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Impaired nodule function in *Medicago polymorpha* L. infected with alfalfa mosaic virus

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SUMMARY

The effects of alfalfa mosaic virus (AMV) on growth, nodule formation and nodule function in the annual burr medic, *Medicago polymorpha* cv. Circle Valley, were investigated in glasshouse pot experiments. Systemically-infected plants from AMV-infected seed produced 21% less shoot dry weight and accumulated 24% less fixed nitrogen in shoots than healthy plants when harvested 53 d after germination. At day 75, infected plants showed similar shoot dry weight losses (22%), but the quantity of nitrogen fixed fell by only 15%. At day 53, soluble sugar, starch and bacteroid concentrations in nodules were unaffected by AMV infection, but nitrogenase specific activity was decreased by 25% and soluble amino acids by 20%.

Although AMV infection resulted in no differences in the number of nodules formed in the first 11 d after germination or at any harvest thereafter, nodule mass was decreased by 23% for virus-infected plants at day 53. However this difference disappeared by day 75. Growth of AMV-infected plants was decreased probably because of impaired N_2 fixation with nodule function affected rather than nodulation. Increased nodule mass relative to plant weight in virus-infected plants, seen at day 75, implied some degree of compensation for the limitation in N_2-fixing capacity. ELISAs for AMV antigen indicated that nodules were active sites of virus multiplication.

Key words: Alfalfa mosaic virus, nodulation, nodule function, annual medic, *Medicago polymorpha*.

INTRODUCTION

Legumes improve pasture quality by contributing nitrogen for animal protein and increase the availability of soil nitrogen both for pasture grasses and subsequent cereal crops grown in the ley farming systems commonly used in Australia (Puckridge & French, 1983). A natural question in the study of virus diseases of annual pasture legumes has therefore been whether viruses interfere with nodulation and the N_2-fixing capacity of infected plants. Many studies using a variety of virus/legume combinations have reported that infected plants produced fewer nodules (Tu, Ford & Grau 1970; Guy, Gibbs & Harrower, 1980; Orellana, Weber & Cregan, 1980; Gibson *et al.*, 1981; Orellana *et al.*, 1983; Khadhair, Sinha & Peterson, 1984; Ohki, Leps & Hiruki, 1986; Wongkaew & Peterson, 1986; Rao & Shukla, 1988; Dall, Randles & Francki, 1989), although in one study increased nodulation occurred (Rajagopalan & Raju, 1972). In studies to date, *Rhizobium* spp. have been introduced into the root rhizosphere prior to inoculation of fully-expanded cotyledons or leaves with virus. However, because seeds harvested from infected plants of burr medic (*Medicago polymorpha* L.) can transmit alfalfa mosaic virus (AMV) to seedlings at rates greater than 60% (Jones & Pathipanawat, 1989), this system is ideal for examining which stages in nodule formation are most affected by virus infection.

Many reports refer to leaf disorders described as mosaics and mottles, indicating that virus infection disrupts chlorophyll formation (Diener, 1963; Tu, Ford & Krass, 1968; Brakke *et al.*, 1988). In squash plants infected with squash mosaic virus, Magyarosy, Buchanan & Schurmann (1973) reported that chloroplast structure and function were not altered, but that fewer chloroplasts were formed indicating that less photosynthate would be available to the

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plant. Decreased photosynthate has been suggested as a likely mechanism whereby virus infection might limit nodule N_2 fixation (Khadhair *et al.*, 1984). Moreover, if nodules are also sites of rapid virus multiplication, this would introduce an additional sink for plant resources of photosynthate and nitrogen and further impair nodule function. Different isolates of AMV produce symptoms in leaves of *Medicago* spp. ranging from no apparent disease to distinct mottling and, in some instances, leaf deformation and dwarfing (Miczynski & Hiruki, 1987; Hajimorad & Francki, 1988; Jones & Pathipanawat, 1989). It may therefore be possible, using an isolate of AMV that produces mild leaf disease, to separate direct effects of virus infection on nitrogen metabolism from indirect effects caused by disrupted carbon metabolism.

