

Inoculation and Nitrogen Affect Herbage and Symbiotic Properties of Annual *Medicago* Species

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ABSTRACT

The interactive effects of *Sinorhizobium* inoculants and soil N status should affect the N contribution of annual medics (*Medicago* spp.) in cropping systems. We determined the effect of N and commercial medic inoculum on nodulation, dry matter, and N yield of annual medics and also determined *Sinorhizobium* strain occupancy in annual medic nodules. Field experiments were conducted on a sandy, mixed Udorthentic Haploboroll and on a fine-loamy, mixed, mesic Typic Hapludalf. More than 75% of annual medic plants (except *M. rugosa* Desr.) were nodulated in the absence of commercial inoculum, and nodulation was due in part to a *Sinorhizobium* strain that frequently nodulates alfalfa (*Medicago sativa* L.). Among the five strains in the commercial medic inoculum, 102G3 and 102A13 had the greatest nodule occupancy. When no N was applied, inoculation improved the percentage of plants nodulated and nodule mass only in *M. rugosa*, compared with no inoculation, but inoculation increased herbage yields of spring-seeded *M. truncatula* Gaertn. cv. Sephi, *M. polymorpha* L., and *M. rugosa* by about 60%, compared with no inoculation. Nitrogen addition reduced the nodule mass of all species when inoculum was applied, and N addition increased only the herbage dry matter yield of spring-seeded *M. scutellata* (L.) Mill. when inoculum was applied. This suggests that a more effective inoculum could be developed for *M. scutellata* so that N would not limit herbage growth. Annual medics fixed from 40 to 80 kg N ha⁻¹ if spring-seeded and grown for 60 d, and from 20 to 50 kg N ha⁻¹ if summer-seeded and grown for 43 d.

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 (Crawford et al., 1989). Medics increase pasture soil organic matter, enhance soil water retention and water availability, and improve soil N status due to symbiotic N₂ fixation (Puckridge and French, 1983). Recently, medics have been evaluated in the midwestern USA as summer annual forages and as intercrops for small grains and corn (*Zea mays* L.) (Zhu et al., 1996; Moynihan et al., 1996; De Haan et al., 1997; Zhu and Sheaffer, 1997).

The symbiotic N₂ fixation capacity of annual medics varies with medic species, *Sinorhizobium* strains, and environmental conditions, especially soil inorganic N concentration and pH (Alston and Graham, 1982; Brockwell et al., 1976). Rates of N₂ fixation by annual medics in Minnesota ranged from 100 to 200 kg ha⁻¹

(Zhu et al., 1998). An inhibitory effect of soil N on nodulation and N₂ fixation, and a differential tolerance of legumes to inorganic N, have been observed frequently (Ewing and Robson, 1990; Hamilton et al., 1991; Hardarson et al., 1984; Harper and Gibson, 1984; Heichel and Vance, 1979). Soil and fertilizer N decreases the number of nodules per root, nodule mass per plant, and nitrogenase activity per unit mass of nodule (Streeter, 1988). Zhu et al. (1998) reported that N derived from fixation for several annual medics averaged 90% for a soil with low organic matter and N content, but only 82% for a soil with higher organic matter and N content. Ewing and Robson (1990) reported that *M. truncatula* had fewer nodules than *M. polymorpha* and *M. murex* Wild. when fertilized with inorganic N in a controlled environment.

The efficiency of inoculation is influenced by the competitiveness and quantity of indigenous sinorhizobia (Brockwell and Holliday, 1988). When a legume is cultivated in a field where it has been grown previously, sufficient effective sinorhizobia are usually present. Studies conducted in western Asia found that, in the absence of inoculation, native medics including *M. orbicularis* Bart. and *M. polymorpha* were nodulated with indigenous sinorhizobia (Radwan et al., 1978).

Annual medic seed has been frequently treated with a commercial inoculum (Liphatech, Milwaukee, WI¹), that contains a mixture of five *Sinorhizobium* strains. However, the effectiveness and competitiveness of each of these strains and possible preference of strains for different annual medics was unknown. To apply the most effective *Sinorhizobium* strain to a specific annual medic, we needed to study the effectiveness of these strains in nodulating medics in the field.

