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USE OF ISOTOPES FOR STUDY OF FERTILIZER UTILIZATION BY LEGUME CROPS

PROCEEDINGS OF A PANEL ON THE
USE OF ISOTOPES FOR STUDY OF FERTILIZER UTILIZATION
BY LEGUME CROPS
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FOREWORD

Protein malnutrition is a serious problem in most developing
countries. With the rapid population increase in these countries, the
need for bridging the protein gap has become a matter of real urgency.
Recognizing this, the FAO, in its "Provisional Indicative World Plan for
Agricultural Development" (1969) emphasized the need to work on the pro-
blem from all possible angles, one of the most important being to in-
crease production of the high protein grain legumes. About 70 % of the
world's total protein production is derived from vegetable sources, which
are also the cheapest, and hence the primary sources of protein in the
developing countries. Of this, more than two-thirds came from the cereals,
and about one-fifth from the grain legumes which contain 18 to 25 % pro-
tein (about double that in cereals). The grain legumes are widely grown,
and often form part of the staple diet in many developing countries. But,
in comparison to the cereals, little research work has been done to im-
prove the productivity of grain legume crops which is very low due to
poor fertilizer and other cultural practices and inherent characteristics
of varieties grown.

The Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture
has already embarked on a research programme to increase the quality and
quantity of protein in grain crops using nuclear techniques. The problem
is being attacked in two directions – breeding high yielding and high
protein varieties through radiation induced mutations, and increasing
yields and protein contents through efficient fertilizer utilization. This
panel was convened to advise the Joint FAO/IAEA Division on the potential
for a research project using isotope techniques on how fertilizer utilization
by grain legumes can be maximised without losing any benefits from their
capacity to fix nitrogen.

The papers presented at the meeting dealt with the numerous factors in-
fluencing symbiotic nitrogen fixation and yield responses to fertilizer
application by legume crops. It is considered that this publication would
be of particular value to research workers in the field of legume ferti-
lization.

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FACTORS AFFECTING THE RESPONSE OF GRAIN LEGUMES TO THE APPLICATION OF FERTILIZERS

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ABSTRACT

The observation that grain legume crops in the tropics do not respond to fertilisers may be largely due to (a) failure to appreciate that legumes can fix nitrogen only when they have an adequate supply of essential trace elements, and (b) failure to ensure that appropriate strains of rhizobium are present on their roots. The trace elements should be present in adequate amounts not only to satisfy the needs of the plant, but also of the nodule organisms. If the latter do not function effectively, applications of P and K fertilizers can only produce a limited response in the absence of adequate combined nitrogen – as any other crop. The paper reviews the various factors which influence the availability of trace elements and effective nodule systems, and it discusses the importance of taking these into account in planning fertilizer experiments with legumes.

Tremendous improvements in the yield of crops in the temperate lands of the world have followed the use of various chemicals containing N,P or K as fertilisers. The most expensive element to apply has normally been nitrogen. As a consequence, the ability of members of the genus Leguminosae to fix atmospheric nitrogen and hence not to need applications of nitrogenous fertilisers has been widely exploited. The view that has been expressed, that the legumes commonly grown for grain in the tropics do not respond to fertilisers is thus at variance with experience in temperate climates. It is the purpose of this article to review factors that have lead to this divergence.

The major reasons appear to have been firstly a failure to appreciate that for the legumes to utilise their ability to fix nitrogen it may be necessary to supply them with a range of nutrient elements only required in traces, and secondly to ensure that appropriate strains of rhizobia are

present on their roots. With the pasture legumes, the practice of adding
trace elements to the major fertiliser has been one of the outstanding
features in post-war agricultural development throughout the world, and par-
ticularly in Australia. The grain legumes have received less attention, but
even for them positive responses have been recorded to molybdenum, vanadium,
manganese, boron, zinc and copper for a wide range of crops. The failure to
obtain responses to fertilisers in experiments on the tropical grain legumes
probably simply reflects the inherent difficulties of such investigations,
for not only is it necessary to satisfy the needs of the plant itself, but
also of the nodule organisms, for without an effectively functioning nodule
system, the legumes are as dependent on combined nitrogen from the soil or
from fertiliser as are any other crops, and applications of P, K or P and
K together could only be expected to produce a limited response.

In any experiment to determine the potential response of grain legumes
to the application of the major fertilisers, the result obtained will be
affected by -

(a) the level of supply in an available form of the nutritional ele-
ments required at trace levels both by the plant and by the nodule
system.

(b) by the presence or absence of strains of rhizobia capable of form-
ing effective nodules on the legume concerned.

(c) by competition between the plant and nodule for nutrient elements
present in limited supply.

(d) by variations in the quantity of trace elements in the seeds used
in the experiment, and

(e) by the methods by which trace elements or bacterial inoculants are
applied.

The trace elements: -

Of the nutrients generally required by plants, the response to P, K,
Mg and S is much the same for legumes as for other plants, with the excep-
tion that the optimum levels for growth for P is higher for the legumes
(Ozanne, Keay and Biddiscombe 1969). Loneragan has already drawn attention
to the higher need for Ca in the nodulated legume.

The absolute level of the trace nutrient may be too low, either due to
a lack of the element in the rocks from which the soil has formed or to
rapid or long continued leaching, the former occurring on coarse textured
soils (e.g. Donald and Riceman 1952) or over drains (e.g. Judy, Lossman &
Rozycka 1964), the latter on the ancient and often lateritized land surfaces
of the tropics.

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Where the quantities of the element present are larger, its availability
for plant growth may be affected by the acidity or alkalinity of the soil, by
specific reaction with certain soil constituents, or by interaction with an-
other nutrient element.

The trace elements required fall into three groups, according to the
extent to which their availability is affected by soil reaction.

(i)   those which become less available in alkaline conditions, i.e.
        Mn, Fe, B and Zn,

(ii)   those which show little change in availability with change in
        pH, i.e. Cu and Co,

(iii)   those which become less available under acid conditions, i.e. Mo
        and V.

On alkaline soils, failure to respond to the phosphatic fertilisers may
consequently be due to fixation of the Mn, Fe, B or Zn in forms unavailable
to the plant. The need for these elements must be tested under such condi-
tions, as well as the possibility that liming an acid soil may induce defi-
ciency of such elements. Examples of this affect for Mn, Fe and B have been
found frequently in the past, and more recently for zinc (Hutton & Fiskell
1963). Copper and cobalt, although showing little change in availability
with change in reaction per se show a fall in availability in certain acid
soils. Copper is strongly chelated, and made less available in highly or-
ganic soils. In many acid soils, the cobalt is adsorbed almost entirely on
the surface of the concretions of manganese oxide (Tiller, Honeysett and
Hallsworth 1969), whilst in neutral and alkaline soils it is adsorbed on the
montmorillonite lattice.

Since the requirements for copper, cobalt, vanadium and molybdenum are
proportionally much higher for the effectively functioning nodule than it
is for the plant itself, the legumes show a better response to nitrogenous
fertilisers on an acid soil than they do on neutral or alkaline soils. The
application of molybdenum or vanadium in quite small amounts to crops grown
on acid soils may produce responses as great as heavy application of lime,
since the effect of the latter may in large part be due to an increase in
availability of the Mo and V already present in the soil.

There are several other interactions that need to be noted. High levels
of manganese may cause a reduction in the quantities of Cu and Fe absorbed
(Hallsworth E.G. et al 1965). The application of high levels of P may be

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associated with evidence of zinc deficiency (Melton, Ellis, Doll 1970; Ambler & Brown 1969). An interaction between zinc and soil temperature has also been reported, a more marked response being obtained at 26°C than at 14° or 20° (Wallace, Romney, Hale 1969), and the effect of such higher applications of P will only be realised if steps are taken to supply additional Zn at the same time. Experiments in sand culture show an interaction between levels of supply of Fe and Cu. At low levels of Fe supply, levels of Cu may be toxic, whilst at a higher level of Fe, those same levels will enhance growth, (Polson, Adams 1970).

The manner in which the trace element is supplied may also affect the balance between nutrient elements, and the application of manganese – E.D.T.A. has recently been shown to increase the uptake of Fe rather than Mn as iron is more strongly chelated by E.D.T.A. than is manganese (Knezek, Greirert 1971).

The number of trace elements that may be required to be added at any particular site varies with the nature of soil. With the pasture legumes in Australia optimum response may be obtained to phosphate on its own, to phosphate with Mo on the New South Wales tablelands, to phosphate with zinc and copper at Esperance in West Australia and to phosphate with zinc, copper and molybdenum in S.E. South Australia, whilst in the York Peninsula in South Australia the optimum response has been obtained only to a fertiliser mixture which as well as phosphate and sulphur contained Fe, B, Mn, Zn, V, Cu, Co, Mo and S (Cartwright and Harris, priv. comm. 1970) and the omission of any one of these elements resulted in a 40 to 60 % reduction in yield.

In the grain legumes, a similar pattern of varying needs is now beginning to show itself, and responses to P + Mo, P + Mo + V, P + Mo + Co etc.have been reported (Table 1). In spite of the importance of Cu for the pasture legumes, very little attention has been paid to its use for the grain legumes. No soil showing a response by a grain legume to the simultaneous application of eight trace elements has yet been reported, but such a field trial does not yet appear to have been attempted!

The need for inoculation: –

The importance of inoculating legume seeds with an effective strain of rhizobium is perhaps best appreciated in Australia where, following British

settlement every cultivated crop that was introduced was being grown in soil
in which it had never grown before and in which, so far as the temperate
legumes except lupins were concerned, rhizobial species or strains capable
of forming effective symbionts were absent. In Europe on the other hand,
inoculation has not been generally practised, in the belief that the appro-
priate rhizobia were widespread, but there has been growing evidence that
the lack of an appropriate host crop over several years can reduce the
numbers of rhizobia to very low levels. Under these circumstances responses
to inoculation are obtained. (Manil, 1958).

In the absence of an appropriate rhizobial population, the legumes
will fail to develop effective nodules. Their response to the addition of
phosphorus, potassium or trace elements as fertilisers in such cases will
be dependent on the level of nitrogen supply from the soil. Since in many
tropical and subtropical areas supplies of combined nitrogen from the soil
are likely to be very low, it seems that in many cases where legumes have
been reported to show little or no response to fertilisers, the plants have
been ineffectively nodulated or not nodulated at all. In several experi-
ments reported from the tropics in which negative results have been obtained,
no attempt was made to inoculate the seeds (Kanwar 1962 with berseem; Jain
and Mehta 1963 with grams). In other cases, even when seed inoculation
has been used, no mention was made of any attempt to see whether an effective
nodule system had been developed (Miyasaka, Freire, Abramides 1966).

The inoculation of the seed with an effective strain, on its own, is
no guarantee of the development of effective nodulation. The lack of such
trace elements as molybdenum, copper, cobalt or vanadium in sufficient
quantities will prevent the nodule system from fixing significant quantities
of nitrogen, even in the present of effective strains of rhizobia.

There is an increasing evidence that leguminous crops in tropical areas
are not as effectively nodulated as they could be, and the inoculation with
specific strains of rhizobia has been shown to produce significant response
even in areas where the crop under test has been traditionally grown, e.g. in
Madagascar (Denarie 1968), in India (Belasemdaram V.R; Iswaran V;
Rao W.V.B.S. 1970)and in Egypt (Loufti, El Sherbini and Ibrahim 1966).

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TABLE I Recorded responses in the last decade where yield of grain legumes has
increased following application of trace elements in the fertiliser mixture.

