THE POTENTIAL OF LEGUMES FOR PROTEIN PRODUCTION

by P. S. NUTMAN, F.R.S., *Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, England*

The world shortage of food proteins is stimulating much basic and applied research on nitrogen fixation, especially by legumes, and on the many factors, genetic, physiological and environmental, that influence it; some of this work is supported by the International Biological Programme.

Agricultural methods have important impacts on all stages of the nitrogen cycle. Some, both traditional and modern, cause serious depletion of soil nitrogen reserves, and highly intensive systems can also lead to net energy losses (full subsidised agriculture).

Nitrogen balances are examined in different agricultural systems, including those of developing countries where legumes could most quickly and massively augment production of food proteins, and some of the attendant problems are discussed.

In this article I shall endeavour to assess the relevance of Rhizobium to the world-wide problem of protein shortage. It is necessary first to determine the scale of the problem in terms of protein production and consumption before discussing the sources of combined nitrogen and their use. This will be done in the context of the nitrogen cycle, and contributions that biological nitrogen fixation may make in different regions and agricultural systems.

FOOD PRODUCTION AND CONSUMPTION

Table I shows the disparities in food consumption in terms of calories and of proteins in a range of countries chosen for their different sizes, populations and systems of agriculture, and which will later be considered in terms of the nitrogen economy of their soils.

TABLE I

Area, population and food (1969)

Country	Area	Population	Food consumption per capita per diem	
	ha $\times 10^{-6}$	$\times 10^{-3}$	Calories	Protein (g)
U.S.A.	919	203	3290	98.6
Australia	523	12	3220	106.4
India	326	537	1940	47.9
Ethiopia	122	25	2150	72.3
Egypt	100	32	2960	76.3
U.K.	24	56	3180	88.0
New Zealand	27	2.8	3380	108.4

Calory and protein intakes are highly correlated but, in this small selection of countries, not linearly. Indians consume less than half the protein and not much more than half the calories of the citizens of the U.S.A. or Australia, moreover, these are average figures and therefore do not reflect the depriva-tion of the poorest section of society.

Table II shows food production per capita in the major regions of the world, using the 1952/56 statistics as a basis for comparison. In spite of increasing population there is a slow but steady improvement, with Europe, the U.S.S.R. and Australia showing the largest increase, partly by extending their areas of cropping and partly from the application of modern methods—increased fertilizer use, irrigation, plant breeding and disease and pest control. India has taken part in these developments, the area of cultivated land increas-ing steadily from 150 million hectares in 1953 to 165 million hectares in 1968.

TABLE II

Per capita food production (4-yr averages)
(1952-53—100)

	—1956	—1960	—1964	—1968	—1972
W. Europe	103	109	118	128	129
E. Europe & USSR	108	121	122	140	141
N. America	96	97	100	100	100
Oceania	98	106	109	114	106
Latin America	101	103	104	101	102
Near East	102	106	109	110	109
Far East (excluding China)	102	107	106	108	110
Africa	100	101	104	103	104
Total	101	106	107	110	109

For very contrasted reasons North America, Latin America and Africa show very little or no per capita increases. These 4-year averages tend to even out seasonal trends and these can be very large. For example, in 1972 the wheat crop of Eastern Europe and the U.S.S.R. was very seriously reduced by adverse weather conditions, and because of successive droughts certain Australian States, for the first time in about twenty years, imported wheat.

THE GREEN REVOLUTION

Undoubtedly, nitrogenous and other fertilizers are very important factors in promoting an increase in world production, and yields per hectare of major crops (excluding legumes) are correlated with the consumption of fertili-zers. This almost overriding factor in increasing yields, especially cereals, will continue and there is good evidence that some legumes may also fix more nitrogen when given some fertilizer nitrogen at particular stages of growth.

The breeding of cereal crops to respond to large applications of fertilizer in intensive systems of agriculture is of great current interest and promise, but there are difficulties in its application quickly on a sufficiently large scale to affect significant changes in world production. Moreover, the "Green Revolution" is industry-based, requiring technical and managerial skill, not everywhere easily organised or executed, and its extension for limited economic ends rather than social needs can have consequences that are not wholly beneficial. For example, in parts of Peru, where beans and maize form a basic diet reasonably well balanced in calories and proteins, the new high-yielding varieties of maize are being increasingly grown as a cash crop rather than for local consumption, displacing beans and leading to a decline in standards of nutrition.