MATERIALS AND METHODS

General procedures

Soil and nutrients. Two experiments were done in an air-conditioned glasshouse with alternating 12 h dark/light periods at 13/21 °C. Pots containing 6 kg sterile coarse river-sand/yellow sand mixed in the ratio 3:1 (pH 6·7, sand 1:5 in water) were used throughout. In expt 1, basal nutrients were applied to the sand in draining pots as a watering nutrient solution (Broughton & Dilworth, 1971). In expt 2, non draining pots were used and basal nutrients were applied to the sand surface at the following rates (mg kg^{-1} soil): KH_2PO_4 (200), K_2SO_4 (160), $CaCl_2.2H_2O$ (71·6), $MgSO_4.7H_2O$ (26·8), $MnSO_4.H_2O$ (10), $ZnSO_4.7H_2O$ (6·6), $CuSO_4.5H_2O$ (2·4), H_3BO_3 (0·8), $CoSO_4.7H_2O$ (0·4), $Na_2MoO_4.2H_2O$ (0·2). Nutrients were allowed to dry and then mixed thoroughly with the sand. Pots were brought to 80 % field capacity (10 % v/w) with deionized water prior to planting and then maintained at field capacity during the experiment. To decrease environmental variation, pot positions in the glasshouse were randomized weekly.

Seed. Certified seed of *M. polymorpha* cv. Circle Valley was supplied from the Western Australian Department of Agriculture. The seed was germinated and cotyledon tissue tested for the presence of seed-borne virus infection with cucumber mosaic virus, subterranean clover mottle virus and AMV by ELISA with the appropriate antisera (Jones & Pathipanawat, 1989; Jones & McKirdy, 1990; Wroth & Jones, 1992); all three viruses are present in Australian pastures and are seed-borne. No virus was detected and this seed then became the source for healthy burr medic treatments in experiments. AMV-infected seed came from four burr medic plants that were infected with the AMV isolate EW (Jones & Pathipanawat, 1989) via aphid transmission. This seed was capable of transmitting virus to seedlings at a rate of 64 %. Separate samples of healthy and AMV-infected seeds were scarified, surface-sterilized with acidified (0·25 % v/v HCl) 0·1 % (w/v) mercuric chloride solution and rinsed five times in sterile water before imbibition in the final rinse overnight (18 h) at room temperature. Seeds were then germinated on inverted 1 % (w/v) water agar plates at room temperature for 48 h. Only seedlings with emerging radicles measuring 5–10 mm were planted.

Rhizobium *inoculum. Rhizobium meliloti* WSM 540, an acid-tolerant strain used commercially in Western Australia for nodulating burr medics, was supplied by J. G. Howieson (Western Australian Department of Agriculture). The strain was grown in minimal salts broth which contained (mM) galactose (10), arabinose (10), sodium glutamate (10), $MgSO_4$ (2), $CaCl_2$ (1), $NaSO_4$ (1), KH_2PO_4 (0·15), K_2HPO_4 (0·15), $MnSO_4$ (0·03), ferric citrate (0·02), Na_2MoO_4 (0·004), $ZnSO_4$ (0·0035), $CuSO_4$ (0·02) and $CoSO_4$ (20 nM). Included also were the vitamins (nM): pantothenic acid (4), thiamine (3), biotin (0·1); 2-(*N*-morpholino)ethanesulphonic acid (20 mM) buffered the pH at 7·0. At planting, each seedling was inoculated with 0·5 ml of a dense suspension of bacteria (approximately 6×10^7 bacteria per root), the soil firmed and the pots brought up to field capacity.

