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Seasonal N_2 fixation by cool-season pulses based on several ^{15}N methods[1]

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Summary Accurate estimates of N_2 fixation by legumes are requisite to determine their net contribution of fixed N_2 to the soil N pool. However, estimates of N_2 fixation derived with the traditional ^{15}N methods of isotope dilution and A_N value are costly.

Field experiments utilizing ^{15}N-enriched $(NH_4)_2SO_4$ were conducted to evaluate a modified difference method for determining N_2 fixation by fababean, lentil, Alaska pea, Austrian winter pea, blue lupin and chickpea, and to quantify their net contribution of fixed N_2 to the soil N pool. Spring wheat and non-nodulated chickpea, each fertilized with two N rates, were utilized as non-fixing controls.

Estimates of N_2 fixation based on the two control crops were similar. Increasing the N rate to the controls reduced A_N values 32, 18 and 43% respectively in 1981, 1982 and 1983 resulting in greater N_2 fixation estimates. Mean seasonal N_2 fixation by fababean, lentil and Austrian winter pea was near 80 kg N ha^{-1}, pea and blue lupin near 60 kg N ha^{-1}, and chickpea less than 10 kg N ha^{-1}. The net effects of the legume crops on the soil N pool ranged from a 70 kg N ha^{-1} input by lentil in 1982, to a removal of 48 kg N ha^{-1} by chickpea in 1983.

Estimates of N_2 fixation obtained by the proposed modified difference method approximate those derived by the isotope dilution technique, are determined with less cost, and are more reliable than the total plant N procedure.

Introduction

Rotations were a necessary agronomic practice in the US until the mid-1940's when low-cost N fertilizers and herbicides permitted farmers to grow cereals in monoculture and to discard the practice of cereal-legume rotations and green manuring. With the recent increases in production and transportation costs of synthetic N, appropriate techniques for estimating the contribution of symbiotically fixed atmospheric N_2 to soil N by legumes in crop rotation should be re-examined.

Appropriate techniques for assessing N_2 fixation can be difficult especially in routine studies when a large number of accessions are under consideration. Although recent reports have addressed the total N difference, acetylene reduction and ^{15}N methods[12,18,24,27], differences in

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opinion still remain regarding the assumptions and appropriate controls for ^{15}N-isotopic methods[3,6,7,8,16,24,27]. One major point of disagreement seems to be the type of controls and the level of fertilizer N applied.

Both isotopic dilution and A_N value methods for estimates of N_2 fixation have been obtained by comparing the uptake of ^{15}N-enriched N fertilizers by a N_2 fixing legume to that of a non-fixing control crop. Minimal rates of ^{15}N-enriched fertilizer applied to the legume were proposed[9] so as not to inhibit symbiotic N_2 fixation. Higher levels have been applied to the non-fixing reference crop to prevent N deficiency[10]. Witty[27] suggested the use of gypsum-pelleted ^{15}N fertilizer, or any other treatment which leads to a more stable soil enrichment to reduce errors caused by mismatched N uptake patterns in the N_2 fixing and non-fixing crops.

The question of the appropriate non-fixing control crop has been investigated[17] where the nodulating soybean in non-fixing mode, *i.e.*, inoculated with an ineffective strain of *Bradyrhizobium japonicum*, was considered the most appropriate control for quantifying N_2 fixation by effectively nodulated soybeans. From greenhouse derived data, Rennie[16,18] concluded that yield-independent estimates of dinitrogen fixation, such as the isotope dilution procedure, were more precise than the yield-dependent A value or difference methods and that the total N balance method cannot be used with confidence to estimate N_2 fixation in field-grown legumes. Contrasting studies[24] conclude that non-nodulating plants are valid controls provided the species exhibit similar maturity dates and similar patterns of fertilizer N uptake.

The choice of the non-fixing crop and appropriate N application level becomes potentially difficult and expensive when estimating N_2 fixation for a large number of legumes. One option would be to monitor the relative depletion of soil N under a fixing and non-fixing crop to estimate N_2 fixation[23]. Another option would be to use an ^{15}N-modified difference method where the contribution of fertilizer N is subtracted from the total plant N of the non-fixing control. Such an approach would provide for some N nutrition to the control as well as reduce the scale and cost of obtaining N_2 fixation estimates.

