THE EFFECT OF NITRATE CONCENTRATION ON THE GROWTH AND NODULATION OF SEVERAL ANNUAL SPECIES OF *MEDICAGO*

M.A. Ewing

Agriculture Western Australia, Baron-Hay Court, South Perth, WA 6151

Summary. Nodulation of legume seedling is generally inhibited by the presence of nitrate. This is likely to be important in annual species of *Medicago* (medics) when they are grow under conditions where nodulation potential is already low, perhaps as the result of an acidic soil. Two experiments where conducted in dilute nutrient solution to evaluate the seedling response of representative cultivars/accessions of commercially important medic species to the presence of nitrate at a range of levels of pH. The experiments confirmed the inhibitory effect of nitrate on medic nodulation. At all levels of nitrate the general order of species nodulation was *M. murex* > *M. sphaerocarpos* = *M. polymorpha* > *M. truncatula*. An evaluation of the response of *M. murex* and *M. sphaerocarpos* to nitrate at low pH indicated substantial intraspecific variation. The techniques used in these experiments could provide a useful basis for a screening procedure to be used in plant improvement programs, especially where ability to nodulate is likely to be a constraint, such as on acidic soils.

INTRODUCTION

Nitrate inhibits the nodulation and nitrogen fixation of many legumes including *Medicago sativa* L. (6) and *M. truncatula* Gaertn. (3). However, legumes vary in their response to nitrate and there is some limited evidence of interspecific variation in the early nodulation response of annual species of *Medicago* (medics) to nitrate (2).

Capacity of medics to nodulate despite the presence of high concentrations of soil nitrate is likely to be important in maximising the quantity of nitrogen fixed by medics for use by subsequent crops. It is also likely to be important for medics growing on light textured and mildly acidic soils. In regions with a mediterranean type climate it is likely that, under such conditions, soil nitrate concentration will be high at the time of the germinating rains in late autumn (7). However, nitrate concentration generally falls rapidly in winter, being depleted by leaching and uptake by plants (5). A medic whose nodulation has been inhibited by a combination of acidity and the presence of nitrate would be poorly placed to compete under such circumstances.

The experiments described were undertaken to quantify the relative response of species and cultivar/accessions of medic to early nodulation inhibition caused by the presence of nitrate. A second objective was to ascertain the conditions which best defined differences in response to nodulation stress with a view to identifying a suitable procedure for a mass screening program.

MATERIALS AND METHODS

In two experiments, medic seedlings were grown in dilute nutrient solution in a glasshouse during winter with solutions maintained at 20^{O}C. In experiment 1, the treatments were the factorial combination of three pH levels (5.5, 6.0 and 6.5) and three concentrations of nitrate (nil, 0.1 mM and 1.0 mM). Four medic species represented by 5 cultivars/accessions (*M. truncatula*, cv. Cyprus; *M. polymorpha* L., cv. Santiago; *M. sphaerocarpos* Bertol. cv. N5655; *M. murex* Willd. cvv. N3172 and 87GRC50) were exposed to nutrient treatments and *R. meliloti* (WSM419) in common 5L containers and replicated 3 times, making a total of 27 pots.

In experiment 2, the treatments were the factorial combination of two pH levels (5.5 and 6.0) and two concentrations of nitrate (0.1 mM and 1.0 mM). Three species of medic represented by 12 cultivars/accessions (*M. polymorpha*, cv. Santiago; *M. sphaerocarpos*, cvv. N5655, 87GRC69, Orion, SEP26.2.7, GRC5659.1.1; *M. murex*, cvv. Zodiac, N3172, 87GRC50, 87FB2.36, 87FOI.28, 87GRC87.1) were exposed to nutrient treatments and *R. meliloti* (WSM540) in common 10 L containers and replicated three times, making a total of 12 pots.