Laboratory procedures

Detection of AMV. The presence of AMV antigen was detected by enzyme-linked immunosorbent assay (ELISA) using the method of Clark & Adams (1977). Polyclonal AMV antiserum was donated by the late R. I. B. Francki, Waite Agricultural Research Institute, University of Adelaide, South Australia. Immunoglobulin preparations made from this antiserum were used at a dilution of 1/600 for the coating and conjugation stages of the ELISA procedure. Seed cotyledons and leaflets were ground, using a sap-extractor machine (Pollahne leaf press, Elektrowerk Hannover, Behncke & Co, Germany), in 1 ml of an extraction buffer (Jones & Pathipanawat, 1989). The extracts were frozen for several days before thawing and testing by ELISA. Absorbance at 405 nm was measured on a Titertek Multiscan photometer (Flow Laboratories, Finland). Absorbance values three times in excess of values recorded for healthy leaf material were considered positive for AMV antigen. Absolute values for virus concentrations were not estimated. However, by diluting plant samples into the linear range of concentrations detectable by ELISA, the absorbance values between different virus-infected tissues could be compared on the assumption that higher absorbances reflect higher virus titres.

Bacteroid isolation and assay. Bacteroids were extracted from weighed fresh nodule tissue taken from five seedlings (Dilworth & Bisseling, 1984). Total bac-

