Dry Matter Accumulation and Dinitrogen Fixation of Annual *Medicago* Species

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ABSTRACT

Assessment of the N_2 fixation potential of annual medics (*Medicago* spp.) in the upper Midwest is important for predicting the net N contribution to cropping systems. Our objectives were to determine the dry matter (DM) accumulation pattern of herbage, roots, and nodules of several annual medic species used as summer annuals and to measure the percentage and amount of N derived from N_2 fixation by annual medics using isotope dilution and difference methods. Experiments were conducted on a Hubbard loamy sand (Udorthentic Haploboroll) at Becker, MN, and a Tallula silt loam (Typic Hapludoll) at Rosemount, MN. The experimental design at both locations was a randomized complete block with six replicates. *Medicago truncatula* Gaertn., *M. polymorpha* L., *M. scutellata* (L.) Mill., and *M. rugosa* Desr. were inoculated with commercial rhizobial inoculant and were seeded in late May. Herbage, root, and nodule DM of N_2–fixing annual medics continued to increase until about 72 d after planting (DAP). The highest maximum herbage, root, and nodule DM yields were 10 669, 648, and 169 kg ha^{-1}, respectively, from *M. polymorpha* in 1993. Based on the estimate from the isotope dilution (ID) method, herbage of annual medics at maximum DM accumulation contained 86% N derived from atmosphere (%Ndfa), using ryegrass (*Lolium multiflorum* Lam.) as a reference crop, or 79% Ndfa, using noninoculated *M. rugosa* as a reference crop. The amount of Ndfa in annual medic herbage ranged from 100 to 200 kg ha^{-1} based on estimates using either the ID method or the difference method (D-method), with *M. polymorpha* producing the highest and *M. rugosa* producing the lowest amount. Estimates of Ndfa from the ID and D-methods were strongly correlated. We conclude that annual medics have the potential to contribute a significant amount of N to cropping systems when seeded in the spring and harvested or incorporated into the soil 2 to 3 mo. later.

Annual medics (*Medicago* spp.) are winter annual legumes used in the Australian ley-farming system, which integrates leguminous pastures into cereal production systems (Unkovich et al., 1997). The ley system has improved soil fertility and subsequent cereal yields (Puckridge and French, 1983; Crawford et al., 1989). Annual medics also contribute symbiotically fixed N_2 to intercropped grasses. The yield of sorghum [*Sorghum bicolor* (L.) Moench] or buffelgrass (*Cenchrus ciliaris* L.) in irrigated grass–medic pastures was more than twice that of nonfertilized grass, and was similar to that of grass fertilized with 100 kg N ha^{-1} yr^{-1} (Clarkson et al., 1987). In the north-central USA, annual medics have been evaluated for potential use as summer annual forage sources (Zhu et al., 1996), as smother plants for weed control in corn (*Zea mays* L.) (De Haan et al., 1997), and as intercrops with small grains (Moynihan et al., 1996). Information on N_2 fixation is important for predicting the net N contribution of annual medics in cropping systems and for selecting species with high N_2 fixation under regional environmental conditions and farming practices.

Symbiotic N_2 fixation may be assessed by several methodologies (Vance, 1991). Isotope dilution is the only method that permits evaluation of the separate contributions of soil, fertilizer, and atmospheric N to total plant N (Danso, 1986; Vose and Victoria, 1986). This method involves labeling the soil N pool with the stable isotope, ^{15}N, and determining the ratio of plant N occurring as ^{15}N vs. ^{14}N (the form most predominant in nature, ≈99.65%) (Hardarson and Danso, 1990). Because they obtain part of their N from the atmosphere, N_2–fixing legumes will have a lower ^{15}N:^{14}N ratio than nonfixing plants. A primary assumption for this method is that the nonfixing crop takes up N from both the ^{15}N labeled tracer and from the soil in the same proportion as the fixing crop ($Ndft_{FIXER}/Ndfs_{FIXER} = Ndft_{NONFIXER}/Ndfs_{NONFIXER}$, where, Ndft = N derived from tracer and Ndfs = N derived from soil). This method is precise and integrates N_2 fixation and plant growth during the season. However, it is an expensive procedure because of the high cost of the stable isotope and instrumentation required to analyze ^{15}N.

