Effectiveness of *Sinorhizobium* Inoculants on Annual Medics

Sindy M. Interrante, Ruchi Singh, M. Anowarul Islam, Jimmy D. Stein, Carolyn A. Young, and Twain J. Butler

**ABSTRACT**
Annual medics (*Medicago* spp.) have potential to be important forage legumes in the southern Great Plains of the United States. Currently there are no commercial medic species-specific rhizobia strains identified for their inoculation. However, commercially available alfalfa (*Medicago sativa* L.) inoculants may be effective at successfully nodulating annual medics. The objective of this study was to determine the relevant symbiotic rhizobial partner in association with seven annual medics. A greenhouse experiment arranged in three replications of a randomized complete block design quantified rhizobium effectiveness index and shoot and root dry matter (DM) of *Medicago arabica* (L.) Huds., *Medicago lupulina* L., *Medicago minima* (L.) Bartal., *Medicago orbicularis* (L.) Bartal., *Medicago polymorpha* L., *Medicago rigidula* (L.) All., and *Medicago rigiduloides* E. Small when inoculated with five *Sinorhizobium* spp. strains (M2, M49, Nitragin A, Nitragin N [a blend of three isolates], and WSM1115) and an untreated control (UTC). Strain M49 was the only strain that effectively nodulated *M. rigiduloides*, while Nitragin A commercial alfalfa strain was only effective on *M. rigidula*. Inoculants M2, Nitragin N, and WSM1115 were able to effectively nodulate *M. polymorpha*, *M. arabica*, *M. minima*, and *M. orbicularis*. To a lesser extent, *M. lupulina*, *M. minima*, and *M. orbicularis* were also effectively nodulated by Nitragin A alfalfa strain. These data indicate that there are differences in rhizobia specificity of annual medic species, and further research is warranted to improve nodulation of these legumes in the field.


**Abbreviations:** DAE, days after emergence; DM, dry matter; UTC, untreated control.

Annual medics (*Medicago* spp.) are cool-season legumes that have potential to be important additions to forage systems in the southern Great Plains of the United States, since they are typically better adapted to drier environments and high soil pH compared to other commercially available annual legumes. They have the potential to increase forage production and extend the grazing season, while reducing the need for N fertilizer. Zhu et al. (1998a) reported that medics have the ability to fix 100 to 200 kg N ha⁻¹ yr⁻¹ with appropriate rhizobia inocula. Certain medics have desirable characteristics such as superior reseeding ability (Krall et al., 2007; Ocumpaugh et al., 2007) that makes them attractive over other annual forage legumes in some areas of the country. Animal toxicity associated with grasses such as tall fescue (*Festuca arundinacea* Schreb. = *Lolium arundinaceum* (Schreb.) S.J. Darbyshire) that contain the toxic endophyte *Neotyphodium coenophialum* (Morgan-Jones & Gams.) Glenn, Bacon, & Hanlin can be minimized with the addition of legumes such as annual medics due to a dilution effect of providing additional high quality forage (Hoveland et al., 1997; Krall et al., 2007; Roberts and Andrae, 2004). Annual medics can produce high quality forage (Muir et al., 2005; Ocumpaugh et al., 2004; Walsh et al., 2001) that is beneficial to livestock producers, as well as aiding in the...
restoration of soil fertility of arable soils (Clarkson et al., 1991). Another potential benefit from annual medics, as utilized in South Australia, will be as “ley” pasture species for dryland cropping systems (Krall et al., 2007; Walsh et al., 2001), which refers to land use where arable crops alternate with vegetation used for livestock production.