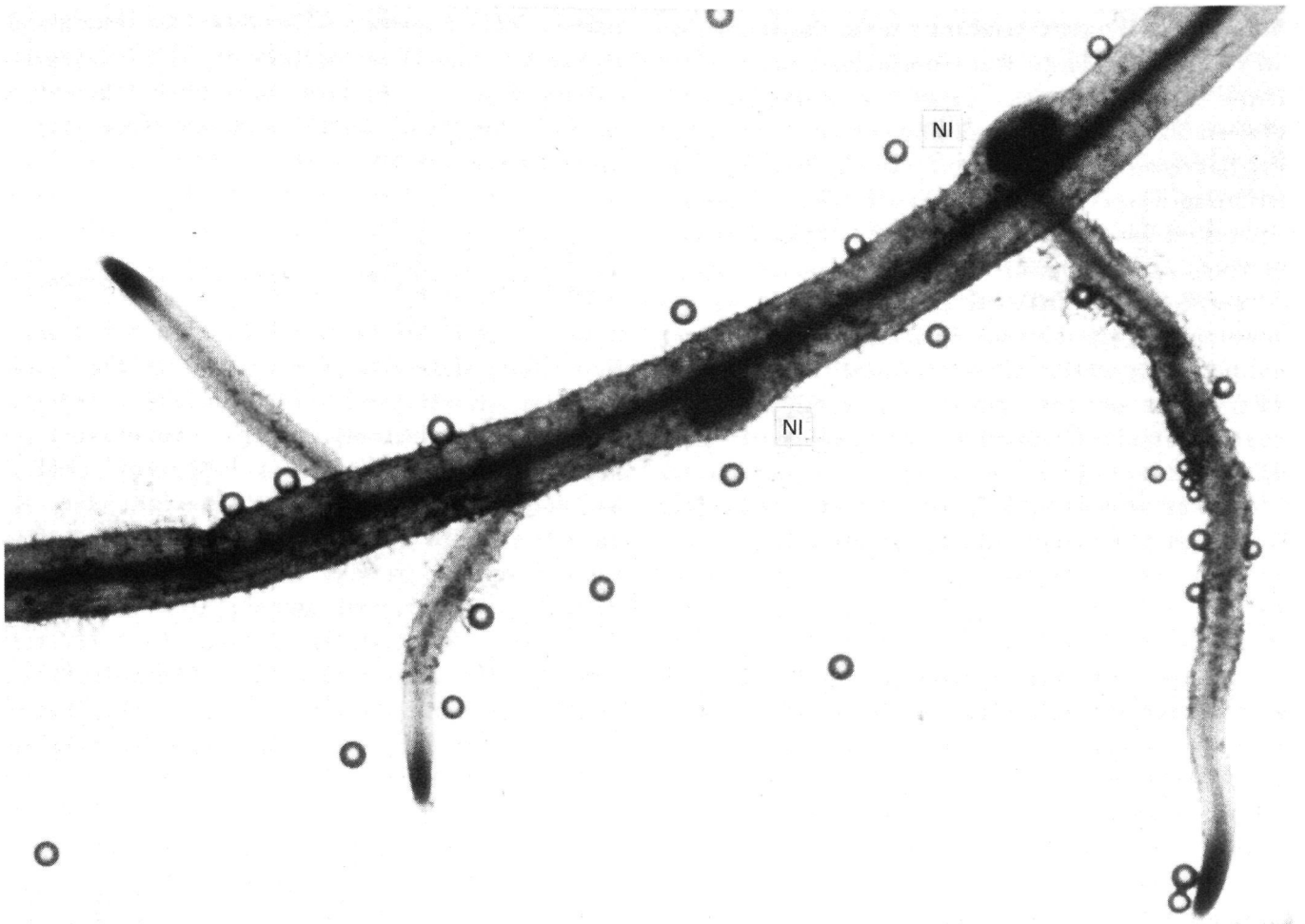

Figure 1. Emerged lateral roots and nodule initials (NI) on a 9-d-old seedling root of *Medicago polymorpha* cv. Circle Valley, grown from an AMV-infected seed.

teroid counts were made by microscopic examination at 400 × magnification using a standard cell counting chamber, 0·01 mm deep.

Nitrogenase activity. Nitrogenase activity in nodules was measured by the acetylene reduction assay (Trinick, Dilworth & Grounds, 1976). Pots containing plants for analysis were not watered on the day prior to harvest in order to assist in the removal of sand during harvest without the need for washing roots, a procedure that can depress measured nitrogenase activity. Plants were decapitated 1·0 cm above the soil level so that roots would fit into the gas assay jars. Gas samples (0·5 ml) were analyzed on a Pye Unicam, GCD Gas Chromatograph, using a column (1·6 m × 2·5 mm diam.) of Poropak T with N_2 as the carrier gas (20–30 ml min^{-1}) and a flame ionization detector.

Nitrogen analysis. Plant samples were dried at 70 °C for 48 h, weighed and then digested using a Kjeldahl procedure (McKenzie & Wallace, 1954). The nitrogen, converted to ammonia, was then measured using the indophenol method described by Isaac & Johnson (1976).

Amino acid analysis. The amino acid content of nodules was measured with ninhydrin (Spiers, 1957).

Carbohydrate and soluble sugar analysis. The polysaccharide and sugar contents of nodules were assayed using an anthrone method modified by C. A. Pierce (personal communication). Sugars were extracted with 25 ml of boiling 80 % (v/v) ethanol from ground nodule tissue (0·2 g approx.). Samples were centrifuged at 1861 ***g*** for 15 min and the supernatant decanted. Extraction was repeated and pooled supernatants were clarified by adding 0·2 ml of 1·2 M lead acetate and shaking for 1 h. Excess lead acetate was precipitated with 1·0 ml of 0·24 M sodium oxalate. Samples were then made up to a standard volume. Polysaccharides were extracted from the pellet remaining after sugar extraction by solubilizing them with 11·5 ml of perchloric acid (29 % v/v). Samples were vortexed for 5 min and then stirred occasionally while standing for 15 min at 25 °C.

Dissolved polysaccharide and sugar samples were centrifuged and 1·0 ml samples of the supernatant were mixed with 10·0 ml of 0·1 % (w/v) anthrone in 75 % (v/v) H_2SO_4. Samples were stoppered, heated for 12 min in a boiling water bath, cooled and their absorbance at 625 nm was related to that of a series of freshly made-up glucose standards.

Experiment 1: *Effects of AMV on early nodulation*

Eight pots to be harvested daily were watered and the surface soil scored with furrows 5 cm apart and

1·5 cm deep. Twenty seedlings from the seed from AMV-infected plants were positioned in the first furrow and ten healthy seedlings in the second. Between 3 and 11 d later, all plants from a single pot were harvested and each plant was numbered before sectioning into cotyledons and root. The cotyledons were tested for the presence of AMV by ELISA and the roots were cleared and stained using chloral hydrate/Nile Blue (Riley & Dilworth, 1985). Meristematic regions at the root tip and in the root cortex (nodule and lateral root initials) stained a deeper blue and for each root the number and position of these were recorded. Distinction between lateral root initials and nodules was only clear by day 7. On day 11, nodules from five AMV-infected and five healthy plants were harvested and their bacteroids counted.

