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Effectiveness of Rhizobia-Legume Associations

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Abstract

Effectiveness of rhizobia-legume associations in dinitrogen fixation depend on susceptibility of the host legume to nodulation, infection by an effective strain of *Rhizobium*, and conditions favorable for plant growth. Effectiveness ratings of rhizobial strains are confounded by variability in host genotype, variability in rhizobial culture, number of inoculated rhizobia, mineral nutrition and photosynthetic process, all of which are usually indicated by dry matter production. Response of legume production to N fertilization indicates opportunity to improve effectiveness of the association. Proper rhizobia-legume associations can apparently obtain from N_2 all the N needed to match photosynthetic activity. Research is needed to provide and maintain proper associations for all legume crops, and to achieve adequate inoculation where needed.

INTRODUCTION

Legumes can express their growth potential without fertilizer or soil nitrogen only if effective nodules are formed by the appropriate rhizobia. These rhizobia are the unseen, but vitally important, partners of the legume plant that provide nitrogen (N) from dinitrogen (N_2). Lack of the appropriate rhizobia has resulted in failures of introduced legumes. Sometimes agronomists have decided certain legumes are not adapted to an area without realising that rhizobia were absent or ineffective. Even native legumes can benefit from inoculation with selected strains of rhizobia. The susceptibility of the host legume to infection and its ability to form an effective symbiosis is also necessary in addition to the presence of a compatible rhizobia strain.

Nutman (1976) summarized the available data on the estimates of N fixed by legumes (Tables 1 and 2). Those estimates which are based on total N in the tops ignore the considerable amount of N in the roots, and the contribution of soil or fertilizer N. Better estimates could be obtained from field plots which include a treatment with an ineffectively nodulated legume or a non-nodulating line of the legume, or a non-legume to give a measure of the soil N contribution.

The potential to increase the N uptake by increasing the yield of legumes is very great. When world yield estimates (Dawson 1970) are considered, the lowest producing areas achieve 60% (49 to 93%) of the world average and only 35% (18 to 45%) of the average of the highest producing area. Limiting factors include mineral nutrient deficiencies, inadequate nodulation and climatic problems.

Table 1. Nitrogen fixed by perennial tropical and subtropical forage and browse plants, green manure and shade trees (kg N/ha/yr).

Plant	Average	Range
Centrosema pubescens (Centro)	259	126-395
Desmodium intortum and *D. canum* (tick clover)	90	---
Leucaena glauca	277	74-584
Lotononis bainsii	62	---
Sesbania cannabina	542	---
Stylosanthes spp. (stylo)	124	34-220
Mixtures of centro and stylo	115	---
Phaseolus atropurpureus (siratro)	291	---
Mikanea cordata	120	---
Pueraria phaseoloides (kudzu)	99	---
Enterolobium saman	150	---

Data from Nutman (1976)

Table 2. Nitrogen fixed by pulses (kg N/ha/crop)

Plant	Average	Range
Vicia faba (broadbean, fababean)	210	45-552
Pisum sativum (garden pea)	65	55-77
Glycine max (soybean)	103	1-168
Lupinus spp.	176	145-208
Vigna aureus (green gram)	202*	61-342
Vigna unguiculata (cow pea)	198	73-354*
Cajanus cajan (pigeon pea)	224	168-280
Cicer arietinum (chick pea)	103	---
Lens esculenta (lentil)	101	88-114
Arachis hypogaea (groundnut)	124	72-124
Canavalia ensiformis	49	---
Cyamopsis tetragonolobus (guar)	130	41-220
Calopogonium mucunoides (calapo)	202*	370-450

Data from Nutman (1976); * Extrapolated from pot experiments.

SUSCEPTIBILITY OF THE HOST LEGUME

All members of the Leguminosae family do not form effective nodules even when exposed to the proper bacteria. Among the non-nodulating legumes, Fred *et al.* (1932) included redbud (*Cercis canadensis*) and *Cercis siliquastrum*; the

Kentucky coffee tree (*Gymnocladus dioica*); several species of Cassia; the honey locust *Gleditsia triacanthos*); and *Acacia brileyana*, the only exception in 24 species of *Acacia* tested.