The difference method (D-method) of estimating N_2 fixation can also integrate N_2 fixation and plant growth and is less expensive than isotope dilution (ID). The only parameters measured are plant N concentration and total plant DM. The difference in the total N content of the fixing and nonfixing counterparts is assumed to be the amount of N_2 fixed (Henson and Heichel, 1984b). A limitation of the D-method is in obtaining representative nonfixing reference crops. For example, Blumenthal and Russelle (1996) recently showed that ineffective Agate alfalfa absorbs about 30% more NO_3 from the soil than Agate, which suggests the D-method is subject to large errors.

Information on the magnitude of N_2 fixation by annual medics is conflicting and incomplete. Materon and Cocks (1988) in Australia reported that 200 kg ha^{-1} of N_2 was fixed by *M. truncatula* cv. Jemalong. Papastylianou (1987) in Cyprus used the *A*-value method (a modified isotope dilution method) and the D-method and reported N_2 fixation by *M. truncatula* cv. Jemalong and cv. Cyprus from 90 to 122 kg ha^{-1}, with 50 to 78% of the total plant N derived from fixation, depending upon the reference crop and soil N status. In the Netherlands, the percent and amount of N_2 derived from fixation by Jemalong averaged only 64% and 23 kg N ha^{-1}, respectively (Materon and Danso, 1991). This 10-fold range in estimates of N_2 fixation by the same cultivar could be due to several reasons, including the rhizobial

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Abbreviations: DAP, days after planting; DM, dry matter; D-method, difference method; ID, isotope dilution; Ndfa, N derived from atmosphere; Ndfs, N derived from soil; Ndft, N derived from tracer.

strains in the nodules, soil nutrient availability, and climatic conditions. Several new cultivars having better disease resistance, higher yield potential, and wider adaptability have been released in Australia, but have not been tested for N_2 fixation (Lake, 1993). Roots may be an important source of N, but their N_2 fixation contribution is unknown.

Our objectives were to (i) describe the biomass accumulation pattern of the above- and belowground organs of spring-seeded annual medics in Minnesota and (ii) estimate the proportion and amount of N derived from the atmosphere by spring-seeded annual medics using the isotope dilution and difference methods.

MATERIALS AND METHODS

DM Accumulation Pattern of Herbage, Roots, and Nodules

Field experiments were conducted at Becker, MN, on a Hubbard loamy sand (sandy, mixed Udorthentic Haploboroll) in 1993 and 1995. Soil pH in the top 10 cm at Becker was 6.6, and extractable soil P (Bray and Kurtz P1) and extractable soil K (neutral ammonium acetate) were about 125 and 260 kg ha^{-1}, respectively.

Four annual medic species, *M. truncatula* Gaertn. cv. Mogul, *M. polymorpha* L. cv. Santiago, *M. scutellata* (L.) Mill. cv. Sava, and *M. rugosa* Desr. cv. Sapo, were planted in the spring (21 May 1993 and 23 May 1995) in rows spaced 15 cm apart within 3 by 6 m plots at a rate of 484 live seeds m^{-2}. Average air temperatures in 1993 were 1.9°C below normal in May and 2.7°C below normal in June and July. In 1995, air temperatures were 1.9 and 1.1°C below normal in May and July, but 1.2°C above normal in June. Total precipitation was 369 and 300 mm in 1993 and 1995, and an additional 150 mm of irrigation was applied each year. All medics were inoculated with commercial inoculant (a mixture of five rhizobial strains) specially designed for annual medics (LiphaTech, Milwaukee, WI).[1] In addition, *M. rugosa* was sown without inoculant. A preplant incorporated herbicide, trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine], was applied and incorporated at a rate of 0.65 kg a.i. ha^{-1} before seeding.