For both experiments, medic seeds of the experimental cultivars/accessions were surface sterilised and then germinated and grown for 7 days on a fibreglass mesh suspended at the surface of a 5 L bucket of nutrient solution. The nutrient solution contained the following; 20 µM KH_2PO_4, 1 mM K_2SO_4, 0.5 mM $CaSO_4.2H_2O$, 0.2 mM $MgSO_4.7H_2O$, 5 µM Fe Sequestrene, 3 µM H_3BO_3, 0.03 µM $NaMoO_4.2H_2O$, 0.75 µM $ZnSO_4.7H_2O$, 0.1 µM $MnSO_4.H_2O$, 0.2 µM $CoSO_4.7H_20$, 0.2 mM KNO_3 and 0.025 mM NH_4NO_3. This solution was adjusted to pH 5 to provide an environment unfavourable for nodulation.

After 7 days, seedlings of each cultivar/accession were suspended through plastic lids into buckets of nutrient solution (8 seedlings of each accessions for experiment 1 and 10 for experiment 2). These solutions were the same as the germinating solution except that they contained 5 mM $CaSO_4.2H_20$, nitrogen treatments with K_2SO_4 added at levels to maintain potassium constant in all treatments and a buffer, 0.5 mM 2-(N-morpholino)ethanesulphonic acid (MES), which had been shown to have no effect on nodulation at the concentration used (1). Containers were titrated to the target pH for each treatment with 0.1 M KOH. After allowing 24 hours for stabilisation, a suspension of *R. meliloti* WSM419 (1 ml/L of nutrient solution containing approximately 10^9 cells/ml) was added. For three days solutions were maintained within 0.1 units of target pH by daily titration. After 3 days, treatment solutions were replaced by the germinating solution adjusted to pH 5 to prevent further nodulation. These solutions were changed every two days for six days with twice daily titration to pH 5. After 6 days, plants were taken from the solution, tops were removed, nodules counted and tops dried (70^0C for 48 hours) and weighed.

RESULTS AND DISCUSSION

Experiment 1

The total dry weight of all cultivars was increased with the addition of nitrate to treatment solutions (nil, 18.0; 0.1 mM, 20.7; 1.0 mM, 21.5 mg/plant; $p<0.001$) whereas pH had no general effect on top growth. Small differences were also observed in top dry weight of the cultivars at harvest (Cyprus, 20.6; Santiago, 18.7; N5655, 18.1; N3172, 19.9 and 87GRC50, 23.1 mg/plant, l.s.d (0.05) = 0.73), due largely to differences in initial seed size.

Increasing the concentration of nitrate had the general effect of reducing the number of nodules/per plant (nil, 13.4; 0.1 mM 10.4 and 1.0 mM, 8.5; $p<0.001$) whereas nodule number increased with increasing pH (pH 5.5, 6.0; pH 6.0, 10.3 and pH 6.5, 16.1; $p<0.001$). The species differed substantially in nodule number with *M. murex* > *M. sphaerocarpos* = *M. polymorpha* > *M. truncatula* (Table 1). The *M. murex* accession 87GRC50 produced more nodules than N3172, the other accession of that species.

The combination of acidity stress (low pH) and the presence of nitrate in solution combine to reduce nodule number. The most stressful treatment (pH 5.5 and 1.0 mM nitrate) completely prevented the nodulation of the most sensitive species (*M. truncatula* cv. Cyprus).

Table 1. The effect of nitrate concentration and pH on the number of nodules/plant on seedling roots of medics in experiment 1.

Nitrate conc. (mM)	pH	Cultivar/Accession					
		Cyprus	Santiago	N5655	N3172	87GRC50	Nitrate*pH mean
nil	5.5	1.0	5.3	6.2	8.6	13.6	6.9

	6.0	1.2	12.9	14.0	18.2	22.2	13.7
	6.5	5.2	21.8	17.6	24.9	29.0	19.7
0.1	5.5	0.3	4.1	6.9	10.9	12.5	6.9
	6.0	0.4	5.4	6.2	13.2	17.0	8.4
	6.5	2.6	14.9	15.1	19.7	26.5	15.8
1.0	5.5	0.0	2.8	2.2	7.3	7.5	4.0
	6.0	0.3	6.5	9.6	14.3	13.6	8.8
	6.5	2.4	13.3	13.4	15.7	19.4	12.8
Cultivar/Accession mean		1.5	9.7	10.1	14.7	17.9	

l.s.d (0.05) for cultivar means = 1.2 and for nitrate*pH means = 2.58

Experiment 2.