EFFECTS OF FERTILIZER ON SOIL NITROGEN CONTENT

Experience in many areas also shows that some soils cannot withstand the heavy applications of fertilizer and mechanisation of intensive production without harmful effects. The contrasted responses of crops on different soils to fertilizers and monoculture is a much studied branch of agricultural science, and can be illustrated by the results of the long-term continuous cereal experiments on Broadbalk field at Rothamsted and Stackyard field at Woburn in the U.K. Broadbalk is a deep, well-structured clay-loam and Stackyard a light sandy soil. At Rothamsted good yields have been maintained for 130 years on mineral and nitrogen fertilizers only, whereas at Woburn in an experiment of much shorter duration yields have declined sharply because of increased acidity and incidence of diseases and pests.

Table III shows the terminal soil-nitrogen contents of four selected treatments only in these two fields (Johnston 1968 ; Mattingly pers. comm). Under rotational cropping neighbouring fields at Rothamsted have a nitrogen content of about 0.145 per cent. Farmyard manure on Broadbalk has increased this by about one-half, whereas the nitrogen content of plots given 53 kg of N/ha as fertilizer each year over this very long period has somewhat declined. On the light soil at Woburn this decline has been much greater. The 1927 analyses given were for when the original experiment was ended. On both these very different soils, therefore, the very large total amounts of fertilizer nitrogen applied over the last century or so have led to no increase in soil nitrogen content.

These are perhaps the best documented records available for an arable crop and represent effects under moist temperate conditions. Changes can be more rapid where climatic stresses are greater. On the other hand, fertilizer nitrogen applied to grass swards is partly retained and can lead to increases in organic matter. This is also shown in Table III by the results of some of the plots of Park Grass experiment laid out in 1856 on Rothamsted clay-loam and cut for hay (Warren and Johnston 1963). Nitrogen contents have risen on plots given nitrogenous fertilizer where none or very few legumes occur, to much above that of similar soils in arable rotation without legumes. Soil nitrogen contents have also increased in the 'nil' and 'minerals only' plots and this is because of nitrogen fixed by the legume component of the mixed herbage and possibly also by free-living nitrogen fixing micro-organisms.

TABLE III

Per cent N in soil (total in 0-6″)

	Rothamsted clay		Woburn sandy soil
	Beadbalk wheat since 1843	Park Grass meadow since 1856	Wheat since 1876 (in 1927)
Nil	0.099	0.28	0.109
Minerals P[1] K. etc.)	0.107	0.23	0.117
Minerals + NH_4	0.115	0.30	0.100
Minerals + NO_3	9.113	0.28	0.104
FYM	0.251	—	0.145
Rotation without legumes	*c.* 0.145		*c.* 0.125

THE NITROGEN CYCLE AND NITROGEN BALANCES

Let us now consider the sources of nitrogen and how they are cycled. There is a superabundance of nitrogen in nature, about 20×10^{16} metric tons; 3.7×10^{14} metric tons in the atmosphere, equivalent to about 35,000 tons above each acre, but Table IV shows that even this vast quantity does not represent the major nitrogen resources.

TABLE IV

*The Earth's nitrogen (in metric tons)**

Primary rocks	19.3×10^{16}
Sedimentary deposits	4.0×10^{14}
Atmosphere	3.7×10^{14}
Hydrosphere	2.7×10^{13}
Soil and sea bottom deposits	14.6×10^{11}
Plants and animals	2.8×10^{10}

*After Hutchinson 1954

In the primary rocks nitrogen occurs in inorganic combination as ammonium salts or substitution compounds in complex silicates, and to a very small extent as nitrides. A very large amount of combined N is contained in sedimentary and sea-bottom deposits and in the soil. The transfer of nitrogen between these sources varies enormously in amounts and rates, measured in some cases against seasonal and in others against geological time scales.

Of most concern to us is cycling between the atmosphere and biosphere and particularly the nitrogen-fixing processes. Hutchinson (1954), by rather indirect means which need not be elaborated, estimated that up to 1×10^8 metric tons of nitrogen was fixed annually in biological systems, and on this basis he calculated that each atom of nitrogen would not have cycled more than about 10 times in the course of evolution. There is now good reason to think that Hutchinson under-estimated fixation by several orders of magnitude at least, and man's activities today in agriculture and industry are affecting nitrogen cycling to an increasing degree.

Amounts of nitrogen fixed may also be crudely inferred from the statistics of nitrogen contents and yields of legumes (assuming certain values for addition to and uptake from the soil), and by summing the products of areas under different forms of land use — crops, grassland and so on—and the best estimate that we can at present make of rates of nitrogen fixation for these areas.