Experiment 2: Effects of AMV on nodule function

The design was a randomized block of 32 pots, with two treatments (healthy plants and plants infected via AMV-infected seed) and two harvest dates (53 and 75 d after germination). Each treatment/harvest date was replicated eight times. Twelve burr medic seedlings were planted in each pot. Single leaflets from the first trifoliate leaf from each seedling were tested for AMV by ELISA before thinning to give five well-spaced plants per pot with the required treatment (healthy/infected) category. The following parameters were measured: nodule number; bacteroid concentration; dry weights of nodules, roots and shoots; nitrogen content in shoots and nodules; relative shoot and nodule virus concentration; nodule amino acid content; nodule sugar and carbohydrate content and the specific activity of nitrogenase. In order to remove the effect of plant size, an index of nodulation (100 × nodule mass/shoot mass) was calculated using the formula of Betts & Herridge (1987). Treatment means were compared using ANOVA analysis. The data set was incomplete in harvest one for nitrogenase activity (one missing value), whilst in harvest two, one virus-infected pot was discarded due to infestation with sciarid fly larvae (Cecidomyiidae).

RESULTS AND DISCUSSION

Experiment 1: Effects of AMV on early nodulation

The use of AMV-infected seed rather than sap inoculation of healthy plants overcame the problem of variable numbers of plants becoming infected as a result of low infectivity and irregular movement of AMV within the plant (Kuhn & Bancroft, 1961; Tu & Tse, 1976). Four days after rhizobia were inoculated onto germinated seedlings, some roots were beginning to show a few areas (0–3) of cell division on the root cortex, the first sign of developing root nodules. By day 5, meristematic zones were clearly visible in the root cortex, forming in a 0·03 m length of the root, from the region of root hairs to a section just before root hairs develop

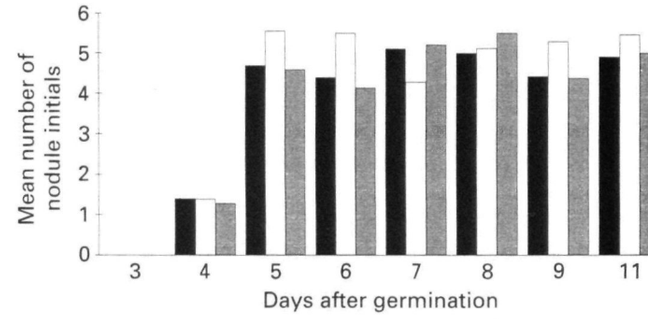

Figure 2. Comparison of time course of nodulation in seedlings grown from seed from healthy and AMV-infected plants of *Medicago polymorpha* cv. Circle Valley. ■, seed from healthy plants (tested AMV −ve by ELISA); □, seed from AMV-infected plants (tested AMV+ve by ELISA); ▨, seed from AMV-infected plants (tested AMV −ve by ELISA).

Table 1. *The effect of AMV infection on productivity of burr medic cv. Circle Valley*

		Number of nodules	Nodules (mg)	Dry weight (g) Shoots	Dry weight (g) Roots	Total plant (g)	Nodulation index
Harvest 1 (day 53)	Mean I	328	164	2·88	0·90	3·94	5·76
	Mean H	276	215	3·65	1·07	4·93	5·83
	LSD ($P = 0·05$)		43	0·51		0·87	
	Significance	n.s.	*	**	n.s.	*	n.s.
	% Reduction		23·7	21·2		20·2	
Harvest 2 (day 75)	Mean I	914	503	12·4	2·69	15·6	4·07
	Mean H	957	554	15·8	3·49	19·8	3·52
	LSD ($P = 0·05$)			1·82	0·38	2·15	0·49
	Significance	n.s.	n.s.	***	***	***	*
	% Reduction			21·7	22·5	21·6	