Single element responses:-

ELEMENT	CROP	REGION	REFERENCE
B	Pea Bean	Estonia	Kalmet 1965
B	Groundnuts	U.S.	Harris & Gilman 1957
B	Beans	Russia	Berzipa 1965
B	Beans	Poland	Tuchoka, Szukalski, Kukurenda 1964
B	Soyabeans	China	Chu, Liang, Chen 1963
Co	Soyabeans	Russia	Piroshnikov 1962
Cu	Soyabeans	China	Chu, Liang, Chen 1963
Fe	Groundnuts	East Africa	Hartzook, Fichman, Karstadt 1971
Fe	Groundnuts	East Africa	Lachover, Fichman, Hartzook 1970
Mn	Peas	England	Rose, Dermott 1962
Mn	Soyabeans	USA (Ohio)	Mederski, Hoff, Wilson 1960
Mn	Groundnuts	India (Madras)	Sanjeevaiah 1969
Mn	Soyabeans	USA (Florida)	Robertson, Thompson 1969
Mn	Pea	Russia	Kamijnine 1965
Mn	Soyabeans	China	Chu, Liang, Chen 1963
Mo	Horse beans	Russia	Bashinskaya 1966
Mo	Soyabean	Siberia	Sakolova 1966
Mo	Pea Vetch Horse beans	Russia	Gladkii 1965
Mo	Pea	USA (Wisconsin)	Hagstrom & Berger 1965
Mo	Pea	Russia	Kamynine 1965
Mo	Groundnuts	Senegal	Martin & Fourrier 1965
Mo	Beans	Bulgaria	Radomirov 1965
Mo	Soyabeans	Siberia	Kurkaev & Golov 1964
Mo	Horse beans	Russia	Musakanov 1964
Mo	Soyabeans	China	Chu, Liang, Chen 1963
Mo	Horse beans	Russia	Silchenko 1963
Mo	Vetch	Russia	Zhorikov 1963
Mo	Soyabeans	France	Bertrand 1962
Mo	Peas	Hungary	Gleria & Tamasy 1962
Mo	Soyabeans	USA	Parker & Harris 1962
Mo	Peas	Russia	Ozolinya & Zhurovskaya 1959
Mo	Groundnuts	Java	Newton & Said 1956
Mo	Peas	Latvia	Peive & Rinko 1957
Mo	Soyabeans	USA (Arkansas)	Joseph, Maples, Hardy 1971
Mo	Soyabeans	USA	de Mooy 1970
Mo	Soyabeans	Brazil	Ruschel, Britto, Carvalho 1969

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ELEMENT	CROP	REGION	REFERENCE
Mo	Soyabeans	China	Lee, Tang, Tsai 1967
Mo	Peas	India (Madras)	Sharga & Jauhari 1970
Mo	Peas	India	Kherde & Yawalkar 1966
Mo	Kidney beans	Bulgaria	Nikolov & Peterburgski 1967
Mo	Phaseolus vulgaris	Bulgaria	Radomirov, Kasarowa, Stoimanov 1969
Mo	Peas	Russia	Muravin, Shumilin 1969
Mo	Peas	Russia	Kamijnine 1965
Mo	Peas	USA (Wisconsin)	Hagstrom & Berger 1963
Mo	Groundnuts	W. Africa	Martin & Fourrier 1967
Mo	Beans (Vicia faba)	Bulgaria	Todorov 1963
Mo	Pea (Pisum sativum)	Russia	Zhorikov 1963
Mo	Soyabeans	USA	Lavy & Barber 1963
V	Kidney beans	Bulgaria	Nikolov & Peterburgski 1967
V	Pea	Russia	Kamijnine 1965
V	Pea	Bulgaria	Peterburgski 1966
Zn	Ragi (Eleusine coracana)	USA (Florida)	Hutton & Fiskell 1963
Zn	Soyabeans	USA (Washington)	Nelson, Roberts, Nelson 1962
Zn	Soyabeans	China	Chu, Liang, Chen 1963
Zn	Peas Beans	USA (Michigan)	Brinkerhoff, Ellis, Davis 1966
Zn	Groundnuts	India (Mysore)	Yadahalli, Radder, Pattil 1970
Zn	Beans (Phassolus vulgaris L)	USA	Melton, Ellis, Doll 1970
Zn	Beans (Phassolus vulgaris L)	USA	Wallace, Romney, Hale 1969
Zn	Beans (Phassolus vulgaris L)	USA	Judy, Lessman, Rozycka 1964

Multiple Effect

B + Co + Mo	Horse beans	Russia	Bērzipa 1965
B + Mo B + Cu + Mo	Horse beans	Latvia	Bambergs 1964
Cu + Mn + Mo Mo + B	Horse beans	Russia	Klincare 1964
Cu + Mo	Lupins Soyabeans	Russia	Zhiznevskaya 1961

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Effect of nitrogen fertilisers: - For all the nutrient elements re-
quired in trace quantities, there is competition between the nodule and the
plant for what is available. It is in terms of this competition that the
effect of supplies of combined nitrogen can best be understood. There are
three aspects. In the past it has been accepted that the effect of nitro-
genous fertilisers has been to depress nodulation. Numerous papers have
demonstrated this and several of these are reported in earlier papers at
this symposium. A second aspect which has been reported on more recently
has been the stimulating effect on nodulation of small additions of nitro-
genous fertiliser. The third aspect is the even more recent demonstration
that additions of nitrogenous fertiliser late in the growing period can
enhance the nitrogen content of the crop.

The effectively nodulated plant, growing in the absence of any extra-
neous source of nitrogen compounds is clearly a system in balance. Some of
the photosynthate produced by the cotyledons and early leaves passes down
the roots to the bacteroids in the nodule, which can utilise it to fix at-
mospheric nitrogen into forms which become available to the plant. The need
of either the plant or the nodule for the supply of any trace element must
consequently remain in balance.

The effect of this balance has been illustrated for copper, which has
been shown on numerous occasions in the past to be essential for plant
growth and more recently to be essential for nitrogen fixation (Hallsworth,
Wilson and Greenwood 1960). The plant and the nodule system can be considered
as two alternative sinks into which the copper supplied to the plant can be
directed. Where the nodulated legume is entirely dependent on the nodules
for its supply of soluble nitrogenous compounds, its growth and hence its
demand for copper is directly related to the quantity of nitrogenous com-
pounds received from the nodule. Any reduction in the supply of copper to
the nodule, because of an increased consumption by the plant, would be fol-
lowed by a reduction in the fixation of nitrogen by the nodule, which would
result in a reduction in the supply of nitrogenous compounds from the nodule
to the plant even though photosynthate was accumulating in the nodule
(Carwright and Hallsworth 1970). This is accompanied by a concomitant
reduction in growth and consequently in a reduction in the demand by the
plant for copper. The two alternative sinks for copper will thus remain
in balance.

Where nitrogenous compounds are added to the plant/nodule system, as occurs when nitrogenous fertilisers are used, the situation is changed. The plant can now continue to grow without reference to the supply of nitrogenous compounds from the nodule. The dimensions of the plant sink are increased relative to those of the nodule sink, and in increasing proportion of the copper available to the system goes to the plant. Where the copper supply is limited, this results in a reduction of the quantity of copper available to the nodules, and as the old ones wither away, the newly initiated nodules will fail to develop. That this is the logical explanation of the classical pattern of reduction or inhibition of nodulation following the application of nitrogenous fertilizers, is supported by the finding that with suitable increases in the level of copper supplied to the plant (Table 2) a 500-fold increase in available nitrogen can be accepted without significant reduction in weight of effective nodules (Hallsworth, Greenwood and Yates 1963).

It can be noted also in Table 2 that increased N-supply leads to an increase in the number of nodules, although in the absence of an increase in the copper supply very few of them become effective. Consequently, under conditions where the copper supply is not limiting, the small addition of nitrogenous fertilisers would be expected to be beneficial to the growth of the nodule system, particularly with the small seeded legumes. The growth of the photo-synthesising system of the seedling depends on the supply of combined nitrogen from the seed, and with a small seed this is limited. The effect of an added supply of combined nitrogen at this stage will be to increase the rate of growth of the leaves, and hence of the supply of photosynthate to the nodule. This in turn would lead to a better supply of combined nitrogen from the nodule system to the plant, always provided that the elements needed for the nodule were not limiting. On soils very low in available trace nutrients, no benefit would be expected to result from early application of a small dressing of nitrogen unless at the same time steps were taken to redress such deficiencies.

Whilst for most trace elements the effects will be analogous to those with copper, for cobalt and perhaps vanadium this interaction between trace element and combined nitrogen may be too slight to show. With cobalt, which has been shown to be stimulating to the growth of the non-nodulated or ineffectively nodulated legume (Hallsworth; Wilson and Adams 1965), a 1000-fold increase in the level of cobalt supplied was without effect on the number or weight of nodules produced (Wilson and Hallsworth 1965).

TABLE 2

Effect of variations in supply of copper and nitrate nitrogen on
the development of nodulation in T. subterraneum (var. Mt. Barker)
Spring/Summer 1959

μM N	0.01 μM Cu	0.1 μM Cu	1.0 μM Cu	5.0 μM Cu

(a) No. of nodules per plant (No. excised given in ( )):

Harvest I

μM N	0.01 μM Cu	0.1 μM Cu	1.0 μM Cu	5.0 μM Cu
1000	37. (1)	123 (45)	106 (56)	155 (59)
	*1.56 (0.20)	*2.08 (1.64)	*1.98 (1.72)	*2.16 (1.77)
500	41 (10)	96 (50)	75 (29)	50 (24)
	*1.60 (0.92)	*1.97 (1.68)	*1.85 (1.46)	*1.70 (1.38)
0	64 (38)	33 (18)	17 (9)	9 (9)
	*1.79 (1.55)	*1.49 (1.22)	*1.20 (0.93)	*0.86 (0.80)

Harvest II

μM N	0.01 μM Cu	0.1 μM Cu	1.0 μM Cu	5.0 μM Cu
1000	252 (2)	215 (128)	232 (86)	143 (102)
	*2.38 (0.30)	*2.26 (1.95)	*2.32 (1.92)	*2.14 (2.00
500	131 (61)	116 (40)	91 (48)	58 (35)
	*2.09 (1.75)	*2.05 (1.60)	*1.96 (1.65)	*1.75 (1.64)
0	51 (44)	32 (22)	31 (25)	10 (8)
	*1.69 (1.62)	*1.49 (1.33)	*1.45 (1.34)	*0.96 (0.88)

*Mean log. $_{10}$ number
Difference for significance in log. numbers Total 5% 0.31; 1% 0.41; 0.1% 0.54
                                                                    Excised 5% 0.38; 1% 0.51; 0.1% 0.66

(b) Dry weight of excised nodules per plant (mg):

Harvest I

μM N	0.01 μM Cu	0.1 μM Cu	1.0 μM Cu	5.0 μM Cu
1000	0.5	17	50	67
	*0.00	*1.16	*1.69	*1.80
500	2	18	19	22
	*0.30	*1.16	*1.69	*1.80
0	26	15	9	19
	*1.38	*1.19	*0.89	*1.18

Harvest II

μM N	0.01 μM Cu	0.1 μM Cu	1.0 μM Cu	5.0 μM Cu
1000	3	151	188	260
	*0.36	*2.10	*2.27	*2.41
500	23	64	92	183
	*1.33	*1.77	*1.68	*2.23
0	62	31	69	106
	*1.72	*1.32	*1.73	*1.95

*Mean log $_{10}$
Difference for significance (log. wt) 5% 0.41; 1% 0.55; 0.1% 0.72

The use of small dressings of nitrogenous fertilisers as starters for leguminous crops would consequently be expected to improve nodulation and enhance yield, and this has been shown to be the case, the optimum level of nitrogenous fertiliser depending on the conditions. (Litynski 1956, Thornton 1956, Young 1958, Dart & Wildon 1970, Ezedinma 1964).