The objectives of this study were to:

1. determine the effect of N application rate and non-fixing crop on estimates of seasonal N_2 fixation by fababean, lentil, pea, Austrian winter pea, blue lupin and chickpea;
2. evaluate a modified difference method for assessing N_2 fixation;
3. determine the seasonal N_2 fixation and the net contribution of fixed N_2 to the soil N pool by the legumes.

Materials and methods

Field studies

The study was conducted on field sites at the Spillman Agronomy Farm near Pullman, WA in 1981 and 1983, and in a cooperating farmer's field near Genesee, ID in 1982. These sites are typical of the Palouse region of eastern Washington and adjoining northern Idaho, characterized by rolling loessial hills. Most of the total annual rainfall of 500 mm occurs during the winter months from November through March. Winter wheat is the dominant cash crop and is typically rotated with either spring barley, pea, lentil, or in some instances with new alternative crops of fababean, chickpea, and lupin.

Soil characteristics of the field sites are shown in Table 1. Randomized complete block designs with four replications were used each year with the number of inoculated and non-inoculated crops and fertilizer rates varied in each year (Table 2). Four-row plots, 1.2 m × 6.1 m, were seeded with a 30-cm interrow spacing on 8 May 1981, 18 May 1982, and 6 May 1983. Fababean (*Vicia faba* cv. Ackerperle), blue lupin (*Lupinus angustifolis* L. cv. Ulyarrie) and chickpea (*Cicer arietinum* cv. UC-5) were seeded at a rate of 10 seeds m^{-1} of row. Seeds of lentil (*Lens culinaris* cv. Chilean 78), Austrian winter pea (*Pisum sativum arvense* cv. Melrose) and pea (*Pisum sativum* cv. Alaska, 1981 and 1982; cv. Tracer, 1983) were sown at a rate of 33 seeds m^{-1}. Spring wheat (*Triticum aestivum* L. cv. Waverly) and barley (*Hordeum vulgare* L. cv. Advance) were planted at a rate of 63 seeds m^{-1}.

Aqueous solutions of ^{15}N-enriched $(NH_4)_2SO_4$ were applied to subplots 0.6 m wide by 1.1 m long within the primary plot area. In 1981 and 1982, rates of 18 or 72 kg N ha^{-1}, with enrichments of 13.55 and 3.39 (1981) or 11.59 and 2.88 (1982) atom percent ^{15}N excess, respectively, were applied in 1 liter of water. In 1983, 12.35 and 5.33 atom percent excess ^{15}N fertilizer solutions were used for the 10 and 80 kg N ha^{-1} rates, respectively. The fertilizer was incorporated into the top 20 cm of soil by manual cultivation or rototilling. To the remaining primary plot, an identical level of non-enriched $(NH_4)_2SO_4$ fertilizer was broadcast and incorporated.

Legume seeds for the inoculated peat treatments were coated with a gum arabic solution (40% w:v), followed by inoculation with the appropriate *Rhizobium* species with approximately 10^6 rhizobia/seed. In 1981, fababean seeds were inoculated with a mix of *R. leguminosarum* strains VM11-295 and VM26-265 (Dr K W Clark, Univ. of Manitoba). Lentil (all years), Alaska pea (1981 and 1982), and Austrian winter pea seeds (all years) were inoculated with *R. leguminosarum* strain C_1-204 (Washington State Univ.). Blue lupin seed was inoculated with a mixture of commercial *R. lupini* strains 3C261 (USDA, Beltsville, MD) and 96Gl (The Nitragin Co., Milwaukee, WI). In 1982 and 1983, fababean and chickpea were inoculated with a commercial granular material (Soil Implant™, The Nitragin Co., Milwaukee, WI) at a rate of 12 kg ha^{-1} by banding in the seed furrow. Pea was nodulated solely by indigenous rhizobia in 1983.

Nodule mass data were obtained in 1982 from 6 plants of fababean, and blue lupin, and 12 plants of lentil, pea, and Austrian winter pea. The roots were separated from the above-ground portion and gently rinsed of all soil. Nodules were stripped from the roots and weighed. As crop maturity approached in early to mid-August and prior to excessive leaf drop, all plants within the ^{15}N

Table 1. Selected characteristics of soils at the experimental sites

Parameter+	Pullman, WA (1981)	Genesee, ID (1982)	Pullman, WA (1983)
Soil type	Latah silt loam	Palouse silt loam	Staley silt loam
Soil classification	Fine, mixed, mesic xeric Argialbolls	Fine-silty, mixed, mesic, Pachic Ultic Haploxerolls	Fine-silty mixed, mesic, Calcic Haploxerolls
Total soil N (%)	0.13	0.24	0.09
Organic matter (%)	2.91	3.90	2.69
NO_3-N (mg kg^{-1})	12.2	8.2	12.6

+To a depth of 30 cm.

subplots were hand-harvested by clipping and gathering all above-ground material. Foliage in plots was sampled intermittently to determine the time of maximum N accumulation. Plant material was dried in a forced-air oven at 65°C, weighed to determine dry matter production, then ground in a Wiley mill to pass a 2-mm sieve. The remaining portion of the plot was harvested to obtain seed grain yields.