These will also tend to be under-estimates because agricultural records for many countries are incomplete and because we can often only make poorly informed guesses about rates of fixation. For example, much of the world's pastures have few or no legumes and produce little. At the other extreme, nitrogen fixation in intensive pastoral systems based on clovers can amount to more than 500kg N/ha, though in parts of the U.K., for example, this is now being sharply reduced by nitrogenous fertilizers.

Such estimates of amounts of nitrogen fixed may then be compared with the total nitrogen yield of all crop production. This gives a very rough estimate of whether nitrogen input and nitrogen output are in balance, as shown in Table V for the countries already considered for per capita food production. Because nitrogen in leguminous crops is mostly fixed it is entered on both the income and expenditure sides of the account and 20 per cent is added to represent a minimum net addition to soil reserves. The table also includes amounts of fertilizer nitrogen used but not the large amounts of combined nitrogen released from power stations, cars and so on, some of which finds its way into soils and crops (Nutman 1971), nor will we consider the nitrogen yield of farm animals or the processes of combined nitrogen loss, such as denitrification and leaching. The source book for these calculations is the F.A.O. Year Book of Agricultural Statistics.

The figures in the table suggest that the nitrogen balance, where achieved, is met in very different ways in different countries, and in areas of most concern there is the severest deficit. The legumes contribution to production is relatively greatest in Australia and least in India. Of more than two thousand million hectares of meadows, pastures and rough grazing in Australia, much of it arid, about one hundred million have been improved by introducing legumes (principally *Trifolium subterraneum*, a self-seeding clover, not before used in agriculture), inoculated with bacteria and usually sown with superphosphate, and sometimes lime and minor elements.

TABLE V

Nitrogen balances 1969 (1000 metric tons N)

	U.S.A.	Australia	India	Ethiopia	Egypt	U.K.	New Zealand
I. N in yield							
Non leguminous crops	4518	365	2841	129	218	326	19
Leguminous crops	1996	6	460	28	21	30	5
Forage and pasture	5180	10700	300	130	—	360	672
II. N input							
Legume crops & forage	8610	12840	912	190	25	468	712
Free-living N-fixation	1400	1000	650	100	14	48	40
Fertiliser nitrogen	6786	172	1360	2	310	692	10
Difference II-I	5102	2941	—679	5	110	552	66

Hutton (1968) considers that at least eighty million more hectares could be similarly developed in the southern areas of Australia and probably more in the sub-tropical regions, where development is also being based on introducing legumes, some new to agriculture. Fertilizer nitrogen is increasingly used but as yet provides a very small proportion only of nitrogen consumed in production. This increase in fertility from pasture legumes is allowing very large areas to be brought into cultivation.

In the U.S.A. legumes also provide the largest amount of nitrogen in primary production, soybeans contributing approximately 1.4 million metric tons of nitrogen. Because of the very large yields of non-leguminous crops, biologically fixed nitrogen is, however, insufficient to balance consumption without the addition of large amounts of fertilizer nitrogen. It is interesting to compare the estimate in this table of the soil nitrogen deficit with the one made by Lipman and Conybeare (1936) for 1930, who calculated, from soil analysis data, a net annual loss of 6.6 million tons of nitrogen. At that time only 300,000 metric tons of fertilizer nitrogen were consumed in the U.S.A.

Fixation of nitrogen by legumes in India would appear to be relatively small, coming mostly from grain legumes. The large quantity of fertilizer nitrogen used is insufficient to attain nitrogen balance, even if a liberal allowance could be made for legume nitrogen not entered in official records and that fixed by other biological agents, such as blue green algae in paddy. Jenny and Raychaudhuri (1960) estimated that the nitrogen content of many Indian soils probably reached steady state within a half-century of being brought into cultivation at between 30-60 per cent of their original contents, and expressed the hope, probably not justified, 'that this will augment again as soon as artificial fertilization is practised on a massive scale'. There appears to be little information on the effect of fertilizers on the nitrogen content of Indian soils, but Viswa Nath (1937) records large losses of total soil nitrogen in plots given sulphate of ammonia over a two year period.

The comparison between Egypt and Ethiopia is interesting. For millenia the soils of Ethiopia, many basaltic in origin, have been subjected to more or less continuous processes of erosion, the soil-laden waters of the Blue Nile eventually depositing their rich burden in the Lower Nile's flood plains and delta. Egypt's consumption of nitrogen must be partly from this source although fertilizer consumption is large. The overall nitrogen reserves of these two countries are declining.