Further studies on the effect of soil N on N₂ fixation of annual medics and on *Sinorhizobium* relationships with annual medics are needed to understand and improve N contribution of medics in cropping systems. Our objectives were to determine the effect of N fertilizer and inoculation on nodulation, dry matter (DM), and N yield of herbage of annual medics and to determine *Sinorhizobium* strain occupancy in nodules of annual medics. A secondary objective was to validate our

¹Names are necessary to report factually on available data; however, the USDA and the Univ. of Minnesota neither guarantee nor warrant the standard of the product, and the use of the name by the USDA and the Univ. of Minnesota implies no approval of the product to the exclusion of others that may also be suitable.

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Abbreviations: DM, dry matter; ELISA, enzyme-linked immunosorbent assay; Ndfa, N derived from atmosphere; NIRS, near-infrared reflectance spectroscopy; NMS, nodule mass score; PPN, percentage of plants nodulated; RCB, randomized complete block. *Treatments:* +I and -I, with and without inoculation; +N and -N, with and without N fertilization.

previous estimates of N derived from the atmosphere (Nd_{fa}) using the difference method.

MATERIALS AND METHODS

Effect of N Fertilizer and *Sinorhizobium* Inoculation

Field studies were conducted in spring and fall of 1990 and 1991 at Becker, MN, on a Hubbard loamy sand (sandy, mixed Udorthentic Haploboroll). The experimental design was a randomized complete block with treatments in a split-plot arrangement. There were three replications. Whole-plot treatments were factorial combinations of inoculation (+I or -I) and N fertilizer (+N or -N). For the +I treatment, the seed was inoculated before planting with commercial medic inoculant, a mixture of five *Sinorhizobium* strains (Liphatech, Milwaukee). For the +N treatment, 100 kg N ha⁻¹ of NH₄NO₃ was broadcast at planting. The subplot treatments were eight *Medicago* spp. consisting of seven annual and one perennial species. The eight species [*M. littoralis* Rhode ex Loisel. cv. Harbinger, *M. lupulina* L. 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] were seeded in spring and summer of 1991 and 1992.

Spring seeding occurred on 9 May 1991 and 29 Apr. 1992 and summer seedings occurred on 2 Aug. 1991 and 24 July 1992. Seeding dates varied somewhat between years because of weather. All legumes were seeded in rows spaced 15 cm apart within 3- by 6-m plots at a rate of 484 live seeds m⁻². Trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine] was applied at 0.65 kg a.i. ha⁻¹ and incorporated before planting. Soil pH, Bray-1 extractable P, and exchangeable K (0 to 20 cm) averaged 6.5, 100, and 220 kg ha⁻¹. Initial soil NO₃-N at the same depth ranged from 5.0 to 16.0 kg N ha⁻¹. The experimental sites were planted to cereal rye (*Secale cereale* L.) in the previous years and had no record of annual medic or alfalfa cultivation.

A single harvest was taken on 2 July 1991 and 26 June 1992 for spring-seeded plots, and 9 Sept. 1991 and 14 Sept. 1992 for summer-seeded plots. Herbage in a 0.5 m² area was cut at ground level. Roots were dug to a 20-cm depth and roots and nodules were manually washed from the soil. A subsample of 20 root systems was evaluated for nodule mass score (NMS) using scale of a 1 (least) to 5 (most). The number of roots with nodules was counted and the percentage of plants nodulated (PPN) was determined. Herbage and roots were dried at 60°C for 48 h and DM yields were recorded. Dry herbage samples were ground to pass a 1.0 mm screen and total N (Kjeldahl N) was determined.