Allen and Allen (1976) state, "nodule formation is more commonly present than absent in the sub-families Mimosoideae and Papilionoideae; the reverse is true in the subfamily Caesalpinioideae .... about 70% of caesalpinoid species lack nodules ... 10% of the genera have both nodulated and non-nodulated species. The legumes that are important and can contribute to tropical agriculture have been reviewed recently (Döbereiner and Campelo 1977).

Even the nodulating legumes have variants that are non-nodulating under most conditions; non-nodulating lines are known for soybeans (*Glycine max*) (Williams and Lynch 1954) and other legumes. The ability to nodulate appears to be controlled by a simple genetic factor. In soybeans, resistance to nodulation was reported to be caused by a single recessive gene, called no (Williams and Lynch 1954), but now named rj_1 (Caldwell 1966). These non-nodulating lines are useful in estimating the N_2 fixed by a nodulating line, and in studying the nodulation process. Dinitrogen fixation cannot be assumed to be large just because the plant is a legume.

Legumes that show a growth response to fertilizer N may lack the ability to form effective N-fixing nodules or to utilize the N fixed in the nodules. Terminology used to describe nodulation responses is not uniform. The system shown in Table 3 (Vest *et al.* 1973) is compared to usage in other literature where the word "effective" has been commonly used to mean the ability of the legume-rhizobium nodules to fix dinitrogen, the word "infective" to describe the ability of the rhizobia to form nodules, and the word "efficient" to relate to the amount of dinitrogen fixed per unit of nodule mass (or, sometimes, per unit of carbohydrate utilized).

As Norris (1962) pointed out, "the symbiosis in the nodule is dependent in the first place on mutual acceptability of bacterial strain and host. Every degree of selectiveness of the host exists, from species like *Lotononis bainesii* that will accept only one peculiar strain of bacterium to so-called 'Promiscuous' species like *Macroptilium lathyroides* that will nodulate with a wide array of strains". In recent reviews (Caldwell and Vest 1977; Vest *et al.* 1973) the role of the macrosymbiont (host plant) in nodulation and N fixation have been described. Little precise genetic information is available on most tropical legumes.

The systems that form nodules, but lack the ability to provide adequate N to the host, are of particular interest because observations of good nodulation can be misleading. The soybean cultivar 'Peking' inoculated with *Rhizobium japonicum*, strain Beltsville serotype 123, formed large nodules with slightly pink, green or white interiors (Caldwell and Vest 1977). Dinitrogen fixation was very

Table 3. Classification and description of nodulation responses in soybeans

Classification	Description*
I. Non-nodulated [or Non-infective]	No nodules, no external evidence on root that nodule formation has been initiated. Plants small and chlorotic symptoms typical of N deficiency.
II. Nodulated [or Infective]	Roots show that nodule formation has been initiated.
A. Ineffective	Roots bear cortical proliferations and/or small nodular-like structures with white green or pink interiors. Plant is small chlorotic. Symptoms typical of N deficiency.
B. Effective	Roots are nodulated.
1. Inefficient [or Low Effectiveness]	Nodules have green or white interior, sometimes pink. Plant is small to medium with degree of chlorosis depending on degree of inefficiency.
2. Efficient [or High Effectiveness]	Nodules have pink-red interior. Plant is dark green and healthy in appearance.

Data from Vest *et al.* 1973; * Descriptions are of plants grown in N limiting medium.

low and the plants showed typical N deficiency symptoms. A similar system was reported for *Trifolium subterraneum* cultivar 'Northam First Early' inoculated with *R. trifolii* strain NA 30. Both the host cultivars and the rhizobial strains in these two examples were able to form effective (efficient) N_2 fixing symbioses with other strains and cultivars, respectively. To obtain completely adequate N_2 fixation, it is critically important to select susceptible host legume genotypes and an effective, efficient strain of rhizobia.

SELECTION OF RHIZOBIAL STRAINS

Selection of highly effective rhizobia has been a major emphasis since the discovery of the symbiotic relationship (Fred *et al.* 1932; Wu 1959; Brill 1974). Schwinghamer (1977) has reviewed the genetic studies relating to the *Rhizobium*. Considerable information has accumulated, but, at this time, genetic studies have neither produced a "super-strain" nor a "key" to indicate greater effectiveness. Tests involving the growth of plants and the measurement of uptake of N are still

required to determine effectiveness, probably due to many interactions between the genetic factors in host plant and bacterial strain.

