

# Fertilizer N and Inoculation Effects on Annual Medics

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## Introduction

Annual medics are important winter annual pasture legumes in southern Australia, where they provide forage for livestock, improve soil fertility, and enhance subsequent crop yield. Medics have been evaluated recently in the North Central USA as summer annual forage crops and as intercrops in small grain and corn.

The symbiotic N<sub>2</sub> fixation capacity of annual medics varies with medic species, *Sinorhizobium* strains, and environmental conditions, especially soil inorganic N concentration and pH. Rates of season-long symbiotic N<sub>2</sub> fixation by annual medics in Minnesota range from about 100 to 200 kg N ha<sup>-1</sup>. There have been no detailed studies of the effect of soil inorganic N supply and the competitiveness of various *Sinorhizobium* strains included in currently available commercial inoculant. The objective of this research was to address both of these questions.

## Methods

**Effect of fertilizer N and inoculation.** Field experiments were conducted at Becker, MN, on a Hubbard loamy sand with no recorded history of annual medic cultivation. Whole plot treatments were factorial combinations of inoculation (+I and -I) and N fertilizer (+N and -N). Seed in the +I treatment received commercial inoculum comprised of 5 *Sinorhizobium* strains before planting. The +N treatment consisted of 100 kg N ha<sup>-1</sup> broadcast as ammonium nitrate at planting. Subplot treatments were 8 *Medicago* species (*M. littoralis* cv Harbinger, *M. lupulina* cv George, *M. polymorpha* cv Santiago, *M. rugosa* cv Sapo, *M. scutellata* cv Sava, *M. truncatula* cv Mogul and Sephi, and *M. sativa* cv Nitro) seeded in spring (late April to early May) and summer (late July to early August) of 1991 and 1992.

Herbage and roots were harvested in late June to early July for spring seedings and in mid-September

for summer seedings. A subsample of 20 root systems was analyzed for nodule mass score, percentage of plants nodulated, and number of roots with nodules. Herbage and root dry mass was determined and herbage was analyzed for total N using near infrared spectroscopy. Apparent symbiotic N<sub>2</sub> fixation was estimated using the difference technique [N derived from the atmosphere = (%N<sub>fixing</sub> X yield<sub>fixing</sub>) - (%N<sub>nonfixing</sub> X yield<sub>nonfixing</sub>), where the nonfixing plant was noninoculated *M. rugosa*]. All data from three field replicates were subjected to analysis of variance, and means were separated using Fisher's protected LSD ( $p < 0.05$ ).

**Nodule occupancy.** Inoculated and noninoculated annual medics (*M. polymorpha*, *M. rugosa*, *M. scutellata*, and *M. truncatula* cv Mogul) were seeded in early May 1993 in single rows spaced 15-cm apart at Becker and St. Paul, MN. The soil at St. Paul was a Hayden fine sandy loam. Peat-based inoculum containing five *Sinorhizobium meliloti* strains (102G3, 102A13, 102Z5, 102H2, and 102B11) was applied to the seeds before planting. Nodules were collected from tap and lateral roots from five randomly selected 50-day-old plants in every plot. Separate antisera were developed against each strain and against 102F51, the primary strain in commercial alfalfa inoculum. Cross reactivity was selectively removed by massive adsorption of the antisera with steamed, washed *Sinorhizobium* cells. The indirect ELISA procedure was used to establish nodule strain occupancy on five replicates, and differences were determined using analysis of proportion.

## Results

**Effect of N fertilizer and inoculation.** Although legumes had not been grown at the site for several years, indigenous *Sinorhizobium* capable of nodulating annual medics were present, and at least 75% of plants in all species were nodulated, except *M. rugosa*, which was poorly nodulated. In -N treatments, inoculation improved nodulation only of *M. rugosa*, but with fertilizer N, inoculation improved nodulation of *M.*

*rugosa*, *M. polymorpha*, and *M. truncatula* cv Sephi compared with –I. Fertilizer N reduced nodulation of most species that received commercial inoculum, but only some species when inoculation was withheld.

Herbage dry matter yield ranged from 1700 to 3100 kg ha<sup>-1</sup> and was not affected by inoculation or N fertilization of summer seedings. This may have been due to presence of sufficient soil inorganic N for the 43-day-long growth period in this treatment. In contrast, inoculation improved herbage yield of spring seedings. Nitrogen addition improved yield of four entries in the –I treatments, but only of *M. scutellata* in the +I treatment, implying that more effective inoculum is needed for this species. Neither N fertilizer nor inoculation affected herbage yield of *M. lupulina*, *M. littoralis*, or *M. sativa*, indicating that indigenous *Sinorhizobium* were as effective as commercial inoculum for these legumes. Root dry matter yields ranged from 70 to 200 kg ha<sup>-1</sup> and were not affected by inoculation or N fertilization.

For spring-seeded plots, estimated N<sub>2</sub> fixation ranged from 40 to 86 kg N ha<sup>-1</sup>, with *M. polymorpha*, both *M. truncatula* entries, and *M. scutellata* having the greatest fixation. For summer-seeded plots, N<sub>2</sub> fixation ranged from 20 to 50 kg N ha<sup>-1</sup>, with *M. scutellata* and *M. truncatula* cv Mogul having highest values. These are lower values than obtained in other research, but only 60 and 43 days were allowed for growth of spring and summer seedings, respectively.

**Nodule occupancy.** Strain occupancy of nodules did not differ between lateral and tap roots, and a high proportion of nodules was occupied by two or more strains, as is typical of multilobed nodules.

*Sinorhizobium* strains serologically related to 102F51, the most common strain nodulating alfalfa in the Midwestern USA, were the most prevalent in medic nodules in the –I treatment. At least 68% of nodules apparently contained this strain, except in *M. rugosa*, which showed only 5% of plants nodulated in the –I treatment. Among the *Sinorhizobium* strains in the inoculum, 102G3 and 102A13 were consistently among the most prevalent strains in nodules of +I and –I plants of all annual medics, whereas 102H2 was not identified in any nodule. Presence of all other strains in at least some nodules of the –I treatment indicates that these strains occur in soils at both locations in Minnesota.

Results of this research indicate that some strains of *Sinorhizobium* capable of nodulating annual medics can be found in Midwestern USA soils. Inoculation may not be warranted when annual medics are to be grown for very short periods. The existence of differences in nodule occupancy among *Sinorhizobium* strains implies that selection for more competitive strains may be possible. Apparent symbiotic N<sub>2</sub> fixation rates of 0.5 to 1.4 kg N ha<sup>-1</sup> d<sup>-1</sup> were found in this research, indicating that annual medics have the potential to add significant amounts of N to cropping systems in relatively short time periods.