I, seed-infected plants; H, healthy plants.
Means are calculated from the mean of five plants over eight replicates.
Nodulation index = (nodule mass)/(shoot mass) × 100.

towards the root tip end. Although nodule initials were generally elliptical in shape, unlike lateral root initials which were more conical, no clear distinction between them could be made until day 7. Furthermore, a number of the meristematic zones were almost certainly sites from which both a nodule and a lateral root formed (Fig. 1). On day 11, the average number of nodules per plant was five and these had developed a faint pink tinge. No difference was detected between healthy and virus-infected plants in the number, positioning or timing of nodule development (Fig. 2). Mean bacteroid numbers per gram wet weight of nodule tissue, scored from plants at day 11, also failed to demonstrate any differences between nodules on healthy ($8{\cdot}3 \times 10^8$) and virus-infected ($8{\cdot}9 \times 10^8$) plants.

Experiment 2: Effects of AMV on nodule function

Disease development and plant growth. Although virus infection was confirmed by ELISA in the first trifoliate leaf of all plants in the AMV-infected treatments, the first visible symptoms of AMV infection to appear on seed infected plants occurred in the fourth trifoliate leaf and were quite distinct in the fifth and subsequent trifoliate leaves. Symptoms were a slight rugosity to the surface and a mild mottle. No leaf deformation was observed but after several weeks infected plants were slightly smaller. AMV infection of burr medic decreased the growth of whole plants by 20 % at day 53 and by 22 % at day 75 (Table 1). At day 53, the dry weight for shoots and for nodules was decreased by 21 and 23 %, respectively, though there was no decrease for root dry weight. At day 75, the loss in dry weight for shoots (22 %) had not changed; in contrast to harvest 1, the dry weight of roots was now lowered by 23 % and there was no decrease in the dry weight of nodules. Although pot experiments have shown greater herbage dry weight losses due to AMV for burr medic (32–36 %, Jones & Pathipaniwat, 1989), barrel medic (50–60 %, Dall *et al.*, 1989) and lucerne (31 %, Ohki *et al.*, 1986), the losses from seed infection of burr medic (22 %) in these experiments were still appreciable.

Virus concentrations. There were significant differences between the virus concentrations in nodule tissue and in the youngest open trifoliate leaf in plants from both harvests (Table 2), 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 appeared to decline between harvests but this fall was only significant at the 10 % level. Virus has previously been detected in nodules by infectivity assays for soybean (soybean mosaic virus (SMV), Tu, 1973), red clover (white clover mosaic virus, Khadhair *et al.*, 1984), peanut (peanut mottle virus, Wongkaew & Peterson, 1986) and *Phaseolus vulgaris* (bean yellow mosaic virus, Orellana & Fan, 1978) and by ELISA for alfalfa (AMV, Ohki *et al.*, 1986), *Glycine max* and *G. soja* (SMV, Orellana *et al.*, 1983). The relatively constant and high concentrations of AMV antigen found by ELISA for the youngest open leaf (YOL) and nodules of burr medic (Table 2) are in contrast to the decline with age in maturing leaves (Kuhn & Bancroft, 1961). However, the mean absorbance values for ELISA assays for SMV in leaf extracts of *G. max* and *G. soja* at the pod-fill stage were two to three times greater than in the corresponding nodule extract. Such single time assays may not reveal the whole situation; in peanuts at the early bloom stage and 20 d after inoculation with peanut mottle virus, leaves had higher levels of infective virus than nodules, but the levels in nodules rose during flowering while those in roots and leaves declined (Wongkaew & Peterson, 1986). As leaves develop from being net importers of both C and N during growth to becoming exporters of C and recycling their N at maturity, their capacity to support virus replication may well decline. On the other hand nodules, which at least until peak flowering are sinks for C and generally high in available N, may continue to support high levels of virus production.