The value of late applications of nitrogenous fertilisers on legume crops to enhance yield or protein content has been investigated more recently (see Hera, this symposium). The theoretical justification of this is that the nodule system becomes less effective at the end of the growing period, partly due to the soil drying out and partly to a diminution of supply of photosynthate, since at this stage the developing seeds constitute a major sink for carbohydrate produced. Late application of N as NH_4NO_3 has been shown to increase considerably the N content of pea shoots (Schalldach and Schilling 1966), and the use of labelled nitrogen showed that 70 % of the late-applied N has been used in synthesis of the lysine-rich seed proteins legumin and vicillin. This, however, does not imply that late application of nitrogenous fertilisers improve the quality of the seed protein, for the total lysine-N content of the seed remained constant at 80.6 % (Schilling and Schalldach 1966).

Whether the practice of late application of N is worth considering in a major co-operative investigation in the developing countries is a moot point. I would suggest that it is a refinement to be introduced later. The potential enhancement of the protein level would seem to be too small to justify the complications that would result from the introduction of such a treatment.

Trace element of seeds used: -
In fertiliser trials in general, the only attention paid to the seed is that it should be of an appropriate variety, disease free and of high viability. No attention is paid to its composition. Since the amounts of P, K, N, Ca and other macro nutrients present in the seed are insignificant in relation to the quantities that the plant will require during its life-time, this neglect is justified, but with the trace nutrients the position is different, for the quantities of molybdenum, vanadium and cobalt required by the crop may be extremely small. The quantity of these elements present in the seed may vary widely, depending on the level available in the

soil on which the plants from which the seed was obtained were grown. I
have found the Cu content of subterranean clover seeds to vary from 7 p.p.m.
in seeds grown on non-deficient soils in New South Wales to 3 p.p.m. on seeds
grown on copper-deficient soils in Western Australia, even where Cu con-
taining fertilisers had been used. Growing lupin seeds on a molybdenum defi-
cient soil in the English midlands reduced the Mo-content 10-fold when
compared with the mother seed. The likelihood that such variations in
seed content will affect the response in fertiliser trials is obvious. For
molybdenum, for example, it has been shown that soya bean seed grown in
six States in U.S.A. varied from 0.6 to 22.4 p.p.m. Mo, and that plants
grown from the seed containing 22.4 p.p.m. Mo failed to show any response
to the application of Mo-fertilisers (Harris, Parkes and Johnson 1965).
An increase in molybdenum in the seed has also been shown to occur follow-
ing liming or applying Mo as a foliar spray, the high molybdenum seeds
supplying the Mo needs when grown on Mo-deficient soils, but there was
very little carry-over into the next crop (Gurley and Giddens 1969).

In field experiments designed to investigate the responses of legumes
to the application of trace elements, it is consequently essential that
the level of trace elements present in the seed it is intended should be
used is known, for high seed content of Mo, V, Co and Cu at least, may
vitiate the results obtained in annual crops.

Method of application of trace elements: -
The response to trace elements added as fertilisers also varies with
the manner in which they are applied. Since the quantities needed are
very small, the distribution of the chemical concerned over the entire
surface of the area to be planted is likely to be unsatisfactory, or to
require larger applications. For the many trace elements applied to
pasture legumes in Australia the technique that has proved very successful
has been to mix the salt, oxide, etc. of the element concerned with super-
phosphate, which may then be broadcast. This technique has been used for zinc,
copper or molybdenum treated superphosphate. For wheat or peas, the copperized,
zincized or manganized superphosphate has been drilled with the seed. In
both of these techniques, the placing of the trace element at the same series
of points at which the phosphate is concentrated has resulted in effective
absorption of the trace element concerned, presumably because the roots of
the plant concentrate around the granules of superphosphate. This treatment,

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however, is used where large areas are to be treated, by farmers accustomed
to the use of large machinery. On the uncultivated pasture lands, success
has generally attended the practice of coating the seeds with superphosphate
containing the element, sometimes coated further with ground limestone and
spreading the fertiliser from aircraft. There have been reports that coating
inoculated weed with superphosphate and ground limestone have had a de-
pressing effect on yield (Mascarenbus, Myasaka, Freire et al 1967).

For seed legume crops, particularly when grown on the smaller areas more
characteristic of tropical agriculture, neither of the Antipodean practices
described above may be satisfactory. As an alternative, the practice of
soaking the seeds in solutions containing the element concerned have been
found successful in several parts of the world. With molybdenum the rate of
.3 to 8 gr per kilo seeds have been found to give satisfactory response, but
when treated seeds were stored at high humidity a considerable loss in ger-
mination resulted. (Johnson, Harris and Parker 1960). Soaking beans in
0.02 % solution of manganese sulphate, ammonium molybdate and zinc sulphate
has also been reported to be satisfactory (Phillipova K.F: Kalatove S.S. and
Ovchara K.E. 1965). With soyabeans, molybdenum applied to the seeds at
sufficient to give a rate of 28 grams/hectare was quite satisfactory, and
gave as good a yield increase at 6-12 times the quantity applied as a foliar
spray (Thomson 1965).

Although soaking the seed in solutions of the trace nutrient before
inoculating with rhizobia has been generally successful, the alternative
of adding the element (Ml) to seeds treated with peat based inoculum has
not, up to 99 % of the rhizobia being killed as a result.

Satisfactory responses have also been reported on numerous occasions
when the trace element has been applied as a foliar spray to the young crops,
but for most purposes this would seem to be the least satisfactory, and only
to be adopted if both seed treatment with the trace element, or mixing the
trace element with another fertiliser such as the phosphate was unsatisfac-
tory. Direct application of the trace element to the soil, by broadcasting
or drilling would seem to be generally unsatisfactory except perhaps for
copper on highly organic soils, when rates of $CuSO_4 7 H_2O$ of up to 48 kg/h
may be required.

Planning: -

In planning for fertiliser experiments with legumes it is consequently necessary to take into account three factors, as well as the levels of the major nutrients P & K. The first is that steps should be taken to secure a strain of rhizobia known to be effective on the legume concerned, and to arrange that inoculation of the seed is carried out in such a manner that an adequate number of viable rhizobia are still on the seed when it is finally sown. Storing seed that has been inoculated and dusted with fertiliser has too often in the past been found to be associated with loss of viability of inoculum.

The second is that an effectively fixing nodule system needs a number of trace elements and unless all are present in the soil in adequate quantities, some or all of the trace elements will need to be added. In the absence of any information about the soil concerned it is necessary that at least one treatment of the experiment should include all the trace elements required. If these mixtures of the salts or oxides of the trace elements are to be compounded some time before application, it will be necessary to check beforehand that the availability to the plant of the elements in the mixture does not fall during storage.

The third is that the method of application of the trace elements to the crop needs to be standardised between the different localities, for the variations in response that can result from the method of application can easily outweigh the other effects.

REFERENCES:

Ambler, J.E; Brown, J.C. 1969   Agron. J. 61   41-43
Belasundaram V.P; Iswaran V; Rao W.V.B.S. 1970 Curr. Sci. 39   328-380
Cartwright B; Hallsworth E.G. 1970 Pl. Soil 33   685-698
Dagis I.K. 1956 Mikroelementy sel. Khoz.Medits. 305-311
Dart P.J; Wildon D.C. 1970 Aust. J. Agric. Res. 21   45-56
Denarie J. 1968 Annale Agron. 19   473-496
de Mooy C.J. 1970 Agron. J. 62   195-197
Elst F.H. van der; McNaught K.J; Rolt W.F. 1961 N₂ture 192, 1315
Ezedinma F.O.C. 1964 Trop.Agric. Trin. 41   243-245
Gleria J. di, Tamàsy I: 1962 Magy.Tud,Akad. agrartud.Oszt.Közl. 21

Govinda Rajan S.V; Gopala Rao H.G. 1964 J. Indian Soc. Soil Sci. 12 355-361

Gurley W.H; Giddens J. 1969 Agron.J. 61 7-9

Hagstrom G.R; Burger K.C. 1965 Soil Sci. 100 52-56

Hallsworth E.G; Greenwood E.A.N; Yates M.G. 1964 Pl. Soil 20 17-34

Hallsworth E. G; Wilson S.B; Adams W.A. 1965 Nature 205, 307-308

Hallsworth E.G; Wilson S.B; Greenwood E.A.N. 1960 Nature 187 79

Hallsworth E.G; Yates M.G; Cansfield P.E; Saul J.T. 1965 Pl. Soil 22

Harris H.C; Gilman R.L; 1957 Soil Sci. 84 233-242

Harris H.B; Parker M.B; Johnson B.T. 1965 Agron. J. 57 397-399

Hartzook A; Fichman M; Karstadt D. 1971 Oléagineux 26 391-395

Hutton C.E; Fiskell J.G.A. 1963 Soil Crop Soc. Fla. Proc. 23 61-70

Johnson B.J; Harris H.B; Parker H.B. et al 1966 Agron. J. 58 517-518

Judy W; Lessman G; Rozycka T. 1964 Mich. Agr. Expt. Sta. Quart. Bull.46 386-400

Joseph L.K; Maples R; Hardy G.W. 1971 Dept. Akansas Un.Agr. Exp.Stn 1971 193

Kharde R.L; Yawalkar K.S. 1966 Ind.J. Agron. 11 198-200

Klincare A. 1964 Miktooth.Tsdy.II Akad.Nauk latvii SSR 23-40

Knesek B.D; Greinert H. 1971 Agron.J. 63 617-619

Lachover D; Fichman M; Hartzook A. 1970 Oléagineux 25 85-88

Lavy T.L; Barber S.A. 1963 Agron.J. 55   154-155

Lee L.T; Tang W.T; Tsai W. F. 1967 J. Agric. Assoc. China 59 65-76

Litynski A. 1956 Acta Agrobot. 4 121-144

Manil P. 1958 "Nutrition of the Legume s" ed. E.G. Hallsworth, Butterworths, London.

Loufti M; El Sherbini M.F; Ibrahim A.N. 1966 J. Microbiol. U.A.R. 161-167 169-177

Martin G; Fourrier P. 1965 Oleagineux 20 287-291

Mascarenhos, H.A.A.; Miyasaka, S; Freire, E.S. 1967. Bragantia 26, 143-154.