Despite these advantages, use of annual medics is limited primarily due to lack of adequate freezing tolerance (Brandsaeter et al., 2002) and hard seed (Ocumpaugh et al., 2007). The establishment of annual medics is affected by factors such as soil type, soil nutrient status, soil pH, rainfall, hard seed (Clarkson et al., 1991), and the appropriate rhizobial symbiotic partner (Rathor and Rajbhoj, 2008; Zharan, 1999). Like other legumes, annual medics form a symbiotic relationship with the α group of proteobacteria commonly known as rhizobia. Biondi et al. (2003) suggested that most diploid annual Medicago spp. form symbiotic relationships with the Sinorhizobium group, specifically Sinorhizobium medicae, while tetraploid perennial species such as alfalfa (Medicago sativa L.) are symbionts with Sinorhizobium meliloti. However, these results are not supported by data fromGarau et al. (2005), in which alfalfa was effectively nodulated by either S. medicae or S. meliloti, while the diploid Medicago littoralis Rohde ex Loisel. was only effectively nodulated by S. meliloti. Sinorhizobium medicae are the dominant micro-symbiotic partner of annual medics historically found growing on coarse-textured moderately acidic soils, while S. meliloti are predominantly associated with medic species adapted to alkaline or neutral sandy soils (Garau et al., 2005).

Establishment of several medic species requires knowledge of their symbiotic partners. The members of Medicago spp. exhibit intrageneric host strain specificity (Brockwell and Hely, 1961). To benefit from biological N fixation, suitable rhizobia must be available, either in the soil or as an added inoculant, but the establishment of desirable rhizobium strains can be hindered by the presence of ineffective rhizobium populations, which can cause parasitic nodulation (Brunel et al., 1996). Alfalfa and Medicago rigidula (L.) All. have been described as promiscuous in that they can be effectively nodulated by many different S. meliloti strains, while Medicago polymorpha L. is more specific and forms effective nodules with only a few strains (Brockwell and Hely, 1966; Brunel et al., 1996). Natural effective nodulation of M. polymorpha and Medicago arabica (L.) Huds. appears to be restricted to S. medicae, while nodulation with S. meliloti resulted in ineffective nodules (Brunel et al., 1996; Rome et al., 1996; Garau et al., 2005). Groose et al. (1996) reported that M. rigidula and Medicago rigiduloides E. Small were effectively nodulated by different S. meliloti strains.

Obstacles with establishment of annual medics are due to limited information available regarding agronomic management and because there are no commercial rhizobium inocula available for annual medics (Bowman et al., 1998). Producers currently utilize the same inocula for annual medics as they use for alfalfa. Research has indicated that a single commercial inoculum strain may be ineffective at successfully nodulating many alfalfa and annual medic cultivars (Howieson et al., 2000). Data on the effectiveness and competitiveness of these inocula and the possible rhizobium preference of different medics is lacking (Zhu et al., 1998b). The objective of this study was to determine the relevant symbiotic rhizobial partner in association with seven annual medics.

MATERIALS AND METHODS

Experimental Site

This study evaluated the host strain compatibility of rhizobium inocula on annual medics and was conducted in 2007 and 2009 at the greenhouse of The Samuel Roberts Noble Foundation in Ardmore, OK (34°19 N; 97°08 W). The greenhouse was maintained at approximately 20°C, 45% relative humidity, and 700 W m⁻² irradiance.

Treatments and Design

The experimental design was a factorial combination of six rhizobium inoculum treatments with seven annual medic species (n = 42) arranged in three replications of a randomized complete block design. In Exp. 1, five peat-based inoculants, M2, M49, WSM1115, Nitragin A, and Nitragin N blend (102A13, 102B11, and 102H2), were evaluated for their specificity and effectiveness toward seven annual medic species. Inoculum M2 was isolated from Medicago lupulina L. (T. Wacek, Plant Pathobiology, personal communication, 2010), WSM1115 was isolated from M. polymorpha (R. Ballard, South Australian Research and Development Institute, personal communication, 2010), and Nitragin N blend was isolated from M. arabica, M. polymorpha, and M. rugosa (J. Kosamke, EMD Crop Bioscience, personal communication, 2010). Inoculum M49 was isolated from M. rigidula (R. Ballard, South Australian Research and Development Institute, personal communication, 2010) and Nitragin A was isolated from M. sativa (J. Kosamke, EMD Crop Bioscience, personal communication, 2010). An untreated control (UTC) was included as an inoculation treatment.