Laboratory analyses

Total plant N and seed N were determined from triplicate subsamples from each plot. Samples were digested by the Kjeldahl procedure[5] in an aluminum block assembly, followed by colorimetric analysis with a Technicon AutoAnalyzer II system[22]. Duplicate samples from each plot were taken from the Kjeldahl digestions and steam-distilled in preparation for mass spectrometric analysis[4]. Conversion of NH_4-N to N_2 was by LiOBr oxidation[15,21]. Ratios of $^{15}N/^{14}N$ in the samples were determined with a Consolidated Electrodynamics Corporation Mass spectrometer model 21-620. Atom percent ^{15}N excess was calculated with reference to natural abundance in the atmosphere (0.366 atom percent ^{15}N). Published equations[19] were utilized for calculating A_N values, fertilizer uptake, and N_2 fixation. When both the controls and N_2 fixing legumes are fertilized at equal rates, the same estimates of N_2 fixation are obtained by using A_N value or isotopic dilution equations.

Results and discussion

Selected soil parameters for the three years are shown in Table 1. The two Pullman sites, although located within one km of each other, were on different soil types. Residual soil NO_3-N levels were similar at all sites, although somewhat higher at the Pullman sites. The cool, moist weather in June 1981 was partially responsible for the relatively high dry matter and seed yields of the legumes, but also intensified a barley yellow dwarf (BYD) disease which reduced the growth and yield of wheat. An aphid and pea leaf weevil infestation combined to reduce plant dry matter production and seed yield of some legume crops in 1982.

Measurements of plant growth parameters

Plant dry matter, total plant N, and grain yields are summarized in Table 2. Total dry matter production for wheat in 1981 and 1982 was comparable, even though BYD was present in 1981. Yields in 1981 of lentil, pea, and Austrian winter pea, and in 1983 of pea and fababean were near average. In all three years, legumes that were inoculated were well-nodulated. The non-inoculated lupin was nodulated by indigenous Rhizobium and consequently could not be utilized as a non-fixing control. Nodule mass values, also reported in Table 2, illustrate that the non-inoculated chickpeas were not nodulated and could be used as a non-fixing control crop.

Plant dry matter production and total N of wheat grown in plots treated at the two fertilizer levels were nearly identical in 1981. An increase in fertilizer N to the non-fixing crops tended to increase total plant N in 1982, although dry matter yields did not increase proportionately. Lower soil NO_3-N levels at the 1982 experimental site may have contributed to the slightly greater response to fertilizer N than in

Table 2. Dry matter production, total plant N, and seed yield of crops grown in 1981, 1982, and 1983

Treatment: Inoculation and crop	Treatment: Fertilizer N rate kg ha^{-1}	Dry matter kg ha^{-1}	Total plant N kg ha^{-1}	Seed yield kg ha^{-1}	Seed N %	Nodule mass g m^{-2}
		Pullman — 1981				
Inoculated						
Fababean	18	7424	161	1106	3.8	
Lentil	18	5433	135	1387	4.1	
Pea	18	5914	140	2462	3.7	
Austrian winter pea	18	5876	142	2316	3.8	
Lupin	18	6679	131	2235	3.5	
Non-inoculated						
Lupin	18	6054	111	2143		
Wheat	18	5004	75	—		
Wheat	72	4862	75	—		
		Genesee — 1982				
Inoculated						
Fababean	18	3139	107	523	3.5	6.0
Lentil	18	4658	158	746	4.0	3.2
Pea	18	4030	122	1390	3.5	10.0
Austrian winter pea	18	3470	116	364	3.5	9.0
Lupin	18	1979	48	155	3.4	4.1
Non-inoculated						
Chickpea	18	2945	53	1053		0.0
Chickpea	72	5074	106	1378		0.0
Barley	0	4587	75	2082		
Wheat	0	4366	75	1453		
Wheat	18	4429	75	1737		
Wheat	72	4522	102	1563		
		Pullman — 1983				
Inoculated						
Fababean	10	5446	107	1876	4.5	
Pea	10	5515	116	2139	3.5	
Chickpea	10	3948	60	1705	2.8	
Non-inoculated						
Chickpea	10	4200	54	1583	2.6	
Chickpea	80	3899	61	1786	2.6	

1981 or 1983. In 1983, non-inoculated chickpea did not respond to applied N indicating that adequate N for plant growth was obtained from the soil.