The considerations involved in strain selection of rhizobia were discussed by Date (1975). Basic methods have been described by Vincent (1970). Strains of rhizobia selected for use in inoculating legumes should be effective on the host plant, able to multiply and form nodules readily in the field. Other characteristics useful in producing inoculants include ease of growth, survival in carrier and on the seed. But abundant, massive nodulation has been challenged as a desirable trait because of the potential greater use of photosynthate by the nodule mass, thereby reducing photosynthate available for top and seed production. Greater amounts of N fixed per unit of nodule would appear to be a desirable attribute. Because this attribute is difficult to measure, less effort has been spent in selecting photosynthate-efficient nodule systems than would seem to be desirable.

Testing rhizobial strains for effectiveness in N_2 fixation appears to be simply a matter of growing plants with different strains and measuring either total N or dry matter production. Unfortunately, several factors can complicate the interpretation and may be part of the reason why field tests do not always correlate well with greenhouse tests.

The number of rhizobia present at inoculation can influence the amount of N_2 fixed (Weaver and Frederick 1972). With soybeans (*G. max*) and each of two strains of rhizobia, the growth of the soybeans for seven weeks in the greenhouse increased as the number of inoculated rhizobia were increased to 10^6 rhizobia per seed. Because many workers inoculate their effectiveness trials heavily, often using a suspension from an agar slant, the effect of rhizobial numbers may not be critical in the greenhouse. But in the situations where the inoculum is put on the seed, or survival is poor, the effectiveness of rhizobial strains can be confounded unless care is taken to assure proper numbers of rhizobia. And, in the field, Ham *et al.* (1971) found commercial inoculants supplied from 0 to 18000 rhizobia per seed. Deficiencies and toxicities of other elements can also reduce the apparent effectiveness of N_2 fixation. Other participants will discuss the relationships with mineral nutrients further.

The importance of carbohydrate supply from photosynthesis cannot be overstated. Light must be adequate and comparable during tests. The technique must provide adequate CO_2; Gibson (1977) found that seedlings growing in test tubes were restricted in their growth by lack of adequate CO_2 and did not give a proper measure of effectiveness. Hardy and Havelka (1973) reported that CO_2 enrichment of the atmosphere around growing soybeans increased N_2 fixation fourfold. The rhizobial strains (unidentified) that were present in the nodules had responded by doubling nodule weight, and nitrogenase acitivity per unit nodule weight, and

prolonging the nitrogenase activity. Similar results but with smaller increases were reported from addition of either CO_2 or straw application by Shivashankar *et al.* (1976). These results indicate that photosynthate is a major limiting factor in symbiotic N fixation. Conditions promoting plant growth also promote N_2 fixation. Increased CO_2 supply to provide greater stress on N_2 fixation might be useful in screening strains. Other conditions should be favorable and consistent to avoid confounding of the results.

Variation in effectiveness of pure cultures of a strain has been reported in a recent report by Roughley (1976). Not only does the same strain obtained from different sources show variation, but isolates from the same culture gave an eightfold variation in plant dry weight of siratro (*Macroptilium atropurpureum*). Large differences in nodule weight and nodule efficiency were also measured as shown in Table 4. Roughley (1976) concluded, "these results strikingly demonstrate both the need for regular tests of strain effectiveness and the possible danger of using single colony isolates as mother cultures for inoculant production." More information is needed to determine the adequacy of methods of maintaining culture collections. The need for testing all cultures to be used as inoculants for effectiveness is more obvious than practiced.

Table 4. Growth of siratro inoculated with seventeen cultures of CB 756 derived from one source.

Isolate of CB756	Mean nodule wt.* (mg)	Mean plant wt.* (mg)	Increase-mg nodule (mg)
1	15	209	12.1
2	11	156	11.6
3	11	138	10.0
4	9	135	11.9
5	10	126	9.8
6	8	119	11.4
7	8	119	11.4
8	8	106	9.8
9	9	102	8.2
10	7	93	9.3
11	6	72	7.3
12	3	65	12.3
13	6	65	6.2
14	6	57	4.8
15	6	58	5.0
16	2	37	4.5
17	-	26	-
Uninoculated	0	28	-

Data from Roughley (1976). * Mean of 10 replicates.