Mederski H.J; Hoff D.J; Wilson J.H. 1960 Agron. J. 52 667

Melton J.R; Ellis B. G; Doll E.C. 1970 Proc.Soil Sci. Soc. Am. 34 91-93

Miyasaka S; Freire E.S; Mascarenhaus H.A.A. 1964 Bragantia 25 65-71

Muravin E.A; Shumilin L.G. 1969 Izv. Timiryazov. Sel'.-khoz. No. 5 151-160

Nelson C.E; Roberts S; Nelson G.D. 1962 Wash. Agr. Expt. Sta. Bull. 642

Newton J.D; Said A. 1957 Nature 186 1485-1486

Nikolov B.A; Peterburgskii A.V. 1967 Izv. timiryazev. sel'.khoz.Akad. No. 4 141-145

Ozanne P.G; Keay J; Biddiscombe 1969 Aust.J. Agric. Res. 20 809-818

Parker M.B; Harris H.B. 1962 Agron. J. 54 480-483

Peive Ya. V; Rin'ke R. S. 1957 Latv. PSR Zinat.akad.Vestis, No. 5 53-62

Peterburgski A.V. 1966 Tagber. dt. Akad. Landwiss. Berl. 85 222-229

Phillipova, K.F; Kolotova S.S: Ovcharov K.E. 1965 Rol.Mikroelem. Protsesse Rosta
                                   Razv.Rast.,Dokl.Nauch. Kong. Vilnyus 1963

15

Pirozhnikov K.D. 1962 Trudy troits. vet. Inst. 8 26-30

Polson D.E; Adams M.W. 1970 Agron. J. 62 557-560

Radomirov P. 1965 Rast. Nauki No. 12   75-83

Radomirov P; Kosarowa M; Stoimenov S. et al Dokl. Akad. Sel'.-Khoz.Nauk Bolgarii 2
                                                                    19-24

Riceman & Donald 1952

Robertson W.K; Thomson I. G. 1969 Proc. Crop. Soil Sci. Soc. Fla. 29   86-92

Rose T.H; Dermott W. 1962 Emp. J. Exp. Agr. 30 263-275

Ruschel A.P; Britto D.P.P de S; Carvallo L.F. de 1969 Pesq. agropec. bras. 4 29-37

Sanjeevaioh R.S. 1969 Mysore J. Agr. Sci. 3 83-85

Schalldach I; Schilling G. 1966 Albrecht-Thaer-Arch. 10 829-839

Schilling G; Schalldach I. 1966 Albrecht-Thaer-Arch. 10 895-907

Sharga A.N; Janhari O.S. 1970 Madris Agri. J. 57 216-221

Tiller K.G; Honeysett J; Hallsworth E.G. 1969 Aust.J. Soil Res. 7 43-56

Thompson L.F. 1965 Ark. Fm. Res. 14   4

Thornton G.D; 1956 Soil Crop Sci. Soc. Fla. Proc. 16 146-151

Wallace A; Romney E.M; Hale V.Q. 1969 Agron. J. 61   567-568

Wilson S.B; Hallsworth E.G. 1965. Pl. Soil 23 260-279

Yadahalli Y.H; Radder G.D; Patil S.V. 1970 Mysore J. of Agric. Res. 4   208-209

Young D.J.B. 1958 J. Brit. Grassl. Soc. 13   106-114

THE SOIL CHEMICAL ENVIRONMENT IN RELATION TO

SYMBIOTIC NITROGEN FIXATION

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ABSTRACT

This paper reviews the principles elucidated in the relations between the soil chemical environment and symbiotic N fixation in legumes.

Several phases of the overall process of N fixation are recognized. The peculiar sensitivity of each phase to particular soil conditions renders the whole process sensitive to that condition. Survival and growth of some nodule bacteria are especially sensitive to soil acidity mainly through effects of H ions but probably also through effects of Ca ions in the rhizosphere. Nodule formation in some species is sensitive to H ions, to combined N, and to B. Nodule function in all species needs more Mo and Co than does plant growth: in at least some species it may need more Ca. Host legumes are also sensitive to adverse soil conditions but variation among legumes is as great as between legumes and other species.

Successful treatment of adverse soil conditions requires an understanding of the fundamental nature of the problems for the particular legume concerned. Specific treatments should be devised to correct the adverse condition and blanket treatments should be avoided. Resolution of the factors involved in responses of temperate

pasture legumes to lime provide outstanding examples of the value of this approach. They provide excellent models of an approach which can profitably be used in the study of the fertilizer needs of all legumes, including those grown for grain production in tropical areas.

I. INTRODUCTION

I have been asked to review the relation of the soil chemical environment to symbiotic nitrogen fixation with special emphasis on the production of legume grain in tropical and sub-tropical regions. I do not propose to make a catalogue of responses obtained by specific crops in certain regions but rather I propose to discuss the principles involved in the relations between soil properties and symbiotic systems.

II. SYMBIOTIC NITROGEN FIXATION

The soil chemical environment may profoundly affect symbiotic N fixation in each of 4 phases of the overall process:-

1. Survival and growth of nodule bacteria
2. Nodule formation
3. Nodule function
4. Growth of the host legume.

Each of these phases has some particular requirement which makes it peculiarly sensitive to the soil chemical environment. These are of special interest since they make the whole process of N fixation peculiarly sensitive to that soil condition. Moreover, an understanding of the relation between soil conditions and the nature of their effects on symbiotic N fixation has frequently assisted in the diagnosis and

treatment of field problems. This paper will therefore consider the relationship of those factors in the soil chemical environment which have special significance to each phase of N fixation.

1. Survival and growth of nodule bacteria

Where they do not already exist in the soil, suitable strains of nodule bacteria must be introduced with the legume crop. Masefield (75) has pointed out that nodule bacteria for tropical annual legumes fall essentially into 2 distinct classes - the cowpea type and other types. Cowpea type bacteria are very widespread and will readily infect appropriate, indigenous or introduced leguminous crops. Problems will arise if the existing bacterial strain is not fully effective. For other leguminous crops grown in the tropics (lentils, French beans, sometimes soybeans and many temperate legumes grown at high altitudes - lupins, peas, field beans), suitable nodule bacteria are usually absent and effective strains must be introduced.

Nodule bacteria of the cowpea type differ so greatly from other types in their physiological behaviour that some workers classify them in a genus, Phytomyxa, separate from Rhizobium (41). Such differences appear to be important in some areas where soil characteristics are associated with differences in the survival of cowpea type bacteria and of Rh. trifolii (74): on grey, siliceous sands Rh. trifolii was more sensitive to heat (73) and to antagonism by indigenous micro-organisms (29, 31). Differences in sensitivity to the soil chemical environment have yet to be shown to be important. However, from a consideration of the origin and evolution of root nodule bacteria Norris (88, 91) has postulated that the cowpea type bacteria should be much more tolerant of soil acidity than other types and that this would be a major factor in their distribution in soils. There is as yet

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little evidence to support this interesting hypothesis and its theoretical basis has recently been challenged (98,99).

Where appropriate, effective nodule bacteria must be introduced into soils with legume crops it is essential that soil conditions permit their survival and growth. Only the soil, chemical environment is discussed here and it must be remembered that the physical and biological environment assume overriding importance in some situations (95, 126).

(a) Soil acidity

Acidity is a particularly important factor in the growth and survival of nodule bacteria in soils. Sensitivity of nodule bacteria to acidity has long been thought to account for nodulation problems in some legumes. For example, Anderson and Moye (10) observed that subterranean clover sown on moderately acid soils (pH < 5.5)[1] in south eastern Australia nodulated poorly in the year of sowing unless soil pH was increased by treatment with calcium carbonate: however, on untreated soils, nodulation improved steadily in successive seasons suggesting that some factor was limiting the rate of growth of nodule bacteria. Increasing the numbers of bacteria at inoculation replaced the effects of lime on nodulation of both subterranean clover (8, 117) and Medicago species (80, 109). Once nodulated, N fixation and growth of the legumes did not respond to lime (10, 109).

The distribution of several Rhizobium species in soils also suggests an important determining role of soil pH. The distribution of Rh. meliloti in soils of Nebraska correlated strongly with soil pH and

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[1]Soil pH determined in a 1/5 suspension of soil/water: soil pH values vary greatly with the technique of determination (130), but unfortunately few workers specify their technique.

the bacteria were virtually absent in soils more acid than pH 6 (102).

In eastern Australia Rh. meliloti and Rh. trifolii were also virtually

absent from soils more acid than pH 6, even when the acid soils were

adjoined by less acid soils carrying dense swards of nodulated host

legumes (127). In New Zealand, Rh. meliloti was restricted to neutral

and alkaline soils: Rh. trifolii occurred more freely on slightly

acid soils (Greenwood 1964 cited by (106)) as would be expected from

its greater tolerance of acidity in agar cultures (55, 56).

Nodule bacteria also grow poorly or not at all when introduced

into acid soils. Although few strains have yet been tested in soils,

cowpea type organisms appear to grow almost as poorly on acid soils as

do other types of nodule bacteria. Thus, not only did Rh. trifolii

fail to grow but 6 strains of cowpea type organisms also grew poorly

when inoculated into acid soils of pH 4.8 to 4.9: when addition of

alkaline earth carbonates or hydroxides increased the soil pH to above

6, all organisms grew vigorously (91, 128). Hence the evidence fails

to support the suggestion that the ubiquitous distribution of cowpea

type nodule bacteria in tropical regions results directly from their

greater tolerance of soil acidity (88, 89). It suggests rather that

all types of nodule bacteria grow poorly in soils more acid than pH 5

and that all respond strongly to increasing soil pH to near neutrality.

Some nodule bacteria appear especially sensitive to soil acidity:

Rh. meliloti disappeared from soils of pH 5.3 to 5.7 in the season

following its introduction with host seed: where treatments raised

soil pH to 6.8, abundant bacteria survived (123).

(i) Components of soil acidity

Acid soils are characterised by several properties potentially

harmful to nodule bacteria. In addition to their high concentrations of

H ions, acid soils often have high concentrations of available Mn and Al and low concentrations of available Mo, Mg, and Ca. Treatment of acid soils with lime will usually modify all of these properties. While there is little critical information on the sensitivity of nodule bacteria to Mo, Mn, and Al, there is evidence that nodule bacteria are less sensitive than other phases of the symbiosis to low Mo and to high Mn and Al. Experiments purporting to show the opposite effect – that Mn toxicity specifically inhibits nodulation (35, 116) – fail to establish this point: application of Mn salt depressed plant growth so severely that it could have caused the concomitant depression in nodule numbers.

Species and strains of nodule bacteria vary widely in their acid tolerance. However, increasing H ion concentration in pure cultures in solution or on agar strongly inhibits the growth of them all – Trifolicae, Vicieae, and cowpea (53, 55, 56). Some strains of nodule bacteria are so sensitive to acidity that their growth may be inhibited at pH values which have no effect on growth of the host plant (62). At very acid pH values the effect of acidity on growth of nodule bacteria is not important in some legumes, since infection is completely inhibited regardless of the number of bacteria present (see below). At less acid pH values it may be very important. It probably explains the marked effects of increasing pH in increasing nodulation of clover and lucerne in moderately acid solutions with relatively low Ca levels: in such solutions, increasing bacterial numbers increased the nodulation of both species (lucerne pH 5.1 and 5.7, (83): subterranean clover pH 5.0, (68)).

The divalent cations, Ca and Mg, have marked effects on growth of nodule bacteria. Their effects are complex. An early

report indicated that strains of <u>Rh</u>. <u>trifolii</u> grew poorly in colloidal

clay suspensions unless relatively large amounts of Ca were added: by

contrast, cowpea type organisms did not respond to Ca (71). Norris (88)

later confirmed this observation and suggested that Viceae-Trifolieae

type organisms had a higher Ca requirement than the cowpea type. His

subsequent extensive researches have failed to confirm any difference

between these groups of organisms in their Ca nutrition on soils or in

solution culture and the reason for their different behaviour towards

Ca in clay suspensions remains unknown (91).

When grown in solution in isolated cultures, all nodule

bacteria require only traces of Ca (22, 62, 90, 91, 118, 124, 125).

They require somewhat larger quantities of Mg (90, 91, 125). In addit-

ion to their specific requirements for Ca and Mg, nodule bacteria have

a relatively large non-specific requirement for divalent cations. Con-

sequently a shortage of either the Ca or the Mg ion will make a

relatively greater demand on the other ion (125). From these studies

of isolated organisms in solution culture, workers have assumed that

Ca would have no effect on growth of nodule bacteria at levels above

those required for plant growth (62). Norris (90, 91) has also

postulated that Mg should be especially important for growth of nodule

bacteria. But Vincent (126) has pointed out that, for growth of

nodule bacteria in the rhizosphere of legumes, some account would need

to be taken of the extent to which plants competed with bacteria for

Ca and Mg. Indeed, recent research has shown that Ca above levels

required for plant growth strongly stimulated the growth of nodule

bacteria in the rhizosphere of subterranean clover seedlings: at low

levels of inoculation, Ca also stimulated nodulation but, surprisingly,

Mg could only partially replace Ca (68). These results indicate either

that Ca and Mg modify the ability of the root to adsorb nodule bacteria

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or that the Ca-Mg environment for the growth of nodule bacteria in the rhizosphere differs greatly from that in solution. Whatever the reason, the growth of nodule bacteria in the rhizosphere of subterranean clover can, under certain conditions, be far more sensitive to Ca or, to a lesser extent, to Mg concentrations in solution than is nodule development or host growth: once initiated, nodule development can proceed at Ca concentrations even lower than those required for plant growth (69, 86).