^{15}N Determined Parameters

Nitrogen derived from fertilizer was generally higher for the non-nodulating legumes and wheat than for N_2 fixing legumes, and increased substantially with an increase in fertilizer rate (Table 3). Consequently, the non-fixing plants had lower A_N values than nodulated legumes. Furthermore, a significant decrease in A_N values was observed with an increase in fertilizer N rate in all years. These data disagree with those of others[1,13] who reported constant A_N values at varying fertilizer N

Table 3. Plant tissue ^{15}N-enrichment, fertilizer N uptake, fertilizer use efficiency, and A_N values obtained in 1981, 1982 and 1983

Treatment: Inoculation and crop	Treatment: Fertilizer N rate kg ha^{-1}	Plant atom % ^{15}N excess %	NDFF^{+} %	FUE^{+} %	A_N value kg N ha^{-1}
		Pullman — 1981			
Inoculated					
Fababean	18	0.484	3.6	31.9	486 a§
Lentil	18	0.689	5.1	38.1	336 b
Pea	18	0.653	4.8	37.5	335 b
Austrian winter pea	18	0.508	3.7	29.5	462 a
Lupin	18	0.656	4.8	35.2	354 b
Non-inoculated					
Lupin	18	0.920	6.8	41.9	247
Wheat	18	1.194	8.8	36.7	186 m§§
Wheat	72	1.235	36.5	38.0	126 n
		Genesee — 1982			
Inoculated					
Fababean	18	0.366	3.2	18.7	553 a§
Lentil	18	0.477	3.9	33.8	449 ab
Pea	18	0.463	4.0	27.1	433 ab
Austrian winter pea	18	0.415	3.6	23.1	484 ab
Lupin	18	0.746	6.4	17.2	262 b
Non-inoculated					
Chickpea	18	1.216	10.5	30.9	154 o§§
Chickpea	72	1.068	37.1	54.6	122 p
Wheat	18	1.209	10.4	43.4	155 o
Wheat	72	1.020	35.4	50.2	131 p
		Pullman — 1983			
Inoculated					
Fababean	10	0.216	1.1	11.4	913 a§
Pea	10	0.306	1.6	18.1	640 b
Chickpea	10	0.580	2.6	12.1	341 c
Non-inoculated					
Chickpea	10	0.628	3.2	16.6	347 r§§
Chickpea	80	0.619	27.0	20.3	197 s

$^{+}$NDFF = Nitrogen derived from fertilizer
$^{++}$FUE = Fertilizer use efficiency
§Within a year and within the group of N_2 fixing crops, A_N value means followed by the same letter are not statistically different ($P < 0.05$) according to a pooled-t analysis.
§§Within a year and within the group of non-fixing controls, A_N value means of the controls fertilized at the lower rate are statistically greater according to a t analysis; $P < 0.06$ (1981), $P < 0.02$ (1982), $P < 0.09$ (1983).

levels in short term greenhouse studies. The lower A_N value of the soil obtained by the non-fixing control fertilized at the higher rate may result in an overestimation of the amount of N_2 fixed by the legumes.

The A_N values in 1982 of the wheat and non-inoculated chickpea plots at the same fertilizer N rates were nearly identical, even though their rooting patterns are radically different. These results are consistent with those of others[26] and suggest that crop-type does not severely affect the soil A_N value when environmental growth factors similarly affect all crops involved and when both crops have a similar N uptake profiles[27]. The consistent decrease in A_N value with an increase in fertilizer N rate suggests a reduced availability of soil N with an increase in fertilizer rate.

The generally lower ^{15}N enrichments in plant tissues and the correspondingly higher A_N values suggest that the effectively nodulated legumes, especially fababean, obtained a large proportion of their total N from symbiotic fixation (Table 3). The fertilizer use efficiency (FUE) ranged from 17 to 38% for the inoculated crops in 1981 and 1982, and decreased slightly in 1983. The FUE values of the inoculated legumes tended to be somewhat less than those of the non-fixing control crops.