INOCULATION OF THE LEGUME

Predicting the need to inoculate the legumes has often been attempted. General relationships, such as the presence of the host plant (Roughley 1976) are often used.

The need to inoculate when a legume is grown for the first time is generally recognized, and inoculation should always be included when introducing a new legume to the field. When a crop has been grown for many years in the area, the situation is more complex. In Iowa from 13 fields not known to have grown soybean, 10 fields had less than 10^3 rhizobia/g. Of 34 fields known to have grown soybeans previously, 3 had less than 100, 5 had between 10^2 and 10^4, and 26 had more than 10^4 rhizobia/g (Weaver *et al.* 1972). The presence of soil rhizobia that will nodulate the legume can present a problem in the successful introduction of a superior strain.

These variations are of concern to the individual farmer. If only one hectare out of 10 hectares responds to inoculation, which hectare is it? The cost of the inoculant is commonly much less than 1/10 of the value of the yield increase for statistical significance. In consideration of these odds, top managers often inoculate legumes to be sure that no part of their field is inadequately supplied with effective rhizobia.

Inadequate performance by soil rhizobia can be due to inadequate numbers of rhizobia or to low effectiveness of the rhizobial strains present. In our experience, it is difficult to get soybean yield responses to inoculation when the soil rhizobial population is greater than 10^3/g. More work is needed to establish such relationships for all legumes.

The data in Table 5 (Weaver and Frederick 1974) show that inoculated strains can successfully form nodules against soil rhizobia competition, but high numbers are required. The number of rhizobia per seed required to obtain 50 percent inoculant nodulation was 1000 times as great as the number of compatible rhizobia per gram of soil. What is the number of rhizobia per seed needed to ensure effective nodulation?

More studies are needed to establish the probability of getting a yield response when high quality inoculants are used for legumes where the numbers and the general effectiveness of the soil rhizobia has been assayed. In field studies the general growth conditions and the need for N fixation to produce the crop must also be considered. Results are needed at many sites over a period of years to assess climatic effects before accurate predictions can be made.

ADEQUACY OF SYMBIOTIC N FIXATION

The critical test of effectiveness occurs in the field. When fertilizer N does increase legume yields, opportunity exists for improving the biological N

Table 5. Effects of inoculation rates on rhizobial population nodule numbers and recovery of inoculated strain.

Initial soil soybean rhizobia/g soil	Location 1 10^2	Location 2 10^4	Location 3 10^5
Inoculum-rhizobia/seed	Rhizosphere-rhizobia/g soil		
0	10^2	10^4	10^5
$10^{2.5}$	10^2	10^4	10^5
$10^{4.5}$	10^2	10^4	10^5
$10^{6.5}$	10^3	10^4	10^5
$10^{8.5}$	10^5	10^5	10^5
	Nodules/plant at 30 days		
0	2	8	20
$10^{2.5}$	2	8	20
$10^{4.5}$	2	8	20
$10^{6.5}$	7	11	20
$10^{8.5}$	10	12	20
	Inoculant strain Recovery - % nodules		
0	0	0	0
$10^{2.5}$	10	1	0
$10^{4.5}$	15	5	0
$10^{6.5}$	70	40	10
$10^{8.5}$	90	50	50

Liquid cultures of *R. japonicum* were applied in rows at planting with soybean seeds to cool, moist Mollisols.
Data from Weaver and Frederick (1974).

fixation system. The question is whether the N_2 fixing system can be improved sufficiently to meet the N need where responses occur to N fertilization. Kang (1975) concluded that 30kg N/ha was required with inoculated soybeans (Table 6). Edje *et al.* (1975) obtained increasing response to fertilizer N on dry beans (*Phaseolus vulgaris*), with an increase of 1600 kg seed beans at 200kg N/ha. It is apparent there was inadequate nodulation. Such results have caused many agricultural agencies to recommend fertilizer N on legumes.

On the other hand, Eaglesham and Dart (1974) reported that inoculated *P. vulgaris*, cv. Seafarer nodulated satisfactorily in field trials on leached greensand soil. The yield of inoculated plots was double that of the uninoculated plots without N fertilizer, and 10 percent greater than uninoculated plots with 220kg fertilizer N/ha. Hinson (1975) reported on four years of yield tests in

Table 6. Mean yields of beans (kg/ha) at 14 per cent moisture content.