Plants were sampled beginning at 14 DAP until maximum DM occurred for all medics, which was in early to mid-August. Harvesting intervals averaged 12 d in 1993 and 18 d in 1995. At each sampling, herbage and roots (to a 20-cm depth) were removed from a 0.2-m^2 area. Herbage was separated from the roots and crown by cutting at a 2-cm stubble height. Roots and nodules were separated from the soil by manual picking and washing on a sieve. Herbage, roots, and nodules were dried at 60°C.

The experimental design in each year was a randomized complete block with treatments in a split-plot arrangement. Medic species were main-plots and sampling times were subplots. There were six replicates. Data from each year (1993 and 1995) were analyzed separately, because of the difference in subplot treatments (stands were harvested seven times in 1993 and four times in 1995). Data analysis for significant treatment effects and interactions ($P < 0.05$) were conducted by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Inst., 1985). When significant treatment effects occurred, means were separated using Fisher's LSD ($P < 0.05$).

Symbiotic Dinitrogen Fixation

Field experiments were conducted at Becker (1993 and 1994) and Rosemount (1993), in Minnesota. The plots at Becker were next to the plots of the DM accumulation study, and the soil (Hubbard) was the same, with soil characteristics as described above. The Becker plots in 1994 were not the same as in 1993. The soil at Rosemount was a Tallula silt loam (coarse-silty, mixed, mesic Typic Hapludoll), with a pH of 6.5 in the upper 10 cm of soil. Extractable soil P and exchangeable soil K in the upper 10 cm at Rosemount were 42 and 189 kg ha^{-1}, respectively. Initial topsoil NO_3–N was 5.2 kg ha^{-1} at Becker and 38 kg ha^{-1} at Rosemount, and organic matter was 17 g kg^{-1} at Becker and 22 g kg^{-1} at Rosemount. The experimental sites were planted to small grains in previous years and had no record of annual medics or alfalfa (*M. sativa* L.) cultivation in the previous 5 yr.

The same annual *Medicago* species that were used in the DM accumulation experiment were evaluated for N_2 fixation. Noninoculated *M. rugosa* was used as a reference crop because it is poorly nodulated by indigenous rhizobia. 'Surrey' annual ryegrass (*Lolium multiflorum* L.) also served as reference crop.

Annual medics and ryegrass were seeded into 3- by 6-m plots at the two locations in spring of 1993 (21 May at Rosemount and 26 May at Becker) and at Becker on 19 May 1994. Annual medic plots were completely randomized. Ryegrass was not randomized with medics but was planted beside of the medic plots, because of the grass-killing herbicide was applied in the medic plots. Annual medic seeds were treated with commercial inoculum of rhizobium specific for medics. All legumes were seeded in 15-cm rows at rates to provide 484 live seeds m^{-2}. Ryegrass was seeded in 15-cm rows at a rate of 1120 live seeds m^{-2}. Trifluralin was applied and incorporated in the medic plots at a rate of 0.65 kg a.i. ha^{-1} before planting. A starter N fertilizer of 20 kg ha^{-1} was applied at each plot. Ammonium sulfate [$(NH_4)_2SO_4$] enriched with 99.4 atom % ^{15}N was applied at the rate of 1.2 kg N ha^{-1} and sprayed as an aqueous solution on a 2-m^2 subplot within each plot 10 d after seeding.

Cumulative growing degree days (GDD) during the 1993 growing season at Becker and Rosemount were 1938 and 1904 GDD, respectively (Sharratt et al., 1987). In 1994, at Becker, the growing degree days were 2319 GDD. Total precipitation during the medic growing season was 364 mm at Rosemount in 1993, and 369 and 252 mm at Becker in 1993 and 1994, respectively. Becker also received 150 mm of irrigation each year.