Herbage total N was predicted using near-infrared reflectance spectroscopy (NIRS). Samples were entered into a NIR-Systems Model 6500 (Infrasoft International, Silver Spring, MD) scanning monochrometer, and reflectance spectra were collected. Total N concentration for calibration was determined by the micro-Kjeldahl method (AOAC, 1975). The reflectance data were related to calibration data using a modified partial least squares regression procedure to develop prediction equations (Shenk and Westerhaus, 1991). The prediction equation for total N was developed from 96 samples. The coefficient of multiple determination (*R*²) was 0.99 and the standard error of cross-validation for the NIRS prediction equation was 6.3 g kg⁻¹.

Seasonal N₂ fixation was estimated by the difference method using noninoculated *M. rugosa* as the nonfixing reference control because it is poorly nodulated by the indigenous *sinorhizobia* at the experiment site. The amount of N derived from atmosphere (Nd_{fa}) was determined by

$$\text{Nd}_{fa} = (\%N_f \times \text{DM}_f) - (\%N_{nf} \times \text{DM}_{nf}) \quad [1]$$

where %N_f = N concentration in N₂-fixing medic, DM_f = dry matter accumulation of N₂-fixing medic, %N_{nf} = N concentration in nonfixing medic, and DM_f = dry matter accumulation of nonfixing medic. Percent of N derived from the atmosphere (%Nd_{fa}) was determined by

$$\%Nd_{fa} = \left\{ \frac{(\%N_f \times \text{DM}_f) - (\%N_{nf} \times \text{DM}_{nf})}{(\%N_f \times \text{DM}_f)} \right\} \times 100 \quad [2]$$

All data were subject to analysis of variance using the statistical analysis system (SAS Inst., 1985). When significant treatment effects occurred, means were separated using Fisher's LSD ($\alpha = 0.05$). Data normality was checked using the UNIVARIATE procedure of SAS and normality assumptions were not violated. The data from spring and summer of 1991 and 1992 for PPN and NMS were combined, because the interactions between year (or season) and legume species were not significant. The DM yield and N production data were combined for years, but not for spring and summer (because of the significant interaction between season and legume species).

Nodule Strain Occupancy

Inoculated and noninoculated annual medics (*M. polymorpha* cv. Santiago, *M. rugosa* cv. Sapo, *M. scutellata* cv. Sava, and *M. truncatula* cv. Mogul) were seeded on 5 May 1993 in single rows spaced 15 cm apart at Becker and at St. Paul (on Hayden fine sandy loam, a fine-loamy, mixed, mesic Typic Hapludalf). Soil (0 to 10 cm) pH, Bray-1 extractable P, and exchangeable K averaged 6.5, 60, and 250 kg ha⁻¹ at the two locations. Peat-based inoculum containing a mixture of five *Sinorhizobium meliloti* strains (102G3, 102A13, 102Z5, 102H2, and 102B11) (Liphatech, Milwaukee) was used to inoculate seeds before seeding. Nodules were collected from 50-d-old annual medic plants. Nodules from the tap and lateral roots were separated for each of five randomly chosen plants from each cultivar and location.

Separate antisera were developed against each of the five strains in the annual medic inoculum and 102F51, the primary strain in commercial alfalfa inoculum (Hardarson et al., 1982). *Sinorhizobium* strains were cultured in yeast extract mannitol media (Graham, 1963) and cells for injection were prepared as described by Olsen and Rice (1983). The cell concentration was adjusted to between 10⁸ and 10⁹ cells mL⁻¹ and stored at 4°C. Antisera were prepared against whole, steamed, washed *Sinorhizobium* cells in New Zealand white female rabbits (*Oryctolagus cuniculus*) using methodologies described by Olsen and Rice (1984), except for modifications in the injection times. Four subcutaneous injections (Days 1, 3, 6, and 24 with a 1-mL mixture of *Sinorhizobium* cells and Freund's complete adjuvant) and two intravenous injections (Days 26 and 36 with 1 mL of *Rhizobium* cells) were given. Antisera were harvested from the rabbits 1 wk after the final injection, divided, and stored at -70°C. Cross-reactivity was selectively removed by massive adsorption of the antisera with steamed, washed *Sinorhizobium* cells (Olsen and Rice, 1984). The indirect ELISA procedure of Ayanaba et al. (1986) was used to establish nodule strain occupancy. Data were analyzed as a completely randomized design with five replications. Significance of differences between percent of nodule strain occupancy was determined using the analysis of proportion procedure (Mead et al., 1993).