Nodulation. Thirty seedlings at the fifth-trifoliate-leaf stage, thinned from pots on day 33, were examined for nodulation; the average number of crown nodules on each plant was 4·9. There were no differences in numbers of nodules formed on healthy plants and those on the 14 plants that tested positive to AMV by ELISA. Later, at day 53 and day 75, there were again no significant differences in nodule numbers between healthy and AMV-infected plants (Table 1). Apart from a few senescing crown nodules by day 75, nodules were in good condition and pink internally.

Although nodule sizes were not visibly different, nodule weight per plant at day 53 (but not day 75), was significantly less (24 %) for virus-infected plants. Mean nodule weights were 0·50 mg and 0·77 mg for virus-infected and healthy plants, respectively. By day 75, when the numbers of nodules on plants had trebled, differences in mean nodule weight were gone and the average nodule weight was 0·55 mg for both treatments, demonstrating that smaller nodules form on healthy plants at later stages of growth. Infection with AMV therefore had no apparent effect on the early events in nodulation as measured by the number of nodule initials formed, even though the actual weight of nodules was decreased at day 53. In fact, relative to plant weight, infected plants at day 75 had more nodules than uninfected plants, as indicated by the higher nodulation index (Table 1).

Nodule function. The specific activity of nitrogenase was measured for nodules at day 53 only. In virus-

Table 2. *Detection of AMV in shoots and nodules of seed-infected* Medicago polymorpha *cv. Circle Valley by enzyme-linked immunosorbent assay (ELISA)*

	Summary of means – absorbance* (405 nm)	
1. Virus concentration in nodules and shoots at harvest 1 (day 53) and 2 (day 75)		
	Harvest 1	Harvest 2
Mean†	464	413
Significance	$P = 0{\cdot}096$	
2. Virus concentration in different plant parts for both harvests		
	Nodules	Shoots
Harvest 1 means	571	358
Harvest 2 means	538	289
Combined means	554	323
Significance	$P < 0{\cdot}001$	

* Absorbance values were the means of samples diluted 10^{-3} and replicated twice on microtitre plates. The values were corrected for the absorbance of virus-free controls. Data were analyzed by ANOVA using a split plot analysis where the main treatment was harvest time and the sub-treatment was plant parts.

† Sample means; nodule and shoot are the mean of 5 plants over 7 replicates.

infected plants, there was a significant decrease (25 %) in the activity of nitrogenase measured as C_2H_2 reduction (Table 3). The nitrogen content in shoots was 24 % lower at day 53 and 15 % lower at day 75 (Table 3). Nitrogen contents for nodules at day 53 are unavailable because of small sample weights, but at day 75 the nitrogen content was 17 % less for virus-infected plants (Table 3). Differences in amino acid concentration between virus-infected (58·2 mM) and healthy (72·4 mM) nodules were significant at day 53 only (Table 3), representing a 20 % decrease in the soluble amino acid pool. Bacteroid numbers per gram wet weight of nodule tissue from both harvests did not differ significantly (range 3·93–4·02 × 10^9 cells), implying that any defect in nodule function was not due to a failure in bacteroid multiplication. Impairment of nodule function at day 53 was indicated by decreases in both the specific nitrogenase activity and in shoot and nodule N content.

In contrast, Dall *et al.* (1989) showed nodulation losses of 31–67 % for three cultivars of barrel medic. Since no significant differences in nitrogenase activity (measured as acetylene reduction) were found between virus-infected and healthy plants, they considered these to be the main cause of decreased N_2 fixation. Differences in nodulation between the two studies may be due to our growing burr medic in steam-treated sand, whereas the barrel medics were grown in an untreated soil mix. Field beans infected with *Dolichos* enation mosaic virus (DEMV) and given the same rhizobial inoculum produced less nodules when grown in untreated soil than in sterile sand (Rajagopalan & Raju, 1972). The nodules on DEMV-infected plants were larger, as also found for soybeans growing in a sand-peat-loam soil mixture and infected with SMV and bean pod mottle virus (Tu, Ford & Quiniones, 1970). Tu & Tse (1976) found that greater numbers of rhizobia were required in order to produce similar numbers of nodules on soybeans infected with AMV to those on healthy plants. While this may explain the nodulation losses of Dall *et al.* (1989), the high inoculum dose used in our experiment may have overcome such an effect.