Plants were harvested in August, when maximum herbage DM was reached. Herbage was harvested by manually cutting a 1-m^2 area in each microplot to a 2-cm stubble height and herbage was dried at 60°C for 48 h. Dry herbage samples were ground to pass a 2-mm screen before subsampling. Roots and nodules were collected at Becker by digging a 0.25-m^2 area to a 20-cm depth. Roots and nodules were separated from the soil by manual picking, aided by rinsing on a sieve. To remove attached rhizosphere soil, roots and nodules were immersed in 0.08 mol L^{-1} phosphate buffer (0.04 mol L^{-1} NaH_2PO_4 plus 0.04 mol L^{-1} Na_2HPO_4), and sonicated in a water bath for 60 s at 210 W (Ultrasonic Cleaner, Mettler Electronic Corp., Anaheim, CA). Clean roots and nodules were dried and ground to pass a 1-mm screen before subsampling. Separate

[1] Names 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.

plant samples were obtained from nonlabeled areas to provide background ^{15}N concentration.

Samples of herbage and roots with nodules were analyzed for total C, total N, and ^{15}N concentration on a Carlo Erba NA1500 analyzer (Carlo Erba, Milan, Italy) interfaced to a Tracermass isotope mass spectrometer (Europa Scientific, Cheshire, UK) at the University of Nebraska, Lincoln.

Proportion and amount of N derived from the atmosphere were determined by comparing the N_2–fixing medic and corresponding reference crop (averaged for each site) for total N and isotopic contents required for the D-method and the ^{15}N ID method, respectively (Talbott et al., 1982). Percent of N derived from the atmosphere (%Ndfa) was determined using the ^{15}N method:

$$\%\text{Ndfa} = \{1 - [(f - a)/(n - a)]\} \times 100 \qquad [1]$$

where f is the atom % ^{15}N in the N_2–fixing medic, n is the atom % ^{15}N in the nonfixing reference crop, and a is the atom % ^{15}N in the control plant. The %Ndfa was determined using the D-method:

$$\%\text{Ndfa} = \{[(\%N_f \times \text{DM}_f) - (\%N_{nf} \times \text{DM}_{nf})]/ (\%(N_f \times \text{DM}_f)\} \times 100\% \qquad [2]$$

where $\%N_f$ is the N concentration in the N_2–fixing medic, DM_f is the dry matter accumulation of the N_2–fixing medic, $\%N_{nf}$ is the N concentration in the nonfixing reference crop, and DM_{nf} is the dry matter accumulation of the nonfixing reference crop. The total amount of N derived from atmosphere (Ndfa) was determined using the ^{15}N method:

$$\text{Ndfa} = \{1 - [(f - a)/(n - a)]\} \times \%N_f \times \text{DM}_f/100 \qquad [3]$$

and was determined using the D-method:

$$\text{Ndfa} = [(\%N_f \times \text{DM}_f) - (\%N_{nf} \times \text{DM}_{nf})]/100 \qquad [4]$$

The experimental design at both locations was a randomized complete block with six replicates. Data at Becker were analyzed with year as repeated measures, and the average of 2 yr was used to evaluate the treatment effects. A combined analysis was conducted for Becker and Rosemount in 1993 to compare the location effects. Significant treatment effects and interactions ($P < 0.05$) were identified by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Inst., 1985). When significant treatment effects occurred, means were separated using Fisher's LSD (0.05). Correlation coefficients for the relationship of the estimates from the two methods were determined for the means of each species.

RESULTS AND DISCUSSION

DM Accumulation Patterns of Herbage, Roots, and Nodules

The maximum DM yields of medics were higher in 1993 than in 1995 (Table 1). The temperatures were lower in 1993 than in 1995, which may be a factor contributing to the yield difference between the 2 yr. The time when maximum DM yields occurred was similar for the 2 yr. The seasonal growth curves discussed later are from 1993 data because in 1995, data were collected only from 45 to 85 DAP.

Herbage, roots, and nodule DM increased for most medics until 72 DAP (Fig. 1). *Medicago polymorpha* had the highest maximum DM accumulation of all fractions in the cooler-than-normal growing season of 1993, whereas in the more normal season of 1995, *M. scutellata* had the highest herbage DM and *M. rugosa* had the highest maximum root DM accumulation (Table 1). Noninoculated *M. rugosa* had much slower growth and

Table 1. Maximum measured dry matter (DM) yield of herbage, nodules, and root + nodule of four annual medics grown at Becker, MN.