N_2 fixation

Estimates of seasonal N_2 fixation in 1981 ranged from 17 to 29% higher when calculated using wheat as the control fertilized at 72 as compared to 18 kg N ha^{-1} (Table 4). This trend was consistent throughout the three-year study. The higher N_2 fixation values were a consequence of the lower reference A_N values obtained at the higher fertilizer N rate (Table 3).

No increase in plant dry matter production, or total plant N of the control was obtained beyond an application rate of 18 kg N ha^{-1} in 1981 (Table 2). However, in 1982, total plant N of chickpea and wheat, and dry matter of the non-inoculated chickpea in 1983 increased at the higher fertilizer rate, suggesting that the controls fertilized at the lower rate were deficient in N. While there may be some rationale for accepting the lower N controls in 1981 and the higher N controls in 1982 and 1983 based on plant growth and yield of N, further verification of the rate of depletion of the ^{15}N soil pool would be required for both the N_2 fixing and control crops. Estimates of N_2 fixation of all legumes were nearly identical when either chickpea or wheat fertilized at the same rate were used as the non-fixing controls.

Total N_2 fixation for 1981 and 1982 was consistently highest for fababean, lentil, pea and Austrian winter pea, ranging from 53 to 115 kg N ha^{-1} over years and different controls. These estimates are comparable to values reported for high N_2 fixing cultivars of *Phaseolus*

Table 4. Seasonal N_2 fixation values (N_2 fixed, kgN h a^{-1}) as determined by A_N value technique using non-inoculated chickpea and spring wheat as control crops

	A_N Value Technique							
	1981*		1982**				1983***	
	Wheat		Chickpea		Wheat		Chickpea	
			N applied (kg N ha^{-1})					
Legume crop	18	72	18	72	18	72	10	80
Fababean	96	115	75	81	74	79	64	81
Lentil	57	80	100	111	99	107	—	—
Pea	63	86	76	84	75	82	53	80
Austrian winter pea	81	99	76	84	76	82	—	—
Lupin	59	80	19	24	18	23	—	—
Chickpea	—	—	—	—	—	—	0	27

*In 1981 the pooled difference in N_2 fixation for all legumes was significantly less ($P < 0.02$) using spring wheat fertilized with 18 kg N ha^{-1} as a reference as compared to wheat fertilized at 72 kg N ha^{-1}.
**In 1982, the pooled difference in N_2 fixation for all legumes was significantly less ($P < 0.06$) using the non-fixing crops fertilized with 18 kg N ha^{-1} as a reference as compared to those crops fertilized at 72 kg N ha^{-1}. Values obtained for N_2 fixation from the reference crops fertilized at the same rate were statistically similar ($P > 0.54$).
***In 1983, the pooled difference in N_2 fixation for all legumes was significantly less ($P < 0.01$) using non-inoculated chickpea fertilized with 10 kg N ha^{-1} as a control as compared to non-inoculated chickpea fertilized with 80 kg N ha^{-1}.

vulgaris L. in southern Alberta[20], considerably below values for soybean under irrigation in the Columbia Basin of Washington[2], and slightly above values for pea reported earlier[14] in the same study area. The percent of plant N derived from symbiotic N_2 fixation varied from 42 to 60% in 1981, and tended to be somewhat higher in 1982 and 1983.

Estimates of N_2 fixation calculated by a modified difference method were comparable to those obtained by the A_N value (or by isotopic dilution at equal N rates) in all years (Table 5). Values for FUE (Table 3) for the fixing crops and the non-fixing control were in a similar range in 1981 and 1983. However, in 1982, FUE values were relatively lower for the N_2 fixing legumes. Estimates of N_2 fixation calculated by the traditional difference method were 17 to 57% lower than those derived by the A_N value (or isotopic dilution). This trend has also been reported for soybeans[10]. In the calculation of the modified difference method, we are assuming that the small amount of N added to the fixing crops (18 kg N ha^{-1}) had little effect on N_2 fixation and total accumulation of N. Unpublished data from this region support this assumption.