The seven annual medics evaluated were M. arabica (L.) Huds. (locally collected), M. lupulina ‘BEBLK’, Medicago minima (L.) Bartal. ‘Devine’, Medicago orbicularis (L.) Bartal. ‘Estes’, M. polymorpha ‘Ueckert’, M. rigidula ‘PI 495552’, and M. rigiduloides ‘PI 227850’. Devine little burr medic (M. minima) was released in 2007 by Texas AgriLife and is commercially available (Ocumpaugh et al., 2007). Ueckert burr medic (M. polymorpha) and BEBLK black medic (M. lupulina) are experimental lines obtained from Texas AgriLife. Estes button medic (M. orbicularis), an accession originally from Ethiopia that was released by the Texas Research Foundation in Renner, TX (Ocumpaugh and Stichler, 2000), is no longer commercially available. Estes seed was purchased in 2000 (the last year it was available), and seed has been propagated in small plots for research purposes. Rigid (M. rigiduloides) and Tifton burr medic (M. rigidula) accessions were obtained through the USDA Agricultural Research Service’s Germplasm Resources Information Network (Beltsville, MD).
Medicago seeds were planted in March 2007 and 2009 in a 1:1 ratio by volume of washed masonry sand and vermiculite that was mixed and autoclaved. Fresh sand and vermiculite were mixed and autoclaved for each experiment. Seeds were planted in 1.9 L volume bottom-watering pots (hereafter called pots) (Apollo Plastics Ltd, Mississauga, ON) to minimize the risks of surface contamination by airborne rhizobia. Before planting, pots were sterilized with 20% NaOCl solution. Seeds were surface sterilized 1 d before planting by treating with 95% ethanol (v/v) for 15 min followed by 0.2% HgCl₂ (v/v) for 60 min and then rinsed with sterilized water. Twenty seeds of a single species were planted per pot and inoculated with a 50 mL suspension of appropriate treatment rhizobial culture grown in yeast extract mannitol broth at 28°C and 180 rpm for 48 h. Every 12 h the inoculated broth was tested for most probable number for viable cell count to maintain the cell number at 10⁹ cells mL⁻¹ for final inoculation. Untreated control pots were treated with 50 mL of sterilized water, and all pots were watered with 350 mL of sterilized water after planting. Subsequent watering occurred approximately once per week and consisted of 350 mL of alternating sterilized water and sterilized N-free nutrient solution (McKnight, 1949). The N-free solution contained 20 g KH₂PO₄, 150 g CaSO₄·2H₂O, 20 g MgSO₄·7H₂O, 30 g KCl, 14 g C₆H₅FeO₇, 2.86 g H₃BO₃, 2.08 g MnSO₄·H₂O, 0.22 g ZnSO₄·7H₂O, 0.08 g CuSO₄·5H₂O, and 0.11 g Na₂MoO₄ L⁻¹. Pots were thinned to 10 plants pot⁻¹ approximately 7 d after emergence (DAE). The pot surfaces were covered with a thin layer of autoclaved heat-treated montmorillonite clay (Profile Products LLC, Buffalo Grove, IL) when seedlings were approximately 3 cm tall to minimize surface rhizobial contamination.

A Medicago accession verification study (Exp. 2) was also conducted in 2009 to evaluate rhizobium effectiveness on additional accessions of four species (M. minima, M. orbicularis, M. rigidula, and M. rigiduloides), using the methodology as previously described. These four species were selected since they have greater freezing tolerance (Brandsaeter et al., 2002; Ocmapaugh et al., 2007; Walsh et al., 2001). Additional inocula included RR1128, a commercial alfalfa strain, and M18, isolated from M. rigidula (R. Ballard, personal communication, 2010). The additional medic entries evaluated were: M. minima (PI 499028, TAM-MM1-local collection, and W6 19015); M. orbicularis (PI 419238, SA 2161, and W6 5206); M. rigidula (PI 498462, PI 495523, and PI 495555); and M. rigiduloides (PI 380919, PI 495464, and PI 495457).

Response Variables

Plants were evaluated and destructively sampled for each experiment at 45 DAE. Plants were removed from pots and separated from the soil by washing with water over a sieve (3 mm mesh). Five plants per pot that were representative of the pot average were selected for evaluation. Individual measurements were averaged across the five plants to obtain a pot average.