Medic	Yield 1993	Yield 1995
	kg DM ha^{-1}	
Herbage		
M. truncatula	9 100	4702
M. polymorpha	10 669	4646
M. scutellata	8 473	6222
M. rugosa	8 025	4666
M. rugosa (noninoculated)†	3 508	869
LSD (0.05)	1 045	860
Nodules		
M. truncatula	87	24
M. polymorpha	169	52
M. scutellata	65	38
M. rugosa	92	65
M. rugosa (noninoculated)†	7	5
LSD (0.05)	26	13
Root + nodules		
M. truncatula	639	470
M. polymorpha	648	291
M. scutellata	410	310
M. rugosa	581	504
M. rugosa (noninoculated)†	374	346
MLSD (0.05)	96	70

† Noninoculated *M. rugosa* was analyzed separately.

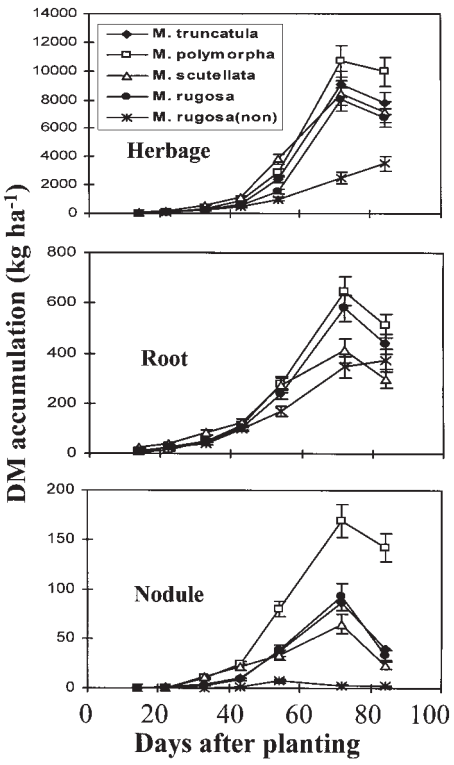

Fig. 1. Herbage, root, and nodule dry matter accumulation of annual medics in 1993 at Becker, MN. Error bars indicate ±1 SD (a measure of dispersion).

did not achieve maximum DM yields until 84 DAP. At maximum yield, *M. truncatula* and *M. rugosa* were flowering, but *M. polymorpha* and *M. scutellata* already had pods. Root data for *M. truncatula* were omitted from Fig. 1, because the root DM accumulation pattern for *M. truncatula* was the same as for inoculated *M. rugosa*.

Nodules were visible on the tap roots of all inoculated medics at the first sampling (14 DAP). At 72 DAP, when total maximum DM accumulation had occurred in the N_2–fixing medics, an average of 103 kg ha^{-1} of nodule DM had accumulated in 1993. The N concentration of alfalfa nodules is about 60 g N kg^{-1}; assuming a similar value, annual medic nodules would on average yield 6.2 kg N ha^{-1} at 74 DAP. With the highest nodule DM yield of 169 kg ha^{-1}, *M. polymorpha* nodules would yield about 10 kg N ha^{-1}, an amount that is similar to the nodule N produced by 'Saranac' alfalfa at 135 DAP at this location (Lory et al., 1992).

About 5% of *M. rugosa* plants in the noninoculated treatment were nodulated. The maximum nodule DM production of these noninoculated plants was about 5 to 7 kg ha^{-1}. Nonnodulated *M. rugosa* plants exhibited general chlorosis, especially in older leaves, a typical symptom of N deficiency, and had much lower total DM accumulation than inoculated plants. Brockwell (1981) documented that *M. rugosa* is highly strain-specific in its requirements for nodulation. We included noninoculated *M. rugosa* in these studies to see if indigenous rhizobia would nodulate this species. We conclude that there were no or very few effective indigenous rhizobia specific for *M. rugosa* at our experimental sites. Nodulation of this control was likely due to contamination of rhizobia from the commercial inoculant.