The nitrogen accounts of the U.K. and New Zealand differ strikingly, especially in their use of fertilizer nitrogen and in the amounts of nitrogen harvested in crops and pastures.

These calculations were made for 1969. Table VI shows trends in nitrogen balances for some of the same areas over the last three decades, calculated similarly but restricted to areas under arable crops and expressed as a percentage of nitrogen provided by legumes.

TABLE VI

Per cent legume N to total N (arable crops only)

	U.S.A	India	Egypt	U.K.	World Total
1948-52	21.3	24.3	10.9	6.5	11.6
1961-62	22.1	24.4	11.1	5.5	14.3
1967	25.2	16.3	7.0	6.7	13.8
1969	30.6	13.9	8.8	8.4	13.8
1970	34.0	15.1	6.8	7.8	14.1

Disregarding minor perturbâtions, nitrogen contributions from legumes seem to be increasing in regions not drawing so heavily on their soil nitrogen reserves and decreasing in those most in deficit. Though the world's soil nitrogen reserves are vast, as Table IV shows, they are not inexhaustible and their immediate availability is often very small.

These relationships again indicate that current levels of primary production are heavily dependent on fertilizer nitrogen (and *inter alia* on fertilizer phosphorus and other plant nutrients). This dependence seems to be increasing at a more than linear rate ; in India from 16,000 metric tons in 1938, through 85,000 metric tons in 1953, 257,000 metric tons in 1958, 429,000 metric tons in 1963, more 1131,000 metric tons in 1968 to 1487,000 metric tons in 1971. The present world production of nitrogen fertilizers is equivalent to 30 million metric tons of nitrogen, whereas ten years ago it was 19.0 million metric tons of nitrogen. In 1975 it will be 45 million metric tons and in 1980, 67 million metric tons. Without this increase there is no prospect of maintaining present rates of production but one questions for how long this can continue. Moreover, these quantities of fertilizer require for their manufacture and distribution quite prodigious amounts of energy—another area of world concern—so that it appears that we are applying remedies that in the long run may not continue to be practicable.

This trend can eventually be redressed by bringing to bear all measures that promote the efficient cycling of resources including fixed nitrogen and which, in their turn, do not create problems of such magnitude. A precipitate return to organic farming, even if practicable, would exacerbate rather than remedy this situation, leading as it inevitably would to catastrophically depressed yields. The contributions from unusual sources of proteins such as leaf protein, bacterial, algal and food-yeast culture, (though useful), offer rather little in the short term, as do the nostrums now seriously proposed by molecular biologists that we should try and put nodules on wheat or nitrogenase into cabbages.

Protein from Legumes

Extending and improving the cultivation of pulses and forage legumes is likely to make the largest and most immediate impact on the problem of protein shortage. Legume nitrogen is fixed by solar energy. Distribution problems are bypassed and under proper management soil nitrogen reserves are added to rather than depleted.

Table VII shows the scope of improvement in terms of the range in 4-year average yields of legumes per hectare in different parts of the world. Whereas they are mostly abysmally low, reflecting low rates of fixation, some legumes in some regions are highly productive. The potential is large and this can also be illustrated by the recorded amounts of nitrogen fixed per ha

Table VII

Yield of some grain legumes (dry) 4-averages 100 kg/ha

	U.S.A	Australia	India	Ethiopia	Egypt	UK	Netherlands
Glycine	18	—	—	6	—	—	—
Arachis	23	12	7	5	21	—	—
Phaseolous	13	7	3	7	15	—	20
Pisum	17	7	8	9	14	27	33
Vicia	—	9	—	9	20	27	24
Cicer	—	—	6	6	16	—	—
Vigna	6	5	—	—	18	—	—

for a range of legumes in field experiments (Table VIII). Very substantial rates of fixation can be achieved by forage as well as grain legumes, under tropical as well as temperate conditions. These data refer to the nitrogen in the harvested crop and make no allowance for that added to the soil. They also show a very large range within as well as between crops.

In promulgating this green counter-revolution, the directions and means of progress need careful consideration. Undoubtedly good agricultural practice in its manifold aspects, including inoculation, are of first importance.