Rhizobium Effectiveness Index

Plants were visually rated and scored on the following color scale: 0 = no nodules present, 1 = pink nodules (effective nodulation), and −1 = white nodules (ineffective nodulation). Rhizobium effectiveness index was calculated as nodule number × nodule color × plant color. Due to the interactions between rhizobia bacteria and medic plants, there needs to be a simple method to quantify the overall effectiveness to determine the best combination. This index gives an indication of overall effectiveness, by accounting for the number of nodules, nodule color, and plant color while avoiding redundancies in describing these three parameters separately.

Plant Component Mass

After visual ratings, plants were cut to separate shoots from roots, dried at 60°C to constant weight, and weighed. If nodules were present they are included in the root mass component. Plant component mass is reported as milligrams per plant.

Statistical Analyses

Statistical analyses were performed using PROC MIXED of SAS (SAS Institute, 2002). Medic species, rhizobium inoculum, and their interaction were considered as fixed effects, and year, replicate, and their interaction were treated as random effects. Medic species was included in the model as a subplot treatment in a split-plot arrangement, with the rhizobium inoculum treatment being the main plot. The PDIF function of the LSMEANS procedure was used to compare means. Significance was determined at P ≤ 0.05.

RESULTS AND DISCUSSION

Experiment 1

Rhizobium Effectiveness Index

Rhizobium effectiveness index was affected by medic species × rhizobium inoculum interaction (Table 1). Most rhizobium inocula positively affected more than one medic species. For example, Nitragin N and WSM1115 effectively inoculated M. arabica, M. lupulina, M. minima, M. orbicularis, and M. polymorpha. Nitragin A effectively inoculated M. lupulina, M. minima, M. orbicularis, and M. rigidula, while M2 effectively inoculated M. lupulina, M. orbicularis, and M. polymorpha. Conversely, M49 effectively inoculated only M. rigiduloides. Consistent with other studies, rhizobia that effectively nodulated M. polymorpha were ineffective at nodulating M. rigidula (Brunel et al., 1996). All medic species responded similarly when uninoculated, resulting in low rhizobium effectiveness indices.

Some medic species were effectively inoculated by several rhizobium inocula, while other species were only effectively inoculated by a single rhizobium. Medicago minima and M. orbicularis were effectively inoculated by M2, Nitragin A, Nitragin N, and WSM1115. However, M. rigidula and M. rigiduloides were effectively inoculated by only Nitragin A and M49, respectively. This response is consistent with results reported by Groose et al. (1996) in which M. rigiduloides was effectively nodulated by M49. Some medic species showed a range of effectiveness across rhizobium inocula. Medicago arabica was most effectively
inoculated by Nitragin N and to a lesser degree by WSM1115 and M2. *Medicago lupulina* was most effectively inoculated by M2 and to a lesser extent by Nitragin A, Nitragin N, and WSM1115. Some rhizobial strains were able to nodulate plants but were not effective in fixing N, as evident from white nodules and subsequent negative rhizobium effectiveness indices. Negative rhizobium effectiveness indices were not statistically different than the UTC, indicating that nodulation by ineffective rhizobia in this experiment does not appear to be more negative than no rhizobia inoculation.

### Plant Component Mass

#### Shoot

Shoot mass was affected by medic species × rhizobium inoculum interaction (Table 2). While most rhizobium inocula positively affected more than one medic species in terms of rhizobium effectiveness index, medic species shoot mass ranking was more variable based on inoculum but generally followed a similar trend. Inocula M2 and Nitragin N were effective on four species (*M. arabica*, *M. lupulina*, *M. orbicularis*, and *M. polymorpha*), while WSM1115 was effective on two medics (*M. arabica* and *M. polymorpha*) and marginally effective on three additional medics (*M. lupulina*, *M. minima*, and *M. orbicularis*). Inocula M49 and Nitragin A strains differed in host specificity. Nitragin A was effective on *M. rigidula*, which was 2.9 times greater than the average of the other inocula, and only moderately effective on *M. lupulina* and *M. orbicularis*. When inoculated with Nitragin N, *M. minima* ranked among the greatest in rhizobium effectiveness index but its shoot mass was not different than the species with the least shoot mass. As observed in rhizobium effectiveness index, *M. rigiduloides* had the greatest shoot mass when inoculated with M49 compared to other medic species, which was 4.7 times greater than the average of the other inocula.