Significant medic species × sampling time interactions occurred for DM accumulation of all plant parts. The interactions were mainly because *M. scutellata* had the highest DM yield during early samplings, but only average yields during late samplings. The herbage and root DM of *M. scutellata* were among the highest until about 54 and 43 DAP, respectively. The high DM yields of *M. scutellata* at seedling stages may have been associated with its large seed size and high seedling vigor (data not shown). The lower DM yield of *M. scutellata* during late samplings may be related to its early maturity and fast senescence. *Medicago scutellata* and *M. polymorpha* both flowered at about 20 to 30 DAP; however, we observed more pod drop and faster root and nodule senescence in *M. scutellata*.

Annual medics had smaller root:shoot ratios, especially at later samplings, than the root:shoot ratio documented for other legumes, such as subterranean clover (*Trifolium subterraneum* L.) and alfalfa (Derkaoui et al., 1990). *Medicago scutellata* had a lower root:shoot ratio than the other medics at 14 DAP (Fig. 2). *Medicago rugosa* (with and without inoculation) had a higher root:shoot ratio than the other medics until 54 DAP. Noninoculated *M. rugosa* had the highest root:shoot ratio after 54 DAP. This may have occurred because noninoculated plants used more resources to develop roots to facilitate N uptake when nodulation was not occurring. Also nodulated plants had much greater herbage production. Lory et al. (1992) reported that the herbage DM of Saranac alfalfa was twice that of Ineffective Saranac, an ineffectively nodulated selection from Saranac, whereas the root DM of the two types of plants was not different. The decrease in root:shoot ratio with time is supported by the results of Derkaoui et al. (1990), who suggested that the decrease in the root:shoot ratio is due to greater use of photosynthate in herbage rather than roots.

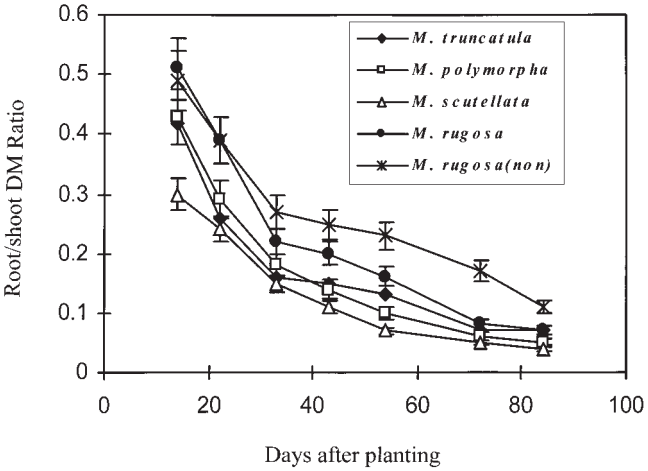

Fig. 2. Root:shoot dry matter ratio of medics in 1993 at Becker, MN. The noninoculated treatment of *Medicago rugosa* is indicated by '(non)'. Error bars indicate ±1 SD (a measure of dispersion).

Symbiotic Dinitrogen Fixation

Percent of N Derived from Atmosphere (%Ndfa)

Based on the isotope dilution technique, herbage of annual medics at maximum DM accumulation contained an average of 86% Ndfa, using ryegrass as reference crop, or 79% Ndfa using noninoculated *M. rugosa* as reference crop (Tables 2 and 3). Our estimates for Ndfa for annual medics are similar to previous studies conducted under field conditions. Materon and Danso (1991) reported values of 91% Ndfa for *M. rigidula* (L.) All. and 64% for *M. truncatula*. Estimates by Papastylianou (1987) for *M. truncatula* varied from 50 to 78%,

Table 2. Percent of N derived from atmosphere (%Ndfa) in herbage and root of annual medics as calculated by isotope dilution (ID) and the difference method (Dm), using ryegrass as the reference crop (grown at two locations in Minnesota).