The effects of divalent cations on nodulation have only been observed in solutions of moderate acidity (pH 4.5 - 5.5 in subterranean clover (62, 68): pH 4.8 - 5.6 in lucerne (86)). Under more acid conditions, as already mentioned, acidity completely inhibits nodulation and Ca has no effect (pH 4.0 in subterranean clover, pH 4.8 in lucerne). At pH values near neutrality, plants achieve maximal nodulation at all levels of Ca above those which are deficient for plant growth. In the pH range between the upper and lower limits where Ca has no effect, increasing Ca and increasing pH replace each other in their effects on nodulation. This phenomenon, formerly so puzzling, now seems readily explained by effects of Ca and H ions on the numbers of nodule bacteria in the rhizosphere. It receives strong support from the evidence already cited that the effects of both high Ca and high pH on nodulation can only occur at low levels of inoculum in solution and can be replaced by increasing bacterial inoculation to very high values. Clearly the relation between the numbers of root nodule bacteria in the rhizosphere and nodulation of subterranean clover and medics in moderately acid media is in a state of delicate balance which can easily be changed by one or more of several interacting ions - H, Ca, and, to a lesser extent, Mg. All could be involved in nodulation problems arising from poor survival and growth of nodule bacteria on moderately acid soils.

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(ii)   Correction of nodulation problems in acid soils

The conclusion that the problem of subterranean clover estab-
lishment on moderately acid soils in eastern Australia resulted from a
peculiar sensitivity of its nodule bacteria to soil acidity led to the
technique of coating seeds with calcium carbonate: apparently trace
amounts of calcium carbonate modify the soil micro-environment near
the seed sufficiently to permit survival and growth there of introduced
nodule bacteria (63). The same effect probably also explains the
success of lime-coated seeds in overcoming nodulation problems in peas
(Sniezko cited by (93)) and clovers (47, 48) in other areas.

Coating seeds with lime can only be expected to overcome
problems of nodulation in legumes on those soils whose acidity retards
growth of introduced strains of nodule bacteria but does not retard
growth of the host plant. It is possible that some species of nodule
bacteria which are very tolerant of high acidity are less sensitive to
acidity than host plant growth. However suggestions that nodule
bacteria of the cowpea group are so tolerant of soil acidity that their
hosts would not benefit from lime pelleting of seeds sown into acid
soils (92, 93) need re-evaluation. Recent evidence suggests that some
cowpea type bacteria may be more sensitive to soil acidity than growth
of their host legume. When sown into sand of pH 5.0 - 5.5, Acacia spp.
failed to nodulate even though growth of the host given combined N was
as good as at pH 6.5 - 7.0 where plants nodulated freely with organisms,
presumably of the cowpea type (43). In some acid soils nodulation of
these species could therefore be expected to respond to lime-pelleting.
Indeed, 4 species of tropical legumes which nodulated poorly with cow-
pea organisms on an acid soil (pH 4.3 in $CaCl_2$) gave 100% nodulated
plants when inoculated seeds were lime-coated: however, lime was not

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essential to this response since other pelleting materials were equally effective possibly by an effect of the adhesive in influencing the survival of bacteria on the seed (94). In other situations the alkalinity of a lime-coat appears to harm cowpea type nodule bacteria as Norris (91, 92) predicted it might. Thus, in near neutral soils, lime-coating of lupins and serradella seeds depressed nodulation of their seedlings by cowpea type organisms (100, 113). The reasons for the variable response of cowpea type organisms to lime-coating are not clear. A better understanding would help in the resolution of nodulation problems of cowpea type host species on acid soils.

Even where the technique of lime-coating seeds overcomes nodulation problems of legume species on acid soils it may not replace liming for maximal legume production. The need to treat many acid soils with Mo is discussed later. In addition, some soils are suffic-iently acid to restrict growth of the host plant through effects of excess H, Mn, or Al ions and on these soils the crop will only achieve maximal yields after amelioration of the acidity of the bulk soil (e.g. 80, 81, 82, 101). Pelleting techniques should therefore only be used to replace other soil treatments when soil conditions are adverse for growth of nodule bacteria but not adverse to other phases of the symbiosis.

(b) Fertilizers

Fertilizers can be toxic to nodule bacteria inoculated on seed. Direct contact should be avoided. Acidity, as in superphosphate (30) and heavy metals (Cu, Zn, (54): Mo, (39)) have been shown to act as toxic agents.

(c) Organic matter

Addition of organic matter to soils has sometimes improved nodulation of legumes (76). However there is no evidence that organic

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matter affects nodulation through chemical factors other than those discussed in this or the next section. In hot climates, mulches of organic matter could critically modify the physical soil environment for survival of nodule bacteria (95).

2. Nodule formation

(a) Acidity

The striking effects of acidity on infection of roots by nodule bacteria have already been mentioned. At pH 4.6 in lucerne (83) and at pH 4.5 in peas (59) no nodules formed even when extremely large numbers of bacteria were added to cultures. The bacteria congregated on roots but root hairs either failed to develop properly, as in peas, or failed to curl as in lucerne. Munns (85) postulated that acidity inhibited infection by inhibiting activity of the exo-enzyme, pectinase: he observed that pectinase, was almost completely inhibited in lucerne at pH 4.5.

Once infected, nodule formation, N fixation, and plant growth can proceed at pH values too low for infection (59, 68, 83, 85).

(b) Calcium

Earlier conclusions that Ca inhibits nodule formation (62) have now been shown to be invalid as the result of experiments, discussed above, demonstrating that Ca can increase the number of nodule bacteria in the rhizosphere. Moreover, once initiated, nodule development in both subterranean clover and lucerne was able to proceed at Ca concentrations in solution too low even for plant growth (62, 86).

(c) Boron

In 1925, Brenchley and Thornton (27) observed that B-deficiency inhibited the development of vascular strands from roots to nodules in beans: bacteria did not form bacteroids and appeared to become parasitic on the cells in the nodule. They suggested that B

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had a specific effect on N fixation. Mulder (79) confirmed this suggestion in peas but only at very low levels of B supply. At low levels of B supply, peas without N salts developed symptoms of N-deficiency and did not develop any symptoms of B-deficiency. Addition of N salts overcame N-deficiency symptoms, promoted growth, and induced symptoms of B-deficiency. By contrast, at higher but still deficient levels of B supply, peas given no N salts fixed N, developed symptoms of B-deficiency, and did not respond to addition of N salts. Mulder concluded that the host plant had a higher requirement for B than did development of the nodule. More likely the results reflect the critical importance to the expression of B-deficiency symptoms of plant development at the time B becomes deficient. All meristems are extremely sensitive to B-deficiency during their development but mature organs are relatively insensitive. As a result, B-deficiency may induce N-deficiency when B becomes deficient during the formation of nodules but not when B becomes deficient after nodule formation has been completed: in this latter stage, B-deficiency would not hinder N fixation and symptoms would develop as B-deficiency of the host plant.

(d) Combined nitrogen

Combined nitrogen depresses nodulation of many legumes by many strains of organism – cowpeas, vetch (33), clovers (40), peas (79), lucerne (105). Very high concentrations of N (c. 4 mM NO_3^-) are required to completely inhibit nodulation. The most effective forms of N are NO_3^- and NO_2^- which possibly act by destroying IAA which, in clovers, is active in root-hair curling and infection. Other forms of N may also suppress nodulation possibly by influencing other stages in the process. Concentrations of N as low as 0.5 mM may delay nodulation when present as NO_3^- but have no effect when present as NH_4^+, asparagine, or urea (40).

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In many situations low concentrations of combined N enhance nodulation as, for example, in lucerne (84) and in cowpeas (33). This appears to be a secondary effect of N treatment resulting from the habit of nodules in this plant to develop in successive crops and from the primary effect of treatment in delaying nodulation: because of the delay in nodulation, N-treated roots have more foci for infection when it does occur. The pattern is repeated in subsequent crops of nodules so that the effects of treatment on nodulation vary with the time of harvest. In all cases the primary effect of combined N appears to be one of depressing nodulation (84).

Combined N also has marked and complex effects on N fixation. As a result it is difficult to assess the likely importance of the effects of combined N on nodule development to symbiotic N fixation in the field.

3. Nodule function

(a) Molybdenum

Field responses of plants to Mo were first observed in subterranean clover growing on acid, ironstone soils in southern Australia (6). Deficiencies of Mo are now known to be widespread in leguminous crops and pastures, on a variety of soils in all continents. In herbage legumes, Mo-deficiency occurs almost invariably as N-deficiency: responses of herbage legumes to Mo may be largely or entirely replaced by N fertilizers even when they depress absorption of Mo from soils. This is not due to any effect of Mo-deficiency on nodulation but to a specific and striking effect of Mo-deficiency in inhibiting the bio-chemical reactions of N fixation (13, 15).

Legumes also require Mo for their growth when given fertilizer N but the amounts of Mo required are very much smaller than

those required for symbiotic N fixation. The amounts of Mo required

by plants for their growth and for N fixation are very small. Indeed

legumes such as beans and peas may contain sufficient Mo in their seeds

to sustain production for a full generation (50, 77) and may prevent

development of Mo-deficiency in crops grown from seeds imported into

low Mo regions (132). This can make diagnosis of potential Mo-deficiency

problems difficult with grain crops (57). The difficulties can be

accentuated by ineffective nodulation of the legume or by tillage

practices which release soil N.

Molybdenum-deficiencies have been reported for several legume

grain crops in temperate areas (see (8)), and for peanuts in Java (87).

All evidence indicates that the special relationship of Mo to N-fixation

in temperate legumes is universal for all legumes, including those of

tropical areas.

(b) Cobalt

There is as yet no satisfactory evidence that plants require

Co for their growth in the presence of N. But nodule bacteria require

Co for their growth in pure culture (67) and nodulated legumes clearly

require Co for symbiotic N fixation (1, 2, 34).

The amounts of Co required for these purposes are extremely

small, being even less than the amounts of Mo required for these

functions. As a result, the presence of Co in seed imported from other

areas may make diagnosis of Co-deficiency in test areas difficult.

Nevertheless Co-deficiency has been reported for subterranean clover

growing on siliceous sands in the field (97, 103). In these cases,

Co-deficient legumes were nodulated and showed symptoms of N-deficiency.

There is as yet no evidence that Co-deficiency induces nodulation

problems in soils although this could be expected on soils extremely

deficient in Co.

(c) Calcium

Calcium-deficiency, like Mo-deficiency, may depress the growth of the host legume when severe, and interfere specifically with the process of N fixation when moderate (20, 42, 60). But unlike Mo-deficiency, Ca-deficiency seldom appears as a simple N-deficiency in legumes: specific symptoms of Ca-deficiency usually appear with those of N-deficiency. How Ca-deficiency interferes specifically with N fixation is not known.

(d) Acidity

Nodulated legumes frequently become yellow and N-deficient on acid soils. In these situations legumes often, but not invariably, respond to lime. Several phenomena may be involved and successful treatment requires an appreciation of the factors responsible.