#### Root

Root mass was affected by medic species × rhizobium inoculum interaction (Table 3) and generally followed the same trends observed in rhizobium effectiveness index and shoot mass, although the responses were not as profound as those observed with shoot mass. Unlike with shoot mass, there were no differences in medic species root mass when inoculated with Nitragin A or Nitragin N. *Medicago arabica*
had greater root mass than all medic species when inoculated with WSM1115, and *M. rigiduloides* had greater root mass than all medic species except *M. polymorpha* when inoculated with M49. *Medicago arabica* and *M. lupulina* had greater root masses than all medic species except *M. orbicularis* when inoculated with M2. Root mass of all medics responded similarly with limited root mass when uninoculated.

Unlike other responses measured, root mass of *M. rigidula* was unaffected by rhizobium inoculation. These results are consistent with the findings of Zhu et al. (1998b) in which root dry matter (DM) yield of eight annual medics including *M. lupulina* and *M. polymorpha* was not affected by commercial inoculant containing five *Sinorhizobium* strains. Aryal et al. (2006) reported no difference in common bean (*Phaseolus vulgaris* L.) root mass among organic fertilizer, organic fertilizer with rhizobial inoculation, chemical fertilizer, and chemical fertilizer with rhizobial inoculation treatments. However, the response of *M. rigiduloides* root mass to rhizobium inoculation was generally consistent with other responses measured in that inoculating with M49 resulted in greater root mass than all inocula. *Medicago arabica* and *M. orbicularis* had the greatest root masses when inoculated with WSM1115 and M2, respectively. Inoculating with M2 generally resulted

### Table 3. *Medicago* root mass as affected by medic species × rhizobium inoculum interaction (*p* < 0.001) at 45 d after emergence. Data are means across three replicates and 2 yr (*n* = 6).

<table>
<thead>
<tr>
<th>Medic species</th>
<th>M2</th>
<th>WSM1115</th>
<th>Nitragin N</th>
<th>M49</th>
<th>Nitragin A</th>
<th>UTC‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago arabica</em> (L.) Huds.</td>
<td>37 a B†</td>
<td>72 a A</td>
<td>12 a C</td>
<td>9 b C</td>
<td>13 a C</td>
<td>15 a C</td>
</tr>
<tr>
<td><em>Medicago lupulina</em> L.</td>
<td>36 a A</td>
<td>23 b AB</td>
<td>15 a BC</td>
<td>9 b C</td>
<td>10 a C</td>
<td>8 a C</td>
</tr>
<tr>
<td><em>Medicago minima</em> (L.) Bartal.</td>
<td>23 bc A</td>
<td>27 b A</td>
<td>18 a AB</td>
<td>7 b B</td>
<td>8 a B</td>
<td>5 a B</td>
</tr>
<tr>
<td><em>Medicago orbicularis</em> (L.) Bartal.</td>
<td>33 ab A</td>
<td>18 b B</td>
<td>17 a B</td>
<td>7 b B</td>
<td>10 a B</td>
<td>8 a B</td>
</tr>
<tr>
<td><em>Medicago polymorpha</em> L.</td>
<td>23 bc A</td>
<td>21 b AB</td>
<td>20 a AB</td>
<td>13 ab AB</td>
<td>7 a B</td>
<td>9 a B</td>
</tr>
<tr>
<td><em>Medicago rigidula</em> (L.) All.</td>
<td>15 c A</td>
<td>8 c A</td>
<td>8 a A</td>
<td>7 b A</td>
<td>14 a A</td>
<td>5 a A</td>
</tr>
<tr>
<td><em>Medicago rigiduloides</em> E. Small</td>
<td>13 c AB</td>
<td>8 c B</td>
<td>8 a B</td>
<td>25 a A</td>
<td>6 a B</td>
<td>9 a B</td>
</tr>
</tbody>
</table>

SE‡

†UTC, untreated control.