To increase legume productivity above present low levels constitutes a formidable challenge to agriculture, the reason for which may lie in the fundamental difference between legumes and other crops. The yields of non-leguminous crop depend upon the interaction of two sets of basic components ; those intrinsic to the crop, ultimately genetic, and those of the whole environment— nutrition, water supply, disease, etc. In legumes the third component, *Rhizobium*, adds a new dimension of complexity. Leguminous crops require the right types of rhizobia to be present in the soil in sufficient numbers, they must be able to form nodules, and in their symbiotic phase to function in a closely integrated and co-ordinated fashion with the host so as to fix nitrogen

TABLE VIII

Nitrogen fixed by legume crops (in herbage or grain)

	No. of reports	kg N $ha^{-1}a^{-1}$
FORAGE LEGUMES GROWN ALONE OR WITH GRASS		
Temperate		
Various clovers (*Trifolium* spp.)	17	23-620
Lucerne and sweet clover	8	164-463
Tropical		
Controi	1	> 112
Stylo	1	30-196
Tick clover (*Desmodum* spp.)	1	700
Glycine wightii	1	160-450
GRAIN LEGUMES		
Temperate		
Veteh and tick beads	4	7-90
Peas	1	46
Lupins	1	128
Tropical		
Fenugreek	1	44
Lentil	2	35-77
Pigeon pea	2	41-90
Cowpea	2	73-240
Soybean	6	17-124
Guar	1	37-196
Groundnut	3	33-111
Canavalia	1	49
Chick pea	2	41-270

as and when required. This is a complex requirement but when fully met, the nodulated legume can out-yield (in terms of protein production) any other crop.

Some Problems Associated with Inoculation

Problems arise at the stage of establishing a nodulated crop legume by seed inoculation, in assessing methods and rates of inoculation and in evaluating strains. Each year some strains of rhizobia are dropped from the recommended lists and replaced by others, so that areas already inoculated with less good strains may be permanently relegated to second best symbiosis. Also, if it is desired to change entirely the type of legume in a particular region, prior inoculation for another species has been known to present problems.

The indirect effects of N-fertilizer on rhizobia in soil presents other problems through the reduction or suppression of nodulation and, sometimes in grass swards to the elimination of the legume (Nutman and Ross 1969) *Rhizobium* species differ in their persistence in the absence of their host and may even disappear, causing a need for inoculation where before none existed. In the field of *Rhizobium* ecology basic studies on competition, variation, lysogeny and genetic interchange can be highly relevant to practice.

Environmental Effects

Because fully effective symbiosis involves the mechanisms controlling growth and physiology of the host plant, as well as of the bacterium, the legume agronomist needs to know something of crop physiology and microbiology so as to diagnose defects and define problems and assess responses to environmental factors. For example, nodule formation in some legumes is known to be strongly influenced by small changes in soil temperature ; a rise of 5°C above optimum can prevent the development of the nitrogen-fixing system (though not affecting the activity of nitrogenase already formed), in *Cicer arietinum*, a further small rise stopping nodulalion (Dobereiner *et al.* 1971).

It is of special interest that such reactions to non-optimal conditions of temperature and other factors of the physical environment are often strongly affected by differences between strains of *Rhizobium* and cultivars of the host.

Genetics

Hereditable host factors affect all phases of the symbiosis between *Rhizobium* and its host legume the initial infection of the root, the growth of the nodule, the amount of nitrogen fixed and the response of the symbiosis to changes in the environment (Nutman 1969). Host factors concerned with nodule development seem to show little specificity with respect to bacterial strain and manyare polygenetic. Some have correlative effects on root growth and these may be important in regulating the amount of nitrogen-fixing tissue produced.

In certain legumes bacteroid development, associated with nitrogen fixation, is strongly influenced by major host genes which, as recessives, stop their formation. These interactions may be specific to rhizobial strain and some have been shown to be circumvented by bacterial mutation.

Some bacterial differences, notably host-range specificity have been studied by transformation but as yet this and other methods of microbial genetics have been little used with practical agricultural aims in view.

As in the other "Green Revolution", genetics may prove to be of central importance in increasing protein yield of legumes. It is, however, a more difficult situation to manipulate because two interacting genomes are involved, and because the techniques for their study, which involve some degree of bacteriological control of plant growth, are more tedious especially with larger seeded species.

It is for this reason that much has so far been done with small seeded pasture species such as white clover (Jones and Burrows 1968) and red clover (Nutman, Mareckova and Raicheva 1971). In red clover large increases in nitrogen-fixing activity have been obtained by breeding from the most effectively nodulated plants. This response involves earlier nodulation, larger nodular weight, possibly increased nodule number and less probably greater nitrogenase activity (unpublished results).

Much basic work needs to be done, especially in unravelling strain-host interactions, before programmes of practical breeding can be properly formulated. But the extensive variation characteristic of legume symbiosis promises great scope for improvement, even in such crops as soybean where yields are already good.

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