Occasionally Ca-deficiency is responsible as, for example, in subterranean clover on acid peaty sands. Here the symptoms were expressed as a combination of symptoms of N-deficiency and of Ca-deficiency of the host legume. Dressings of calcium sulphate were ineffective on such acid sands and the problem was only cured by treatment with heavy dressings of lime (37). On low-Ca, high-Na or high-Mg soils, liming would not be needed.

More frequently, Mo-deficiency causes legumes to be N-deficient on acid soils. Liming may or may not overcome this problem. Where liming overcomes the problem, it does so as a result of the release of Mo from soil colloids by increasing pH (11). The extent of the lime response varies with the amount of Mo released from Mo-deficient soils by liming: where soils are low in native Mo, liming may have no effect, but where soils are high in native, unavailable Mo liming may increase Mo content of legumes to levels harmful to livestock (8).

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Many Mo-deficient soils are also sufficiently acid to induce
nodulation problems in the absence of lime. However, the effects of
lime in releasing Mo from soils are quite distinct from those in improv-
ing nodulation (8, 10). Failure to recognize this point may result in
failure to diagnose Mo-deficiency because of unsatisfactory nodulation
(e.g. by the failure to nodulate crops with effective strains of nodule
bacteria at sowing) or in failure to diagnose nodulation problems
because of an unsatisfactory Mo supply (e.g. by expecting lime to
release Mo from soils in which Mo is totally deficient). Unfortunately,
a great deal of research has failed to recognize the need to correct
both problems on acid soils and as a result, interpretation of lime
responses, especially in tropical legumes, has often been confusing.

(e) Combined nitrogen

The amount of N fixed by various legumes seems to vary
directly with the plant's demand for N. Consequently soil N can
influence N fixation in a number of ways (see reviews 121, 126). The
primary effect of combined N is to suppress N fixation. For example,
the amount of N fixed by peanuts, soybeans, lucerne, and clovers
decreased with increasing levels of combined N beyond those necessary
for growth (4, 5). However, the extent of the inhibition varies
greatly with plant species, strain of nodule bacterium, growth condit-
ions, and the time and level of N application. In some cases, as with
vigorously growing cowpeas, combined N may stimulate the amount of N
fixed: this may indicate that judicious use of N fertilizers at
strategic times during plant growth and nodule development could be
beneficial to N fixation in soils of low N status (33).

4. Growth of the host legume

Even when soil conditions permit the formation and the
function of nodules to proceed satisfactorily, the symbiotic system may
fail to fix much N because soil conditions do not permit satisfactory
growth of the host legume. Soil conditions which limit N fixation
through effects on the growth and metabolism of the host plant are
characterised by the failure of the legume to respond to fertilizer N.
In their response to these soil conditions, legumes thus behave in the
same way as do plants which do not fix N. Consequently, in the present
context, no special interest attaches to these soil conditions unless
legumes are unusually sensitive to them.

Before discussing the sensitivity of legumes to adverse soil
conditions, it is useful to consider briefly how nutrients affect the
growth and the N metabolism of the host plant itself.

(a) Nature of interference with legume growth

Soil conditions which interfere with host plant growth may be
divided into 2 categories - those which restrict N metabolism of the
host plant thereby causing its N concentrations to fall and those which
restrict some other process of growth in the host thereby frequently
causing N concentrations to rise.

(i) Interference with metabolism of fixed nitrogen

Several nutrient deficiencies restrict host plant growth by
interfering with its metabolism of fixed N. Deficiency of S in
subterranean clover behaves in this way. Anderson and Spencer (12, 14),
noted that S-deficient plants had low concentrations of N and appeared
pale green as if N-deficient. But, unlike pale green, Mo-deficient
subterranean clover, S-deficient subterranean clover failed to respond

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to fertilizer N. Deficiency of S prevented the conversion of non-protein N to protein and did not interfere primarily with the process of N fixation. In other legumes and in non-legumes, S-deficiency interferes with N metabolism in a similar way (see 36).

Deficiencies of Cu and of P under some circumstances may also depress the percentage N in subterranean clover plants (28, 42, 72). However, no responses to N have been reported and it is not yet clear whether these deficiencies interfere with N metabolism of the host or specifically with reactions of N fixation. More generally, P-deficiency increases the N concentrations in the host legume, indicating a prime effect on some other aspect of host plant growth as discussed below.

(ii) Interference with other processes

Most nutrient deficiencies and soil toxicities do not inter-fere primarily with N metabolism but with some other process of growth. For example, Ca-deficiency may develop in the host before soil N has been depleted. In both soybean (3, 52) and in subterranean clover (60) severe Ca-deficiency depressed N-fixation manyfold by inhibiting host growth. By contrast with the effects of moderate Ca-deficiency which interfered with the functioning of the symbiotic process itself, severe Ca-deficiency actually increased the concentration of N in plants and addition of fertilizer N failed to increase their yield. Many other nutrients behave in a similar way - Zn (79), K (24), P (120).

Toxicities of Mn (e.g. various tropical and temperate pasture legumes (17), beans (115), peanuts (25)) and of Al (e.g. lucerne (49, 81, 82), soybeans (38)) may also severely depress host plant growth: there is no evidence that excess concentrations of either of these elements interfere with other phases of the symbiotic system before they affect host plant growth.

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(b) Sensitivity of legumes

Phosphorus and S deficiencies are emerging as major problems to growth of legumes and other crops in tropical and sub-tropical regions (96, 114, 119, 129). Phosphorus-deficiency is widespread and is frequently severe. Some tropical soils have notoriously high capacities for fixing P and most crops, including grain legumes, require very large fertilizer dressings to achieve maximal production. Sulphur-deficiency has also been recognised in many areas. Its importance may be expected to increase for 2 reasons. Firstly, high P fertilizers of low S content are replacing superphosphate for treatment of P deficiency in crops. Secondly, in many soils in high rainfall regions, the residual value of S in fertilizers is much lower than that of P (21, 46).

In addition to P and S, many other nutrients can be expected to affect growth of tropical grain legumes on particular soils. But, apart from their insensitivity to low levels of combined N in soils, legumes as a group do not behave towards adverse soil conditions in ways which are very different from all other species. Suggestions that legumes have especially high requirements for P and for Ca do not seem valid for reasons discussed below.

Legumes have frequently responded dramatically to P-fertilizers while grasses in the same pasture have shown little or no response. Experiences of this nature may have led to erroneous conclusions that legumes have unusually high P requirements. Usually they indicate that the soils under investigation are N-deficient as well as P-deficient. When N is added to such soils, grasses respond strongly to P-fertilizers and may even suppress legume growth by competing with legumes for P (120). Similar differences between legumes and grasses may be expected in their response to many nutrients on soils with concomitant N-deficiency (e.g. S-deficiency in subterranean clover (14), and in medics (51)).

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The high Ca content of legumes compared with grasses has led to suggestions that they have unusually high Ca requirements. Legumes do indeed seem to need about twice as much Ca in their tissues as do cereals and grasses. But since they absorb Ca much faster than do cereals and grasses, legumes are no more sensitive to Ca-deficiency where the deficiency develops as the result of low but rapidly replenished Ca concentrations in solution. But where Ca-deficiency develops as the result of depletion of a limited amount of Ca in the root zone, legumes will be much more sensitive to Ca-deficiency than grasses and cereals. However, herbaceous dicotyledons also absorb Ca very rapidly and would be as sensitive to Ca-deficiency as most legumes (64, 65, 66). Legumes which bury their fruits show an exceptional sensitivity of fruit development to Ca: this is discussed later.

Suggestions have also been made that tropical legumes are much more tolerant of low Ca than are temperate legumes (18). The evidence indicates rather that both groups of legumes vary widely in their response to dressings of Ca fertilizers (126). Moreover, response to Ca fertilizers frequently involves factors other than Ca nutrition. Thus dressings of Ca salts have complex effects on both the concentrations of other ions in and the absorption of other ions from the soil solution. For example, because Ca stimulates P absorption strongly, plants may respond to Ca in solutions containing low concentrations of P but may fail to respond to Ca in solutions containing high concentrations of P: the effect is more marked in some species (e.g. medics) than in others (e.g. subterranean clover (107)). Calcium can also suppress Mn absorption and in this way can prevent Mn toxicity from developing (e.g. in medics (108)). Such indirect effects of Ca may explain some of the apparent differences in the Ca requirements of legumes as for example, between Mt. Barker strain of _Trifolium subterraneum_ which

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grew well with 2.5 μM Ca in nutrient solutions and 0.2% Ca in its tops and Medicago truncatula which grew poorly at these Ca levels and responded strongly to increasing Ca up to 1000 μM in solution and 2.5% in its tops (64, 66, 66a).

In soils, interpretation of responses to Ca fertilizers are further complicated by changes in concentrations of ions other than Ca in the soil solution. Thus applications of Ca sulphate or chloride increase the acidity of the soil solution and often also increase Mn and Al and decrease Mo concentrations. Applications of calcium carbonates or oxides have opposite effects on the concentrations of these ions. For growth of the host legume on acid soil, Mn and Al concentrations may be particularly important, since these ions may be toxic to legume species. Treatment of Mn and Al toxicities is expensive since it generally requires the treatment of soil to some depth with heavy dressings of lime. It may be preferable to grow alternative species or cultivars with greater tolerance of high concentrations of Mn and Al in the soil solution: for example, subterranean clover is much less sensitive to Mn and to Al toxicities than are medics (49, 108) and soybean cultivars vary in their sensitivity to Al toxicity (38).

As with other factors which affect host plant growth, there is no evidence that legumes are any more sensitive to Mn and Al toxicities than are plants from other groups. Host plant growth of individual genera, species, or cultivars within the legumes shows a vast range of sensitivity to various soil conditions. Their behaviour probably encompasses the extremes in behaviour of all plant groups. Therefore, there is no reason to treat the response of the host legume to fertilizers any differently from the response of any other crop on a soil well supplied with N. Indeed it is often desirable to consciously treat the legume as a non-legume and to supply it with an adequate

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supply of fertilizer N. In this way the response of the host legume
to other fertilizers can be examined independently of any special
problems associated with the symbiotic system (111).

III. GRAIN PRODUCTION

Much of the N fixed by legumes during their vegetative growth
is transported from other organs to flowers and to grain during their
development. Fixed N may also be supplied directly from the nodules
to the reproductive organs. But the extent to which symbiotic fixat-
ion can supply all the N required for maximal grain production in
legumes is not clearly established. Some experiments show no response
to N fertilizers while others show large responses (122). However, it
is important to emphasize that in no case showing large effects of
fertilizer N on grain production has the effectiveness of the bacteria
in the nodules been unequivocally established: the responses could
have resulted from an inefficient N fixing system. More critical work
is needed on this question.

Other nutrients affect grain production in legumes in much
the same way as they affect seed production in all species. Nutrients
which move in plant phloem (P, S, K) move readily from other organs to
developing seeds. However, nutrients, such as Ca, B, and probably Cu
and Fe, which do not move in phloem, can not move to developing fruits
from leaves even when they are present there in luxury amounts (23).
As a result, the plant can only supply these nutrients to developing
flower and seed via the transpiration stream. Seed development is
therefore usually dependent upon a continuous external supply of all
phloem-immobile nutrients. This makes seed production much more sens-
itive to deficiencies of phloem-immobile nutrients than to phloem-

mobile nutrients. When phloem-immobile nutrients become deficient in the later stages of growth, vegetative growth may be unaffected but seed development may fail completely as reported, for example, in B-deficiency of clover on some soils (58). Nutrients such as Zn, Mg, and Mn appear to be intermediate in their ability to move from leaves to developing flowers and fruits. For example, in plants containing luxury amounts, Zn moved in appreciable quantities from leaves to flowers and fruits but none moved in plants with deficient or marginal Zn supplies (104).