Applied N (kg/ha)	Soybean Yields Uninoculated	Inoculated
0	1110	1140
30	1140	1700
60	1640	1800
120	1760	2010

L.S.D. (P = 0.05): inoculation means, 210; nitrogen means, 280.

Data from Kang (1975).

Florida on three soil types, a range of planting dates, and various rates and dates of N applications. Only one positive response to N was obtained, and the same variety did not respond in an adjacent area of the same field the next year. He concluded, "Care should be taken to provide a favorable soil environment for symbiotic N fixation" and "Occasional responses to N fertilizer on soybeans should not be considered evidence that N should be used on all soybean fields." In evaluating legume responses to fertilizer N, information is needed on quality of inoculation, nodulation, N_2 fixing ability of the legume, and other limiting factors.

Relatively few definitive field experiments are available to assess the nitrogen fixation capability for many of the legumes. The IBP (International Biological Program) studies reported by Nutman (1976) were most helpful in adding both to our information and to our awareness of the inadequacy of the information available.

Rather complex trials with 32 treatments and minimal replication were used in the IBP experiments to determine N_2 fixation. Several replicates of three treatments (uninoculated, inoculated, inoculated plus N) may be adequate to determine inoculation effectiveness (Date 1976) but an application of other nutrients in factorial combination (to give six treatments) would give a better measure of the yield potential of the legume. For inoculation trials, a series of rates of inoculation giving at least a 100-fold difference in rhizobial populations should replace the trials where only the presence or absence of inoculation is tested.

Nitrogen fixation is always limited to the amount required by the crop produced. The idea that a legume has the ability to supply all the nitrogen needs of the crop at maximum production by N_2 fixation is still controversial.

The capacity of the legume N fixing system has been considered by some to be too low to achieve maximum yields, or even maximum economic yields (Harper 1974). The IBP data (Nutman 1976) indicated that the inoculated lucerne (*Medicago sativa*) plots nearly always had a greater N uptake when N fertilizer was added, indicating that N was a limiting factor or that luxury consumption occurred. These yield increases are usually not economic, as is true for the similar small (1 to 5%) increases with fertilizer N that were obtained by Ham *et al.* (1976) with soybean. The symbiotic system must provide economical yields consistently, or farmers will use fertilizer N to insure yields. Reliability in crop production is often more important to a farmer than high yields. Many workers have suggested that a small amount of N (20 to 50 kg N/ha) is desirable to hasten early growth. Most of the studies indicating the benefit of these small N additions do not clearly determine whether the fertilizer N itself is important, or whether the fertilizer N increases the availability of other important nutrients, especially phosphorus. If small amounts of fertilizer N are beneficial, what is the minimum quantity needed?

Another aspect of N_2 fixing capacity is the effectiveness during the growing cycle. The CO_2 enrichment studies clearly showed that the capacity of the N_2 fixing system can be increased tremendously when greater photosynthate is available. It is tempting to conclude that the effectiveness of the legume-rhizobia nodules can match any rate of photosynthesis that can be produced, but experimental proof for such a conclusion is not available.

The third aspect of N_2 fixing capacity is the effectiveness during the maturation period for non-perennial legumes. Garcia and Hanway (1976) proposed that the transfer of photosynthate to the seed sink during maturation could result in nutrient deficiencies due to inadequate uptake by the starved roots. To overcome these deficiencies, nutrients were applied to the leaves. Yield increases were observed under some conditions. Generally, conditions very favorable for high production appear to be most favorable for yield increases by this late foliar fertilization. The implications of this theory of limited carbohydrate transfer to the root nodules are obviously detrimental to N_2 fixing capacity. Efforts to extend root activity during maturation must avoid concommitant yield decreases of the crop.

Continued research is needed to develop compatible and capable genotypes of both the host legume and the nodule-forming rhizobia to minimize the need for N fertilizer, and to eliminate N fertilizer use where possible. The fertilizer N available should be used on non-legumes where it can efficiently and economically contribute to food, fiber and fuel production. Research on fertilizer nutrients other than N is needed to determine the proper amount,

time and place of application to develop the plant and provide photosynthate so N fixation can be more effective.

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