	%Ndfa					
	Herbage					
	Becker†		Rosemount		Root (Becker)	
Medic	ID	Dm	ID	Dm	ID	Dm
	%					
M. truncatula	91	85	83	72	78	—‡
M. polymorpha	88	87	87	83	80	—
M. scutellata	89	85	81	75	75	—
M. rugosa	90	80	81	68	76	—
LSD (0.05)	4	3	5	6	6	—

† Becker data are averaged over 1993 and 1995; Rosemount data are for 1993.
‡ Negative values.

Table 3. Percent of N derived from atmosphere (%Ndfa) in herbage and root of annual medics as calculated by isotope dilution (ID) and the difference method (Dm), using noninoculated *M. rugosa* as the reference crop (grown at two locations in Minnesota).

	%Ndfa					
	Herbage					
	Becker†		Rosemount		Root (Becker)	
Medic	ID	Dm	ID	Dm	ID	Dm
	%					
M. truncatula	86	87	74	71	77	60
M. polymorpha	82	88	81	82	79	52
M. scutellata	83	86	72	72	73	38
M. rugosa	83	82	72	67	74	63
LSD (0.05)	6	3	8	6	7	14

† Becker data are averaged over 1993 and 1995; Rosemount data are for 1993.

depending on the reference crops used (oat, *Avena sativa* L.; barley, *Hordeum vulgare* L.; and annual ryegrass) and on soil N status. At Becker, the %Ndfa was similar for all annual medic species. At Rosemount, %Ndfa was higher for *M. polymorpha* than for *M. scutellata* and *M. rugosa*. Average %Ndfa was higher at Becker than at Rosemount, which likely was attributable to the lower soil organic matter and N content at Becker. This interpretation is supported by twofold higher N yields of annual ryegrass and noninoculated *M. rugosa* at Rosemount than at Becker (Table 4).

Estimates of %Ndfa using the ID method based on annual ryegrass were higher than those based on noninoculated *M. rugosa*, possibly because ryegrass had shallower and more fibrous roots than medics (data not shown), which could have led to an over estimation of N_2 fixation if ^{15}N was not evenly distributed through the root zone (Vose and Victoria, 1986). In addition, even a small amount of nodulation on noninoculated *M. rugosa* may have reduced the estimates of %Ndfa. Noninoculated *M. rugosa* is probably a better reference crop if nodulation can be prevented. We were fortunate that few or no indigenous strains of *Rhizobium meliloti* capable of nodulating *M. rugosa* were present at our experimental sites; other experimental sites should be tested before this approach is used for measuring symbiotic N_2 fixation.

Table 4. Herbage and root dry matter (DM) and N yield of annual medics and ryegrass (grown at two locations in Minnesota).

	Yield					
	Herbage					
	Becker†		Rosemount		Root (Becker)	
Medic	DM	N	ID	Dm	DM	N
	kg ha^{-1}					
M. truncatula	5977	155	6257	165	395	7.9
M. polymorpha	5839	172	8009	255	308	6.9
M. scutellata	6390	157	7752	181	222	4.2
M. rugosa	4498	119	5154	141	410	8.9
M. rugosa (Non‡)	1236	20	2796	45	208	3
L. multiflorum	2515	23	5102	44	939	6
LSD (0.05)	824	28	1068	38	73	1.8

† Becker data are averaged over 1993 and 1995; Rosemount data are for 1993.
‡ Non: Noninoculated (grown without commercial inoculant added).

The %Ndfa in roots of annual medics was lower than that in herbage, similar to results obtained with alfalfa by Lory et al. (1992). However, Henson and Heichel (1984a) reported that alfalfa roots contained a higher proportion of fixed N_2 and soybean [*Glycine max* (L.) Merr.] roots contained a lower proportion of fixed N_2, compared with whole plants. Despite the inconsistency in intraplant distribution of fixed N_2 in legumes, N_2 fixation products from the nodule are transported out of the root and redistributed among the plant organs. The %Ndfa in the root did not differ among annual medic species when the ID method was used, but the %Ndfa of *M. scutellata* root was lower than roots of other medic when the D-method was used. There were negative values of %Ndfa because ryegrass roots had higher N yield than the medic roots.