Special problems are encountered with phloem-immobile nutrients in legumes such as peanuts which bury their seed. Seed burial eliminates transpiration to developing peanut fruits (131). As a result, any phloem-immobile nutrient such as Ca must be absorbed directly from the soil surrounding the developing peanut fruit (44). Since the fruits and roots of peanut plants differ in their characteristics of Ca absorption from soil colloids (78) seed production and vegetative growth may respond differently to soil Ca levels. Indeed it has long been known that, whereas a low level of Ca is adequate for vegetative growth of peanuts, a relatively large supply is necessary for proper development of fruit (32).

Other phloem-immobile nutrients may also need to be supplied directly from the soil to developing underground fruits. Where the buried fruit is more sensitive to adverse soil conditions than is symbiotic N fixation or growth of the host plant, the requirements of the fruits will determine the soil treatments for optimal grain production. Fertilizers would need to be incorporated into the surface layer of soil and would be ineffective if drilled. Once again, the peculiar sensitivity of a particular phase of growth is seen to dominate the response of the legume to the soil environment.

IV. CONCLUSION

This review has surveyed the problems which can arise in legume production from various factors in the soil chemical environment. It has stressed the value of understanding how each factor influences each phase of the symbiotic system for the legume crop under study. It has shown how this knowledge can result in the solution of problems by use of specific treatments which are much more reliable than blanket treatments. This approach has proved particularly valuable on acid soils where lime dressings were shown to have several effects which could be replaced by simple, specific treatments. It has the added advantage of avoiding possible harmful effects of blanket treatments as, for example, the inducement of Zn deficiency in legumes by liming (112).

While most of the examples cited involve work with temperate, pasture legumes the general principles should be equally valid for growth of all legumes. But because plant species and nodule bacteria vary in their sensitivity to soil conditions, the relevance of specific findings for one crop may not be directly applicable to another. They must be assessed critically for each crop.

Diagnosis of adverse factors in the soil chemical environment can be difficult and complex in legume crops. It is especially difficult where several factors operate simultaneously as, for example, in the acid soils of eastern Australia where conditions are unfavourable for growth of nodule bacteria and where Mo, P, and S may all be deficient (10). Where such multiple problems exist, satisfactory diagnosis of each problem can only be achieved when no other problem limits plant growth, Anderson (9, 61) has pointed out that this is a direct consequence of Blackman's law of limiting factors and has rightly emphasized its pre-eminence in diagnosis of nutritional deficiencies of plants. In these

situations, applications of fertilizers in traditional factorial com-
binations are sometimes less useful than are applications of fertilizers
in complex basal mixtures from which individual nutrients are omitted in
turn (7).

The problem of diagnosis may sometimes be simplified by using
fertilizer N to differentiate those factors influencing nodulation and
the symbiotic process from those affecting host plant growth. A sub-
stantial response of legumes to N fertilizers indicates that some factor
is limiting nodulation or N fixation: further definition of the limit-
ing factors may then proceed in the absence of fertilizer N. In this
case it is essential to have available a satisfactory source of effect-
ive nodule bacteria. This has often proved unusually difficult as, for
example, in many of the early attempts to establish subterranean clover
on problem soils in Australia which failed because inoculants were
ineffective (126): the resulting deficiency of N can limit plant growth
so severely that it prevents diagnosis of other nutrient problems.
Similar problems are likely to arise with grain legume crops in develop-
ing countries. They must be resolved before the limitations of the soil
chemical environment on the production of grain legumes can be completely
assessed.

IV. REFERENCES

(1)   Ahmed, S., and Evans, H.J. 1959. Effect of cobalt on the growth of
        soybeans in the absence of supplied nitrogen. Biochem. and
        Biophys. Res. Commun. 1:271-275.

(2)   Ahmed, S., and Evans, H.J. 1960. Cobalt: a micronutrient element
        for the growth of soybean plants under symbiotic conditions.
        Soil Sci. 90:205-210.

41

(3)   Albrecht, W.A. 1937. Physiology of root nodule bacteria in relation
        to fertility levels of the soil. Proc. Soil. Sci. Soc. Amer.
        2:315-27.

(4)   Allos, H.F., and Bartholomew, W.V. 1955. Effect of available
        nitrogen on symbiotic fixation. Proc. Soil. Sci. Soc. Amer.
        19:182-184.

(5)   Allos, H.F., and Bartholomew, W.V. 1959. Replacement of symbiotic
        fixation by available nitrogen. Soil Sci. 87:61-66.

(6)   Anderson, A.J. 1942. Molybdenum deficiency on a South Australian
        ironstone soil. J. Aust. Inst. agric. Sci. 8:73-75.

(7)   Anderson, A.J. 1952. Diagnosis of nutrient deficiencies in
        pastures. Rural Res. C.S.I.R.O. Australia. 2:9-10.

(8)   Anderson, A.J. 1956a. Molybdenum as a fertilizer. Adv. Agron.
        8:163-202.

(9)   Anderson, A.J. 1956b. Effects of fertilizer treatments on pasture
        growth. Proc. 7th. Int. Grassl. Congr., 323-33.

(10)   Anderson, A.J., and Moye, D.V. 1952. Lime and molybdenum in clover
        development on acid soils. Aust. J. agric. Res. 3:95-110.

(11)   Anderson, A.J., and Oertel, A.C. 1946. Plant responses to molybdenum
        as a fertilizer. 2. Factors affecting the response of plants
        to molybdenum. Council Sci. Ind. Res. Bul. 198:25-44.

(12)   Anderson, A.J., and Spencer, D. 1949. Molybdenum and sulphur in
        symbiotic nitrogen fixation. Nature (Lond.) 164:273.

(13)   Anderson, A.J., and Spencer, D. 1950a. Molybdenum in nitrogen
        metabolism of legumes and non-legumes. Aust. J. sci. Res.
        Ser. B. Biol. Sci. 3:414-430.

(14)   Anderson, A.J., and Spencer, D. 1950b. Sulphur in nitrogen
        metabolism of legumes and non-legumes. Austral. J. sci. Res.
        Ser. B. Biol. Sci. 3:431-449.

(15) Anderson, A.J., and Thomas, Margaret P. 1946. Plant responses to

       molybdenum as a fertilizer. 1. Molybdenum and symbiotic

       nitrogen fixation. Council Sci. Ind. Res. Bul. 198:7-24.

(16) Andrew, C.S. 1962. Influence of nutrition on nitrogen fixation and

       growth of legumes. In A Review of Nitrogen in the Tropics with

       Particular Reference to Pastures. Commonwealth Bur. Pastures

       and Field Crops. Bul. 46. pp. 130-146.

(17) Andrew, C.S. and Hegarty, M.P. 1969. Comparative responses to

       manganese excess of eight tropical and four temperate pasture

       legume species. Aust. J. agric. Res. 20:687-96.

(18) Andrew, C.S., and Norris, D.O. 1961. Comparative responses to

       calcium of five tropical and four temperate pasture legume

       species. Aust. J. agric. Res. 12:40-55.

(19) Andrew, C.S. and Robins, M.F. 1969. The effect of phosphorus on

       the growth and chemical composition of some tropical pasture

       legumes. 1. Growth and critical percentages of phosphorus.

       Aust. J. agric. Res. 20:665-74.

(20) Banath, C.L., Greenwood, E.A.N., and Loneragan, J.F. 1966. Effects

       of calcium deficiency on symbiotic nitrogen fixation. Pl.

       Physiol. (Lancaster) 41:760-3.

(21) Barrow, N.J. 1970. Comparison of the adsorption of molybdate,

       sulfate and phosphate by soils. Soil Sci. 109:283-8.

(22) Bergersen, F.J. 1961. The growth of Rhizobium in synthetic media.

       Aust. J. biol. Sci. 14:349-360.

(23) Biddulph, O. 1959. Translocation of inorganic solutes. In Plant

       Physiology. (ed. F.C. Steward). Vol. 2, pp. 553-603.

       (Academic Press : New York).

(24) Blaser, A.E. and Brady, N.C. 1950. Nutrient competition in plant

       associations. Agron. J. 42:128-35.

(25) Boyd, H.W. 1971. Manganese toxicity to peanuts in autoclaved
      soil. Pl. Soil 34:133-44.

(26) Bornemisza, E. 1972. Fertilizer response and current fertilization
      and nutritional problems of grain legume crops in Central and
      South America. This conference.

(27) Brenchley, W.E., and Thornton, H.G. 1925. The relation between the
      development, structure and functioning of the nodules of Vicia
      faba as influenced by the presence or absence of boron in the
      nutrient medium. Proc. Roy. Soc. (Lond.) B. 98:373-398.

(28) Cartwright, B. and Hallsworth, E.G. 1970. Effect of copper defic-
      iency on root nodules of subterranean clover. Pl. Soil 33:685-698.

(29) Cass Smith, W.P., and Holland, A.A. 1958. The effect of soil
      fungicides and fumigants on the growth of subterranean clover
      on new light land. J. Agric. Western Australia (3rd. ser.)
      7:225-231.

(30) Cass Smith, W.P., and Pittman, H.A.J. 1938. The influence of methods
      of planting on the effective inoculation and establishment of
      subterranean clover. J. Agric. Western Australia. 16:61-73.

(31) Chatel, D.L., Greenwood, R.M., and Parker, C.A. 1968. Saprophytic
      competence as an important character in the selection of
      Rhizobium for inoculation. 9th. Int. Congr. Soil Sci. 2:65-73.

(32) Colwell, W.E. and Brady, N.C. 1945. The effect of calcium on yield
      and quality of large-seeded type peanuts. J. Am. Soc. Agron.
      37:413-28.

(33) Dart, P.J. and Wildon, D.C. 1970. Nodulation and nitrogen fixation
      by Vigna sinensis and Vicia atropurpurea: the influence of
      concentration, form, and site of application of combined
      nitrogen. Aust. J. agric. Res. 21:45-56.

44

(34) Delwiche, C.C., Johnson, C.M., and Reisenauer, H.M. 1961. Influence
of cobalt on nitrogen-fixation by Medicago. Pl. Physiol.
(Lancaster) 36:73-8.

(35) Dobereiner, J. 1966. Manganese toxicity effects on nodulation and
nitrogen fixation of beans (Phaseolus vulgaris L.) in acid
soils. Pl. Soil 14:153.

(36) Eaton, S.V. 1966. Sulphur. In Diagnostic criteria for plants and
soils. (ed. H.D. Chapman). Univ. Calif. Div. Agric. Sci.

(37) Fitzpatrick, E.N. 1956. A study of the problems of establishment
and nutrition of subterranean clover on acid peaty sands.
M.Sc. Thesis, University of Western Australia.

(38) Foy, C.D., Fleming, A.L. and Armiger, W.H. 1969. Aluminium toler-
ance of soybean varieties in relation to calcium nutrition.
Agron. J. 61:505-511.

(39) Gartrell, J.W. 1969. The effect of sodium molybdate mixed in the
lime pellet seed on nodulation, nitrogen content and growth of
subterranean clover. Aust. J. exp. Agric. An. Husb. 9:432-436.

(40) Gibson, A.H., and Nutman, P.S. 1960. Studies on the physiology of
nodule formation. 7. A reappraisal of the effect of preplant-
ing. An. Bot. 24:420-433.

(41) Graham, P.H. 1964. The application of computer techniques to the
taxonomy of the root-nodule bacteria of legumes. J. gen.
Microbiol. 35:511-7.

(42) Greenwood, E.A.N., and Hallsworth, E.G. 1960. Studies on the nutrit-
ion of legumes. II. Some interactions of calcium, phosphorus,
copper, and molybdenum on the growth and chemical composition
of Trifolium subterraneum L. Pl. Soil. 12:97-127.