As with experiment 1 the nitrate treatments influenced growth of tops (0.1 mM, 22.4; 1.0 mM 24.9 mg/plant; $p<0.01$) but not pH. Variation amongst accessions in top dry weight ranged from 19.4 mg/plant to 28.2 mg/plant for 87GRC50, again reflecting differences in seed size. However, varietal differences in plant growth were not related to effects on nodulation (Table 2).

Table 2. The effect of nitrate concentration and pH on the number of nodules/plant on seedling roots of medics in experiment 2.

Species	Cultivar/accession	pH nitrate conc (mM)				
		5.5		6.0		
		0.1	1.0	0.1	1.0	Mean
M.polymorpha	Santiago	16.9	12.1	22.6	15.5	16.8
M.sphaerocarpos	N5655	15.8	8.5	23.9	17.9	16.5

	GRC5659.1	11.3	6.0	16.5	13.2	11.7
	Orion	18.9	15.7	26.7	23.7	21.2
	SEP26.2.7	13.9	10.5	14.8	13.3	13.1
	87GRC69	14.6	9.3	19.9	17.1	15.2
M.murex	Zodiac	14.7	13.4	33.9	28.7	22.7
	N3172	17.9	14.2	38.1	31.9	25.5
	87GRC50	31.7	16.6	44.5	30.9	30.9
	87GRC87.1	7.6	6.4	13.5	15.3	10.7
	87FOI.28	12.2	12.5	25.7	16.6	16.8
	87FB2.36	27.1	19.1	43.1	39.1	32.1
Mean		16.9	12.0	26.9	21.9	

l.s.d. (0.05) for variety means = 4.19 and for variety*pH = 8.21 (5.93 for means with the same pH).

Of particular significance are the differences between species and accession in response to the combined stresses imposed by acidity and the presence of nitrate. Although some accessions of *M. murex* produced very high numbers of nodules, other accessions produced fewer nodules than accessions of both *M. polymorpha* and *M. sphaerocarpos*. Although the general hierarchy of ability to nodule under the combined stress of acidity and the presence of nitrate demonstrated in experiment 1 was maintained in experiment 2, it is clear that considerable intraspecific variation exists.

Increased acidity generally reduced nodule numbers ($p<0.05$) but increasing nitrate concentration from 0.1 mM to 1 mM did not generally reduce nodule number. Neither were there differences in cultivar response to nitrate. Cultivars did display differences in response to pH with several accessions (87GRC50 and 87FB2.36) showing outstanding nodulation at low pH.

The current study indicates that both acidity and the presence of nitrate inhibit nodulation. It seems likely that the plant characteristics giving rise to a high level of seedling nodulation under acidic conditions will also benefit the plant when exposed to nitrate.

The order of nodulation potential among medic species in dilute nutrient solution is generally in line with than demonstrated for acid soils under field conditions (4). The experimental system used for the current experiments could form a basis for screening large numbers of accessions as part of breeding and selection programs for medics. The current experiments which used similar general procedures gave similar results in each case indicating that these procedures are sufficiently repeatable to form the basis of a screening procedure. Those accessions included in both experiments produced similar numbers of nodules/plant in each experiment and their relative ordering was the same (i.e. 87GRC50 > N3172 > N5655 = Santiago). The similarity in medic varietal response to the nitrate treatments in experiment 2 indicates that, as a screening system, the procedure could be simplified to involve the use of a single nitrate concentration.

ACKNOWLEDGMENTS

I acknowledge the financial support of the International Wool Secretariat in funded this project.

REFERENCES

1. Ewing, M.A. and Robson, A.D. 1990. Plant Soil 131, 199-206.

2. Ewing, M.A. and Robson, A.D. 1990. Aust. J. Agric. Res. 41, 489-497.

3. Harper, J.E. and Gibson, A.H. 1984. Crop Sci. 24, 797-801.

4. Howieson, J.G. and Ewing, M.A. 1989. Aust. J. Agric. Res. 40, 843-850.

5. Mason, M.G., Rowley, A.M. and Quayle D.J. 1972. Aust. J. Exp. Agric. 12, 171-175.

6. Munns, D.N. 1968. Plant Soil 29, 33-47.

7. Simpson, J.R. 1962. Aust. J. Agric. Res. 13, 1059-1072.