RESULTS AND DISCUSSION

Effect of N and *Sinorhizobium* Inoculation

Nodulation

Indigenous sinorhizobia capable of nodulating annual medics apparently occurred in the soil, although legumes had not been grown at the sites for several years. Nodulation of annual medics with indigenous sinorhizobia varied with species and, for the $-I-N$ treatment, PPN for all species except *M. rugosa* exceeded 75% (Fig. 1). *Medicago rugosa* was poorly nodulated by indigenous sinorhizobia. Our findings are in agreement with Brockwell et al. (1976), who reported that annual medics exhibit varying degrees of specificity in their symbiosis with sinorhizobia, and that *M. rugosa* is highly specific. The NMS of annual medics nodulated by indigenous sinorhizobia also varied among species (Fig. 2). For the $-I-N$ treatment, *M. scutellata* and *M. polymorpha* were among the medics with the highest NMS, whereas *M. rugosa* was among the medics with the lowest NMS.

The effect of inoculation (+I) on nodulation of annual medics and alfalfa varied with species and N treatment. When no N was applied ($-N$), +I improved the PPN and NMS only of *M. rugosa*, compared with the $-I$ treatment, but when N was applied (+N), +I improved the PPN of *M. rugosa*, *M. polymorpha*, and *M. truncatula* cv. Sephi, compared with $-I$. The lack of response to inoculation by some medics was probably due to the effectiveness of indigenous sinorhizobia.

Without inoculum ($-I$), +N reduced the PPN of all medics except *M. lupulina* and *M. rugosa*, compared with $-N$. Similarly, when medics were inoculated (+I) at seeding, +N reduced PPN of *M. sativa*, *M. rugosa*, *M. scutellata*, and *M. truncatula* cv. Mogul, compared with $-N$. Nitrogen fertilization (+N) also reduced the NMS of all species when inoculum was applied (+I), compared with the $-N+I$ treatment. In contrast, without inoculum ($-I$), +N reduced the NMS only of *M.*

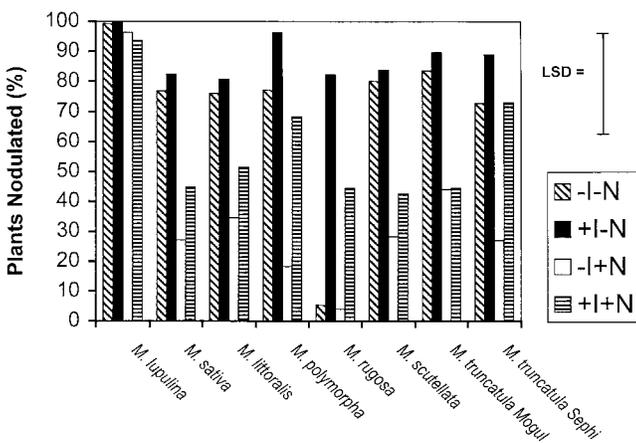


Fig. 1. Effect of inoculation and N fertilizer on percentage of legume plants nodulated. Data are averaged over years (1991 and 1992) and seedings (spring and summer). $-I-N$: no inoculation and no N fertilizer treatment, $+I+N$: inoculation and N fertilizer treatment, $-I+N$: no inoculation with N fertilizer treatment, $+I-N$: inoculation and no N fertilizer treatment. LSD (0.05) is for comparison of two treatment means within a legume.

littoralis, *M. scutellata*, and *M. truncatula* cv. Mogul, compared with the $-N-I$ treatment.