The correlation coefficient (r) between the estimates from ID and D-methods for herbage samples was 0.88 ($P = 0.01$, $N = 12$), using noninoculated *M. rugosa* as reference crop. Correlation between the methods was poor ($r = 0.44$; $P = 0.01$, $N = 12$) when root data were included. When annual ryegrass was used as the reference crop, correlation between the methods ($r = 0.76$ for herbage samples, $r = 0.30$ for all data; $P = 0.01$, $N = 12$) was not as large and estimates from the D-method were consistently lower than estimates from ID. This may be due to ryegrass recovering ^{15}N-labeled N disproportionally compared with N_2–fixing medics, resulting in an overestimation of N_2 fixation by ID. A general agreement between the estimates based on D-method and ID methods for %Ndfa has been also observed by other investigators (Henson and Heichel, 1984b; Papastylianou, 1987).

Amount of N Derived from Atmosphere (Ndfa)

Medicago truncatula, *M. polymorpha*, and *M. scutellata* had higher herbage DM and N yield than *M. rugosa* across locations (Table 4). The overall herbage yields of medics at Rosemount were higher than those at Becker, which was attributable to soil type and fertility level. Herbage DM and N yields of medics in our study were in agreement with the reports of other investigators who evaluated medics as winter annuals (Papastylianou, 1987; Mohammad and Qamar, 1988). The contribution of medic roots to total biomass and N yield was very small at the time of maximum herbage DM accumulation.

The amount of Ndfa in herbage ranged from 101 to 205 kg ha^{-1}, based on isotope dilution and using noninoculated *M. rugosa* as the reference crop (Table 5). Our estimates are in agreement with results of Materon and Cocks (1988) and Papastylianou (1987), but are higher than estimates by Materon and Danso (1991). In addition to differences in reference crops and rhizobial strains, higher soil inorganic N in the latter study might have resulted in lower N_2 fixation. *Medicago polymorpha* had the highest Ndfa, whereas *M. rugosa* had the lowest Ndfa in our experiment. The Ndfa from roots was agronomically insignificant, mainly because of the low root DM and the low N yield. Our results

Table 5. The amount of N derived from atmosphere of annual medics as calculated by isotope dilution (ID) and the difference method (Dm), using noninoculated *M. rugosa* as reference crop (grown at two locations in Minnesota).

	Ndfa					
	Herbage					
	Becker†		Rosemount		Root (Becker)	
Medic	ID	Dm	ID	Dm	ID	Dm
	kg N ha^{-1}					
M. truncatula	133	135	124	119	6.1	4.9
M. polymorpha	142	152	205	210	5.5	3.9
M. scutellata	131	137	129	136	3.1	1.3
M. rugosa	101	99	102	96	6.7	5.9
LSD (0.05)	26	28	32	38	1.6	1.8

† Becker data are averaged over 1993 and 1995; Rosemount data are for 1993.

suggest that the herbage could account for about 95% of total plant N_2 fixation, avoiding the need for difficult root recovery in estimating annual medic N_2 fixation.

There was a significant correlation between the estimates for amount of Ndfa from ID and D-methods. Using noninoculated *M. rugosa* as reference crop, the correlation coefficient (r) for Ndfa was 0.998. The correlation between the two methods were greater for Ndfa than for %Ndfa, which is supported by other investigators (Henson and Heichel, 1984b; Papastylianou, 1987; Talbott et al., 1982). The high correlation between the methods in our study suggested that the D-method could substitute for the more accurate but much more expensive ID method, where relative rankings of Ndfa are primary concern. However, the ID method would be advantageous when the soil N content is high and the total N taken up by the reference crop and N_2–fixing crop is different but the ratio of N derived from ^{15}N and from soil for the reference crop and N_2–fixing crop is similar (Blumenthal and Russelle, 1996).

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