‡Means followed by the same letter—lower-case letter within column and upper-case within row—do not differ by the LSMEANS test (*p* > 0.05).

§Standard error of an interaction mean.

### Table 4. Rhizobium effectiveness summary presented by medic species and rhizobium treatments at 45 d after emergence compared to an untreated control (UTC).

<table>
<thead>
<tr>
<th>Rhizobia inoculum†</th>
<th>Medicago entry</th>
<th>M2</th>
<th>WSM1115</th>
<th>Nitragin N</th>
<th>M18</th>
<th>M49</th>
<th>Nitragin A</th>
<th>RR1128</th>
<th>UTC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago minima</em> (L.) Bartal.</td>
<td>Devine ‡</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>499028</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TAM-MM1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>W6 19015</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td><em>Medicago orbicularis</em> (L.) Bartal.</td>
<td>Estes ‡</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>419238</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>SA 2161</td>
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<td>-</td>
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<td></td>
<td>W6 5206</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td><em>Medicago rigidula</em> (L.) All.</td>
<td>495552</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>495462</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>495553</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>495555</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td><em>Medicago rigiduloides</em> E. Small</td>
<td>227850</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>0</td>
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<td></td>
<td>495464</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

†Nitragin A and RR1128 are commercial alfalfa inocula.

‡+, effective pink nodules; –, ineffective white nodules; 0, no nodules.
in greater root mass of *M. lupulina*. Inoculating with M2 and WSM1115 generally resulted in greater root mass of *M. minima*.

**Experiment 2: Medicago Accession Verification**

The rhizobium effectiveness responses of all additional entries evaluated in the verification experiment were consistent with the representative medic species in Exp. 1 (Table 4). Complete data is not shown in an attempt to reduce repetitive discussion. All entries within a medic species responded similarly to rhizobium treatments as did the representative entry in Exp. 1. For example, all entries of *M. minima* and *M. orbicularis* were effectively nodulated by M2, Nitragin A, Nitragin N, and WSM1115, which was consistent with results obtained in the previous experiment. *Medicago rigidula* 495552 was positively affected by only Nitragin A in Exp. 1, as were 495462, 495523, and 495555 in the accession verification experiment. *Medicago rigiduloides* 227850 was positively affected by only M49 in Exp. 1, as were all other *M. rigiduloides* entries in the accession verification experiment. The additional RR1128 inocula was similar to Nitragin A, which was expected since both are commercial alfalfa strains, while the M18 strain was ineffective on all accessions from all four species.

**SUMMARY AND CONCLUSIONS**

This study was conducted to determine the relevant symbiotic rhizobial partner in association with seven annual medics adapted to Oklahoma and Texas. There were distinct differences in specificity of rhizobia strains on medic species. Based on rhizobium effectiveness index and dry weight, it appears that M49 is the most effective rhizobia strain for nodulation of *M. rigiduloides*, and Nitragin A was most effective for *M. rigidula*. Inocula M2, Nitragin N, and WSM1115 were able to effectively nodulate several medic species, including *M. polymorpha*, *M. arabica*, *M. minima*, and *M. orbicularis*. Inocula M2 and Nitragin N were most effective on *M. lupulina*. The performance of these rhizobia strains under field conditions needs to be verified. Naturalized or indigenous rhizobia in the soil have been shown to reduce the effectiveness of inoculated rhizobial strains by competing for infection sites and ultimately nodulating the host plant (Zeng et al., 2007). Soil rhizobia may be highly competitive for nodule formation, resulting in either effective or ineffective nodules, in part because they have adapted to local environmental conditions (Winarno and Lie, 1979). Meade and O’Gara (1985) suggested the use of environmentally adapted indigenous rhizobia strains as inoculants for use in soils containing indigenous rhizobial populations. Future research is recommended to evaluate the performance and competitiveness of inoculated rhizobia in the field.

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