There was no significant difference in the soluble sugar (2·2 %) or carbohydrate (1·4–1·5 %) concentration between nodules of virus-infected or healthy plants. The high variability in the values for the soluble sugars in nodules at day 53 may indicate

Table 3. *The effect of infection with AMV on nitrogen (content and concentration) in burr medic cv. Circle Valley*

		Nitrogen shoots (%)	Total nitrogen shoots (mg)	Nitrogen nodules (%)	Total nitrogen nodules (mg)	Amino acid concentration in nodules (mM)	Acetylene reduction rate†
Harvest 1 (day 53)	Mean I	4·32	122			58·2	161
	Mean H	4·32	158			72·4	215
	LSD ($P = 0{\cdot}05$)		21			11·4	8·5
	Significance	n.s.	***			*	***
	% Reduction		23·7			19·6	25·1
Harvest 2 (day 75)	Mean I	4·13	510	6·99	35·2	44·8	
	Mean H	3·79	597	7·65	42·3	55·3	
	LSD ($P = 0{\cdot}05$)	0·16	78	0·59	7·2		
	Significance	***	*	*	*	n.s.	
	% Reduction	8·1	14·6	8·5	16·7	—	

I, seed-infected plants; H, healthy plants.
Means are calculated from the mean of five plants over eight replicates.
† Specific activity for nitrogenase = nmol $C_2H_4 min^{-1} g^{-1}$ nodule tissue.

further metabolism between picking and processing. Values at day 75 were less variable, the carbohydrate concentrations (1·3–1·4 %) were less, but again not significantly different. Though the flux of photosynthate from shoots to nodules was not measured in our study, the similar concentrations of sugars and starch in AMV-infected and healthy nodules suggest that carbohydrate metabolism in nodules remained normal despite virus-infection.

The lower rates of acetylene reduction in AMV-infected plants at day 53 could be one factor leading to decreased nodule growth. A second may well be the higher rate of production of virus particles in nodule tissues compared to other plant parts; both may result in lower plant and nodule weight gain due to AMV infection, particularly as in their early development many legumes suffer a period of nitrogen starvation before nodule N_2 fixation meets the demand for N.

When N_2 fixation in cowpeas was prevented by altering the N_2/O_2 atmosphere to Ar/O_2, shoot growth was arrested; on restoring N_2/O_2 the plants responded by forming more nodules than untreated plants (Pate *et al.*, 1984). A similar compensation operates in cobalt deficient lupins (Robson, Dilworth & Chatel, 1979). AMV infection of burr medic also seems to elicit a nodulation response, with a higher nodulation index at day 75 for infected plants than for uninfected plants. Such an ability of virus-infected legumes to compensate for losses in nodule function can also be seen in the results of Rajagopalan & Raju (1972) with DEMV-infected *Dolichos lablab*.

The increase in nodulation index for burr medic at day 75 is accompanied by a decrease in root weight, as it was for DEMV-infected field beans (Rajagopalan & Raju, 1972), perhaps implying a reallocation of photosynthate by the infected plant. The increased nodulation in AMV-infected burr medic was nevertheless ineffective in restoring shoot weight (Table 1), though it did improve the relative N-content. The fall in N concentration (% N) that occurred in healthy plants between day 53 and day 75 (and which probably occurs because legumes appear to use fixed N most efficiently for late vegetative stages of growth; Pate & Herridge, 1978), was much less evident in AMV-infected ones (Table 3). Possible explanations could be (i) that the virus content of leaves represented a significant part of shoot N, or (ii) that photosynthate supply for plant growth was limited by energy consumed in AMV multiplication or lowered rates of translocation.

Because early nodulation and bacteroid concentration were similar in both virus-infected and healthy plants, loss in nodule function may follow alterations in nodule metabolism associated with virus multiplication. The consequences for shoot growth of such a loss in nodule function would then be overcome by adding fertilizer N as Ohki *et al.* (1986) found for AMV-infected lucerne.

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