Herbage and Root Dry Matter Yield

Herbage DM yields averaged across inoculation and N treatments ranged from 1709 to 3126 kg ha⁻¹ for the spring seeding (Table 1). Yields were from 25% (*M. truncatula*) to 50% (*M. scutellata*) less than in our previous field trials (Zhu et al., 1998), probably because of the shorter growing period in this study. Inoculum and N effects on herbage DM yields varied with seeding date and legume species. For spring seedings when no N ($-N$) was applied, +I increased herbage DM yields of *M. truncatula* cv. Sephi, *M. polymorpha*, and *M. rugosa*, compared with $-I$. When N was applied, +I increased the yields only of *M. scutellata*. Nitrogen (+N) increased the herbage yields of $-I$ treated *M. truncatula*, *M. polymorpha*, *M. rugosa*, and *M. scutellata*, compared with the $-N-I$ treatment. For the +I treatment, however, +N increased the yield only of *M. scutellata*, compared with $-N$. The yield enhancement effect of +N on inoculated *M. scutellata* may indicate that a more effective inoculum could be developed to improve herbage DM yield of some annual medics. Neither +I nor +N affected herbage DM yield of *M. lupulina*, *M. sativa*, and *M. littoralis* with spring seeding, compared with the $-I-N$ treatment. Apparently, indigenous *Sinorhizobium* strains were as effective as commercial inoculum and added N for these legumes.

For the summer seeding, N and inoculum treatments had no effect on September herbage yields. The DM yields from summer planting were lower than from spring planting, ranging from 703 to 1700 kg ha⁻¹ (Table 2). The growing period for summer-seeded plots was about 43 d, compared with about 60 d for spring-seeded plots. *Medicago lupulina*, *M. sativa*, and *M. littoralis* were among the lowest-yielding legumes, whereas *M. scutellata* was the highest-yielding legume.

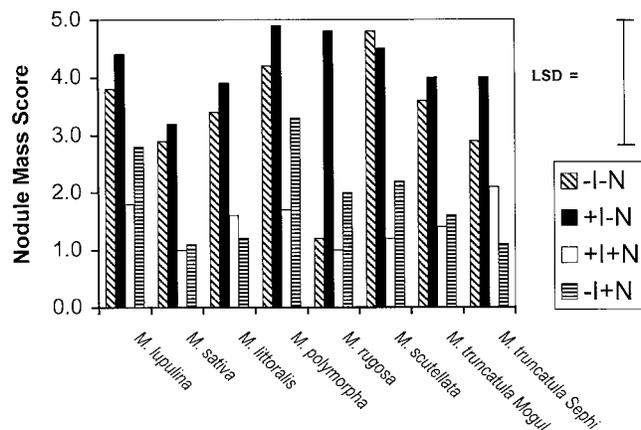


Fig. 2. Effect of inoculation and N fertilizer on nodule mass score (scale of 1 = least and 5 = most) of legumes. Data are averaged over years (1991 and 1992) and seedings (spring and summer). $-I-N$: no inoculation and no N fertilizer treatment, $+I+N$: inoculation and N fertilizer treatment, $-I+N$: no inoculation with N fertilizer treatment, $+I-N$: inoculation and no N fertilizer treatment. LSD (0.05) is for comparison of two treatment means within a legume.

Table 1. Effects of inoculation and N treatments on herbage dry matter (DM) and N yield of spring-seeded legumes (averaged over 2 yr).†

Legume	DM yield‡				N yield§			
	-I-N†	+I-N	-I+N	+I+N	-I-N	+I-N	-I+N	+I+N
	kg ha ⁻¹							
<i>M. lupulina</i>	1404	1838	1924	1749	55	70	74	68
<i>M. sativa</i>	1846	1711	2018	2085	66	61	74	75
<i>M. littoralis</i>	1846	1779	1581	1629	74	69	66	76
<i>M. polymorpha</i>	1965	2944	3176	3176	60	107	114	113
<i>M. rugosa</i>	1024	1756	2683	2369	21	61	101	89
<i>M. scutellata</i>	2309	2668	3303	4222	77	90	111	141
<i>M. truncatula</i>	2167	2533	3714	2885	72	87	123	104
<i>M. truncatula</i>	1663	2855	2563	2810	58	101	95	102

† -I-N: no inoculation and no N fertilizer treatment; +I-N: with inoculation but no N fertilizer treatment; -I+N: no inoculation with N fertilizer treatment; +I+N: with inoculation and N fertilizer.