From the 1982 data calculation of N_2 fixation by modified difference using wheat rather than chickpea would lower the estimate for N_2

Table 5. Comparison of N_2 fixation values (N_2 fixed, kg N ha^{-1}) for three years as determined by A_N value, the modified difference method and by the difference method

Legume crop	1981		1982			1983	
	A_N*	MD**	A_N*	MD+	D++	A_*	MD+++
Fababean	96	93	75	60	32	64	53
Lentil	57	67	100	111	83	—	—
Alaska pea	63	72	76	75	47	53	62
Austrian winter pea	81	75	76	69	41	—	—
Lupin	59	63	—	—	—	—	—
Chickpea	—	—	—	—	—	6	7

*Estimates were obtained by A_N value (isotopic dilution) using the low rate of N to wheat and non-nonulated chickpea, respectively in 1981 and 1983. The mean value for chickpea and wheat was used in 1982 since these respective values were nearly identical.
**Modified difference method: The difference in total plant N of the N_2 fixing legume and plant N of spring wheat fertilized with 18 kg N ha^{-1} after subtracting the contribution of fertilizer N to the wheat.
+Modified difference method: The difference in total plant N of the N_2 fixing legume and plant N of non-inoculated chickpea fertilized with 18 kg N ha^{-1} after subtracting the contribution of fertilizer N to chickpea.
++The difference in total plant N of the N_2 fixing legume and plant N of unfertilized spring wheat.
+++Modified difference method: The difference in total plant N of the N_2 fixing legume and plant N of the non-inoculated chickpea fertilized with 10 kg N ha^{-1} after subtracting the contribution of fertilizer N to the chickpea.

fixation, since total plant N was greater for wheat and nitrogen derived from fertilizer values were similar (Table 3). However, chickpea is the preferred choice since FUE values for N_2 fixing legumes were more similar to chickpea than to wheat. Using chickpea rather than wheat as a control is further justified, since most legume crops and not wheat were affected by the aphid infestation in 1982.

Estimates of the contribution of fixed N_2 to the soil N pool are summarized in Table 6. Total seed N was subtracted from the estimate

Table 6. Net effects of legume crops on the soil N pool in 1981, 1982, and 1983+

Crop	Pullman — 1981++	Genesee — 1982*	Pullman — 1983**
Fababean	+54	+57	−20
Lentil	0	+70	—
Pea	−28	+27	−22
Austrian winter pea	−7	+64	—
Lupin	−19	+14	—
Chickpea	—	—	−48

+Calculated as the difference between N_2 fixed using the appropriate A_N value, and N removed in the seed at harvest.
++Calculated using the spring wheat (18 kg N ha^{-1}) as a non-fixing control.
*Calculated using the mean of the spring wheat and non-inoculated chickpea (18 kg N ha^{-1}) as a non-fixing control.
**Calculated using non-inoculated chickpea (10 kg N ha^{-1}) as a non-fixing control.

of N_2 fixed to obtain a value of net N addition or removal by each legume crop. Except for fababean in 1981, the legumes removed from 0 to 48 kg N ha^{-1} from the soil in 1981 and 1983. These levels are significant when estimating N budgets in crop rotation management systems. The lower-than-average seed yields in 1982 after an aphid infestation late in the season resulted in a general net contribution of symbiotically fixed N_2 to the soil N pool.

These data support the conclusion that residue incorporation of normal-yielding legumes often results in less net N input than previously assumed. It has been reported that soybeans would have the effect of supplying about 16.7 mg N per kg seeds produced per hectare to a following corn crop[25]. However, this response was suggested as a rotational effect[11,25]. In studies on N_2 fixation, the choice of the non-fixing crop may be influenced by the rotation. Using a cereal as a non-fixing crop may not be desirable where cereals have been grown continuously or frequently in the rotation, since cereal performance and production may be reduced by monoculture. Furthermore, a cereal may be less suitable as a control than a non-nodulating legume when disease or insect infestations reduce legume productivity and not cereal production. This was observed in 1982.

In summary, estimates of N_2 fixation by A_N value at higher N application to the non-fixing controls were substantially greater than by isotope dilution. Very similar estimates of N_2 fixation were obtained using either spring wheat or chickpea as controls. Estimates of N_2 fixation by difference were substantially below those values obtained by A_N value and are not considered suitable. By using a modified difference method, where labeled ^{15}N-fertilizer was applied to the control, estimates of N_2 fixation were nearly equal to values obtained by A_N value (and isotopic dilution) when the contribution of fertilizer N in the control was subtracted. This approach may be useful when the type of controls and N rates are verified from previous studies on N_2 fixation using isotopic dilution or A_N values. The modified difference method is extremely attractive and less expensive in routine studies in which a large number of legumes are evaluated since ^{15}N need be added only to the non-fixing control.

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