoot are the sum of five plants with 7 replicates.

** Absorbance values are the means of samples diluted 10^{-3} and replicated twice on microtitre plates.

Nodulation

No differences were detected between virus-infected and healthy plants in the number, positioning or timing of nodules developing on newly germinated seedlings in the first 11 days after inoculating with rhizobia. Plants thinned from pots on day 33 when the fifth trifoliate leaf had formed showed no significant difference in numbers of nodules formed on virus-infected and healthy plants, either. This indicated that virus infection was not interfering with the formation of nodules in the early stages of growth for burr medic, before nitrogen fixation by the plant was fully operational.

Nodule numbers per plant were not significantly different between healthy and AMV-infected plants at either harvest 1 or harvest 2 (Table 8). A significant decrease (24%) in nodule weight was measured in virus-infected plants at the first harvest but not the second harvest. The nodulation index which corrects for differences in shoot weight was the same for infected and uninfected plants at the first harvest, but by the second harvest had increased in AMV-infected plants. This indicated that AMV-infected plants had produced more nodules than healthy plants of comparable size.

Table 8. The effect of AMV infection on productivity of burr medic cv. Circle Valley

	Number of nodules	Nodule dry wt (mg)	Shoot dry wt (g)	Nodulation index
Harvest 1				
Mean I	328	164	2.88	5.76
Mean H	276	215	3.65	5.83
LSD			43	0.51
Significance	ns	*	**	ns
% Reduction		23.7	21.2	
Harvest 2				
Mean I	914	503	12.4	4.07
Mean H	957	554	15.8	3.52
LSD			1.82	0.49
Significance	ns	ns	***	*
% Reduction			21.7	

I Seed-infected plants.
H healthy plants.

Means are calculated from the sum of five plants per pot with eight replications.

Plant Growth

AMV infection of burr medic reduced the overall growth of plants by approximately 20% for both harvest. However, the loss in nodule dry weights was only significant at the first harvest (23%) and by the second harvest root growth was depressed in virus-infected plants by a similar amount (23%). This indicated that virus-infected plants, as they continued to grow, were deploying plant reserves into nodulation at the expense of root development.

Nitrogenase Activity

The specific activity of nitrogenase was measured for nodules from the first harvest only. In AMV-infected plants the reduction in nitrogenase activity was 25% as measured by the acetylene reduction assay.

Nitrogen Content

Corresponding to the reduced N_2-fixing capacity of virus-infected plants the total nitrogen content in shoots was 24% lower at the first harvest and 15% lower at the second. The improvement in nitrogen concentration in virus-infected plants at the second harvest corresponds to increased nodulation on these virus-infected plants.

The soluble amino acid concentration in nodules was 20% less in virus-infected plants when compared to healthy plants. The bacteroid, soluble sugar and polysaccharide concentrations in nodules were not significantly different between healthy and AMV-infected plants or between harvests. These results

indicate that nodules from virus-infected plants had similar numbers of resident rhizobia to those in healthy plants and that carbon utilization was not different. However the influx rate of photosynthate from shoot to the nodule was not measured and it is possible that in virus-infected plants the translocation rate may be different. The disease response to AMV in burr medic is mild however, and plants develop only a faint mosaic in leaves. In contrast, many of the other virus diseases of legumes produce severe mottles and mosaics in leaves which results in severe disruption to chloroplast structure with corresponding limitations to carbon metabolism. The fact that nodule formation in burr medic infected with AMV is not impaired suggests that carbon metabolism may not be the limiting factor to plant growth and nitrogen fixation.

The exact nature whereby virus infection limits nitrogenase activity in the nodule cytosol was not determined in this experiment. However, nodules are sites of virus multiplication, requiring energy and nitrogenous products for virus protein and nucleic acid synthesis. Measurement of the proportion of cellular nitrogen sequestering into virus particles would indicate the size of this additional sink within nodules of virus-infected plants and give an idea whether the requirement for photosynthate for virus synthesis was significant and might impact on the supply available for N_2-fixation.

CONCLUSION:

Three major conclusions resulted from this study.

1. Growth of burr medic infected with AMV is limited by nitrogen fixation which results from loss in nodule function and not diminished nodule formation.

2. Increased nodule formation in virus-infected plants relative to plant weight at the second harvest indicated plants were able to compensate to some degree for limitations to nitrogen availability.

3. Nodules are sites of active virus multiplication (42% greater concentrations than in shoots) and the concentration of virus persists during the vegetative stage of legume growth when nodules are actively fixing nitrogen.

PUBLICATIONS:

1. Thesis: The effect of alfalfa mosaic virus on the formation and function for burr medic. Murdoch University.

2. Farmnote: Virus diseases of subterranean clover. In press.