‡ LSD (0.05) = 708 for comparing two DM yield treatment means within a legume.

§ LSD (0.05) = 25 for comparing two N yield treatment means within a legume.

Root DM yield of annual medics was not affected by inoculum or N treatments and was similar to yields reported in our previous study (Zhu et al., 1998). For spring and summer seedings, *M. sativa* had higher average root yields than annual medics (Table 3). Annual medic average yields ranged from 94 to 209 kg ha⁻¹ in the spring and from 70 to 136 kg ha⁻¹ in the summer. *Medicago rugosa*, *M. polymorpha*, and *M. truncatula* cv. Mogul had the highest root DM yields, whereas *M. littoralis* had the lowest root DM yield.

Herbage N Yield

Herbage N yield of annual medics averaged over the four treatments (two N and two inoculation) ranged from 67 to 105 kg N ha⁻¹ for spring seeding and from 33 to 68 kg N ha⁻¹ for summer seeding. The effects of N and inoculation treatments on herbage N yield were similar to those observed for herbage DM. For spring seedings, when no N was applied, +I improved the herbage N production of *M. truncatula* cv. Sephi, *M. polymorpha*, and *M. rugosa*, compared with -I. Nitrogen (+N) improved the herbage N production of both *M. truncatula* cultivars, *M. rugosa*, *M. polymorpha*, and *M. scutellata*, compared with -N when no inoculum (-I) was applied. The herbage N production of *M. lupulina*, *M. sativa*, and *M. littoralis* was not affected by inoculation or N. For summer-seeded plots, neither inoculation nor N treatment altered herbage N accumulation. Species ranking for N yield was similar to that observed for DM yield.

Table 2. Herbage dry matter (DM) and N yield of summer-seeded legumes (averaged over 2 yr).†

Legume	DM yield	N yield
	kg ha ⁻¹	
<i>M. lupulina</i>	703	33
<i>M. sativa</i>	966	43
<i>M. littoralis</i>	906	45
<i>M. polymorpha</i>	1239	52
<i>M. rugosa</i>	1007	39
<i>M. scutellata</i>	1700	68
<i>M. truncatula</i> cv. Mogul	1478	61
<i>M. truncatula</i> cv. Sephi	1321	53
LSD (0.05)	300	20

† Values are averaged over inoculation and N treatments because of a nonsignificant interaction ($P > 0.05$).

Symbiotic Dinitrogen Fixation

For spring-seeded plots, estimates of N₂ fixation (Ndfa) using the difference method ranged from 40 to 86 kg N ha⁻¹, with *M. polymorpha*, both *M. truncatula* cultivars, and *M. scutellata* among the entries with the greatest N₂ fixation and *M. sativa*, *M. rugosa*, and *M. lupulina* among the entries with the least N₂ fixation (Table 4). For summer-seeded plots, N₂ fixation ranged from 20 to 50 kg ha⁻¹, with *M. scutellata* and *M. truncatula* cv. Mogul among those entries with the greatest fixation and *M. sativa*, *M. lupulina*, and *M. rugosa* again among the entries with the least N₂ fixation. These estimates are lower than those from other studies (Papastylionou, 1987; Zhu et al., 1998), possibly due to the low herbage and N yields associated with the shorter growing period in this study. These estimates of Ndfa are conservative, because about 5% of the noninoculated *M. rugosa* plants were nodulated.

The %Ndfa averaged 70%, in agreement with other studies (Materon and Danso, 1991; Papastylionou, 1987), but somewhat less than we previously reported with these same medics (Zhu et al., 1998). Annual medics did not differ in %Ndfa in either spring or summer seedings.

Nodule Strain Occupancy

Each unadsorbed antiserum reacted with cells of its homologous strain and in varying degree with cells of other strains, indicating that a common antigen or antigens existed among the strains (Olsen and Rice, 1983).

Table 3. Root dry matter (DM) yield of spring- and summer-seeded legumes.†

Legume	Root DM yield	
	Spring	Summer
	kg ha ⁻¹	
<i>M. lupulina</i>	143	94
<i>M. sativa</i>	572	190
<i>M. littoralis</i>	94	70
<i>M. polymorpha</i>	181	130
<i>M. rugosa</i>	209	136
<i>M. scutellata</i>	133	107
<i>M. truncatula</i> cv. Mogul	181	133
<i>M. truncatula</i> cv. Sephi	152	115
LSD (0.05)	52	43

† Data are averaged over 2 yr, over N and inoculum treatments.

Table 4. Symbiotically fixed N in herbage of spring- and summer-seeded annual medics and alfalfa as calculated by the difference method using noninoculated *M. rugosa* as the reference crop.†

Legume	Ndfa‡		%Ndfa	
	Spring	Summer	Spring	Summer
	kg ha ⁻¹		%	
<i>M. lupulina</i>	49	26	70	62
<i>M. sativa</i>	40	20	66	56
<i>M. littoralis</i>	48	31	70	66
<i>M. polymorpha</i>	86	35	80	69
<i>M. rugosa</i>	40	28	66	64
<i>M. scutellata</i>	69	50	77	76
<i>M. truncatula</i> cv. Mogul	66	45	76	74
<i>M. truncatula</i> cv. Sephi	80	36	79	69
LSD (0.05)	20	15	NS	NS

† Data collected at Becker, MN, and averaged over 2 yr.

‡ Ndfa: N derived from atmosphere via dinitrogen fixation.

Adsorption of antisera with cells of cross-reacting strains reduced cross-reactivity and enabled the identification of sinorhizobia in nodules. Occupancy of a high proportion of medic nodules by several strains probably occurred because of the multilobed nodules produced by medics. *Sinorhizobium* strain occupancy of nodules on lateral roots was not different from the occupancy of nodules on the tap roots (data not shown); consequently, we present results as an average of both lateral and tap root nodule locations.

Strain 102F51, the most common strain nodulating alfalfa in the midwestern USA (Hardarson et al., 1982), was the most prevalent strain in medic nodules when no inoculum was applied (Table 5). This strain was positively identified in every nodule of *M. rugosa* and *M. truncatula*, in 86% of nodules on *M. polymorpha*, and in 68% of nodules on *M. scutellata*, averaged over two locations. This is an indication that the indigenous sinorhizobia nodulating medics were either the same or were serologically related to strain 102F51. About 95% of *M. rugosa* plants were not nodulated when no inoculum was applied; therefore, it appears that strain 102F51 was not effective in nodulating this species. Inoculation decreased the frequency of strain 102F51 in nodules of annual medics, but 102F51 was still identified in 61, 79, 40, and 52% of *M. polymorpha*, *M. rugosa*, *M. scutellata*,

and *M. truncatula* nodules, respectively, averaged over two locations.

Among the *Sinorhizobium* strains in the inoculum, 102G3 was consistently among the most prevalent strains in nodules of +I and -I plants of all annual medics. In contrast, strain 102H2 was not positively identified in any nodule. We did not determine the frequency of strain 102H2 in the initial inoculant; however, assuming reasonable representation, it appears to be much less competitive than the other strains in our environment. Studies with single- and double-strain inoculation would provide more information on the relative competitiveness of the *Sinorhizobium* strains. Nodule occupancy by several *Sinorhizobium* strains in -I medics indicates that they now occur in soils at both locations.

Some strains showed a pattern of preference for specific medic species. For example, nodule occupancy by 102Z5 occurred more with *M. rugosa* and *M. scutellata* than with *M. truncatula* or *M. polymorpha*. Also, at Becker, reaction with 102B11 was more frequent with *M. polymorpha* and *M. rugosa* than with *M. truncatula* and *M. scutellata*.

Summary

Although legumes had not been grown in the test plot areas for several years, all medics except *M. rugosa* nodulated in the absence of commercial medic inoculum. Nodule occupancy analysis indicated that nodulation of noninoculated medics was due to a *Sinorhizobium* strain serologically similar to 102F51, a common strain nodulating alfalfa, as well as to strains 102A13 and 102G3. With application of commercial inoculum, the frequency of strain 102F51 in nodules decreased. Of the strains applied in the inoculum, strains 102G3 and 102A13 were the most prevalent occupants of nodules and strain 102H2 was not found in nodules. Consistent with reports on other legumes, N application reduced the NMS of all inoculated medics, but when medics were not inoculated the N application reduced NMS only of *M. littoralis*, *M. scutellata*, and *M. truncatula* cv. Mogul. Nitrogen addition also reduced the PPN

Table 5. Percent of nodules on four annual medics occupied by five *Sinorhizobium* strains and by strain 102F51 in inoculation (+I) and noninoculation treatments (-I).†

Strain	<i>M. truncatula</i>		<i>M. polymorpha</i>		<i>M. rugosa</i>		<i>M. scutellata</i>	
	-I	+I	-I	+I	-I	+I	-I	+I
	%							
Becker, MN								
102A13	67b‡	50b	67b	55b	100a	25c	63b	40b
102B11	0d	38c	100a	64b	83a	75ab	0d	20c
102G3	83a	88a	100a	100a	100a	100a	88a	100a
102H2	0	0	0	0	0	0	0	0
102Z5	0b	0b	0b	0b	33a	0b	0b	10ab
102F51	100a	75a	100a	82a	100a	88a	75a	40b
St. Paul, MN								
102A13	0b	38a	14b	50a	22b	40a	20b	20b
102B11	0c	25b	0c	30b	33b	50a	20b	60a
102G3	29b	88a	29b	90a	100a	90a	80a	100a
102H2	0	0	0	0	0	0	0	0
102Z5	0b	0b	0b	10b	22a	30a	0b	20a
102F51	100a	29b	71a	40b	100a	70a	60ab	40b

† Commercial inoculant contains all strains except 102F51.

‡ Within rows, means not followed by the same letter differ at the 0.05 probability level.

of all medics except *M. lupulina* and *M. rugosa* grown without inoculum (–I) and the PPN of *M. sativa*, *M. rugosa*, *M. scutellata*, and *M. truncatula* cv. Mogul grown with inoculum (+I). Inoculation improved PPN and NMS only of *M. rugosa*, compared with –I; when N was applied, however, +I increased PPN of *M. truncatula*, *M. polymorpha*, and *M. rugosa*, compared with the –I–N treatment.

Herbage and N yields of some spring-seeded medics were influenced by inoculum and N treatments, but yields of summer-seeded medics were not. Dry matter and N yield response to treatments were similar. When no N was applied, +I increased herbage DM yield of *M. truncatula* cv. Sephi, *M. polymorpha*, and *M. rugosa*, compared with –I; with N addition, however, +I increased yield only of *M. scutellata*. Legumes also differed in their response to +N. For the –I treatment, +N increased herbage yield of *M. truncatula* cv. Sephi, *M. polymorpha*, *M. rugosa*, and *M. scutellata*; with inoculation, however, +N increased herbage yield only of *M. scutellata*. The lack of response to N or inoculum by some species may have been due to effective nodulation by indigenous soil sinorhizobia. Because inoculated *M. scutellata* responded to +N, there may be potential to increase yields by selecting more effective *Sinorhizobium* strains. Lack of response to *Sinorhizobium* and N treatments for fall seedings may indicate the adequacy of the soil to supply N to medics during a short period of growth.

Levels of apparent N₂ fixation determined by the difference method ranged from 40 to 86 kg ha⁻¹ for spring-seeded and from 20 to 50 kg ha⁻¹ for summer-seeded medics. These amounts were less than in our previous research, when plants were grown longer; however, they indicate that annual medics have potential to fix a significant amount of N₂ in a short growing period. A portion of this fixed N should be available for use by subsequent crops.

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