(43) Habish, H.A. 1970. Effect of certain soil conditions on nodulation
of Acacia spp. Pl. Soil. 33:1-6.

(44) Harris, H.C. 1949. The effect on the growth of peanuts of nutrient

deficiencies in the root and pegging zone. Pl. Physiol.

(Lancaster) 24:150-62.

(45) Harris, H.C. and Gilman. 1957. Effect of boron on peanuts. Soil

Sci. 84:233-42.

(46) Harward, M.E. and Reisenauer, H.M. 1966. Reactions and movement

of inorganic soil sulfur. Soil Sci. 101:326-35.

(47) Hastings, A., and Drake, A.D. 1960. Inoculation and pelleting of

clover seed. New Zealand J. Agric. 101:619-621.

(48) _____ and _____ 1962. Further tests with materials for

pelleting inoculated clover seeds. New Zealand J. Agric.

104:331-3.

(49) Helyar, K.R. and Anderson, A.J. 1970. Some effects of soil pH on

different species and on the soil solution for a soil high in

exchangeable aluminium. Proc. XIth. Int. Grassl. Congr. 431-4.

(50) Hewitt, E.J., Bolle-Jones, E.W. and Miles, P. 1954. The production

of copper, zinc, and molybdenum deficiencies in crop plants

grown in sand culture with special reference to some effects

of water supply and seed reserves. Pl. Soil 5:205-22.

(51) Hilder, E.J. and Spencer, K. 1954. The influence of sulphur on a

natural Medicago pasture. J. Aust. Inst. agric. Sci. 20:171-6.

(52) Horner, G.M. 1936. Relation of the degree of base saturation of a

colloidal clay by calcium to the growth, nodulation and com-

position of soybeans. Mis. Agric. Expt. Stn. Res. Bull. No. 232.

(53) Ishizawa, S. 1953. Studies on the root nodule bacteria of legumin-

ous plants. I. Characters in artificial media. Part 4.

Effect of pH. J. Sci. Soil, Tokyo. 23:169-72.

(54) Jenkins, H.V., Vincent, J.M., and Waters, L.M. 1954. The root-

nodule bacteria as factors in clover establishment in the red

basaltic soils of the Lismore District, New South Wales. 3.
Field inoculation trials. Aust. J. agric. Res. 5:77-89.

(55) Jensen, H.L. 1942. Nitrogen fixation in leguminous plants. I.
General characters of root-nodule bacteria isolated from
species of _Medicago_ and _Trifolium_ in Australia. Proc. Linnean
Soc. New South Wales. 67:98-108.

(56) Jensen, H.L. 1943. Nitrogen fixation in leguminous plants. 4.
The influence of reaction on the formation of root-nodules
in _Medicago_ and _Trifolium_. Proc. Linnean Soc. New South
Wales. 68:207-220.

(57) Johnson, C.M., Pearson, G.A. and Stout, P.R. 1952. Molybdenum
nutrition of crop plants. II. Plant and soil factors concerned
with molybdenum deficiencies in crop plants. Pl. Soil. 4:178-96.

(58) Johnson, W.C. and Wear, J.I. 1967. Effect of boron on white clover
(_Trifolium repens_ L.) seed production. Agron. J. 59:205-6.

(59) Lie, T.A. 1969. The effect of low pH on different phases of nodule
formation in pea plants. Pl. Soil. 31:391-406.

(60) Loneragan, J.F. 1959. Calcium in the nitrogen metabolism of
subterranean clover. Aust. J. biol. Sci. 12:26-39.

(61) Loneragan, J.F. 1964. "The nutrition of grasslands". _In_ Grasses
and Grasslands. pp. 206-220. Macmillan : London.

(62) Loneragan, J.F., and Dowling, E.J. 1958. The interaction of calcium
and hydrogen ions in the nodulation of subterranean clover.
Aust. J. agric. Res. 9:464-472.

(63) Loneragan, J.F., Meyer, D., Fawcett, R.G., and Anderson, A.J. 1955.
Lime pelleted clover seeds for nodulation on acid soils.
Jour. Aust. Inst. agric. Sci. 21:264-265.

(64) Loneragan, J.F. and Snowball, K. 1969a. Calcium requirements of
plants. Aust. J. agric. Res. 20:465-78.

(65) _____ and _____ 1969b. Rate of calcium absorption by plant
roots and its relation to growth. Aust. J. agric. Res. 20:479-90.

(66) _____ , _____, and Simmons, W.J. 1968. Response of plants
to calcium concentration in solution culture. Aust. J. agric.
Res. 19:845-57.

(66a) Loneragan, J.F., Rowland, I.C., Robson, A.D., and Snowball, K. 1970.
The Ca nutrition of plants. Proc. XIth. Int. Grassl. Congr.

(67) Lowe, R.H., and Evans, H.J. 1962. Cobalt requirement for the growth
of rhizobia. J. Bact. 83:210-211.

(68) Lowther, W.L. 1970. Calcium in the nodulation and growth of legumes.
Ph.D. Thesis : Univ. W. Australia.

(69) Lowther, W.L. and Loneragan, J.F. 1968. Calcium and nodulation in
subterranean clover (Trofolium subterraneum L.). Pl. Physiol.
(Lancaster) 43:1362-6.

(70) Lowther, W.L. and Loneragan, J.F. 1970. Calcium in the nodulation of
legumes. Proc. 11th. Int. Grassl. Congr. 446-50.

(71) McCalla, T.M. 1937. Behaviour of legume bacteria (Rhizobium) in
relation to exchangeable calcium and hydrogen ion concentrat-
ion of the colloidal fraction of the soil. Res. Bull. Mo.
agric. Exp. Sta. No. 256.

(72) McLachlan, K.D., and Norman, B.W. 1961. Phosphorus and symbiotic
nitrogen fixation in subterranean clover. J. Aust. Inst.
agric. Sci. 27:244-245.

(73) Marshall, K.C. 1964. Survival of root-nodule bacteria in dry soils
exposed to high temperatures. Aust. J. agric. Res. 15:273-81.

(74) Marshall, K.C., Mulcahy, M.J., and Chowdhury, M.C. 1963. Second-
year clover mortality in Western Australia - a microbiological
problem. J. Aust. Inst. agric. Sci. 29:160-4.

48

(75) Masefield, G.B. 1958. Some factors affecting nodulation in the tropics. In Nutrition of the Legumes (ed. Hallsworth). pp. 202-215. (Butterworths. London).

(76) Masefield, G.B. 1965. The effect of organic matter in soil on legume nodulation. Exp. Agric. 1:113-119.

(77) Meagher, W.R., Johnson, C.M., and Stout, P.R. 1952. Molybdenum requirement of leguminous plants supplied with fixed nitrogen. Pl. Physiol. (Lancaster) 27:223-230.

(78) Mehlich, A. and Reed, J.F. 1947. The influence of type of colloid and degree of calcium saturation on the fruit characteristics of peanuts. Proc. Am. Soc. Soil Sci. 11:201-5.

(79) Mulder, E.G. 1948. Investigations on the nitrogen nutrition of pea plants. Pl. Soil. 1:179-212.

(80) Munns, D.N. 1965a. Soil acidity and growth of a legume. I. Interactions of lime with nitrogen and phosphorus on growth of Medicago sativa L. and Trifolium subterraneum L. Aust. J. agric. Res. 16:733-41.

(81) _____ 1965b. ibid. II. Reactions of aluminium and phosphate in solution and effects of aluminium, phosphate, calcium, and pH on Medicago sativa L. and Trifolium subterraneum L. in solution culture. ibid. 743-55.

(82) _____ 1965c. ibid. III. Interactions of lime and phosphate in growth of Medicago sativa L. in relation to aluminium toxicity and phosphate fixation. ibid. 757-66.

(83) Munns, D.N. 1968a. Nodulation of Medicago sativa in solution culture. I. Acid-sensitive steps. Pl. Soil 28:129-46.

(84) _____ 1968b. ibid. II. Compensating effects of nitrate and of prior nodulation. ibid. 246-57.

(85) Munns, D.N. 1969. Enzymatic breakdown of pectin and acid-inhibition of the infection of _Medicago_ roots by _Rhizobium_. Pl. Soil 30:117-9.

(86) Munns, D.N. 1970. Nodulation of _Medicago sativa_ in solution culture. V. Calcium and pH requirements during infection. Pl. Soil 32:90-102.

(87) Newton, J.D. and Said, A. 1957. Molybdenum deficiency in latosols of Java. Nature (Lond.). 180:1485-6.

(88) Norris, D.O. 1956. Legumes and the _Rhizobium_ symbiosis. Empire J. exp. Agric. 24:247-270.

(89) Norris, D.O. 1958a. Lime in relation to the nodulation of tropical legumes. _In_ Nutrition of the Legumes (_ed_. Hallsworth), Butterworths. London. pp. 164-182.

(90) Norris, D.O. 1958b. _Rhizobium_ needs magnesium, not calcium. Nature (Lond.) 182:734-735.

(91) Norris, D.O. 1959. The role of calcium and magnesium in the nutrition of _Rhizobium_. Aust. J. agric. Res. 10:651-698.

(92) Norris, D.O. 1967. The intelligent use of inoculants and lime pelleting for tropical legumes. Trop. Grassland 1:107-21.

(93) Norris, D.O. 1970. Nodulation of pasture legumes. _In_ Australian Grasslands. (_ed_. R.M. Moore) pp. 339-348. Aust. Nat. Univ. Press : Canberra.

(94) Norris, D.O. 1971. Seed pelleting to improve nodulation of tropical and sub-tropical legumes. 2. The variable response to lime and rock phosphate pelleting of eight legumes in the field. Aust. J. exp. Agric. An. Husb. 11:282-9.

(95) Nutman, P.S. 1972. Influence of physical environmental factors on the activity of _Rhizobium_ in soil and symbiosis. This Conference.

(96) Nye, P.H. 1952. Studies on the fertility of gold coast soils.
Pt. III. The phosphate status of the soils. Emp. J. exp.
Agric. 20:47-55.

(97) Ozanne, P.G., Greenwood, E.A.N., and Shaw, T.C. 1963. The cobalt
requirement of subterranean clover. Aust. J. agric. Res.
14:39-50.

(98) Parker, C.A. 1968. On the evolution of symbiosis in legumes. In
"Festskrift til Hans Lauritz Jensen" (ed. Henriksen) pp. 107-16.
(Gadgaard Neilsens Bogtrykkeri, Lemvig).

(99) Parker, C.A. 1971. The significance of acid and alkali production
by rhizobia on laboratory media. 4th. Austral. Legume
Nodulation Conference, Canberra.

(100) Parker, C.A. and Oakley, A.E. 1965. Reduced nodulation of lupins
and serradella due to lime pelleting. Aust. J. exp. Agric.
An. Husb. 5:144-6.

(101) Paton, D.F. 1960. Problems in legume establishment with special
reference to nodulation and nutrition. J. Aust. Inst. agric.
Sci. 26:32-47.

(102) Peterson, H.B., and Goodding, T.H. 1941. The geographic distribut-
ion of Azotobacter and Rhizobium meliloti in Nebraska soils in
relation to certain environmental factors. Univ. Nebraska
Agr. Exp. Sta. Res. Bul. 121. 24 pp.

(103) Powrie, J.K. 1960. A field response by subterranean clover to
cobalt fertilizer. Aust. J. Sci. 23:198-199.

(104) Riceman, D.S. and Jones, G.B. 1958. Distribution of zinc and copper
in subterranean clover (Trifolium subterraneum L.) grown in
culture solutions supplied with graduated amounts of zinc.
Aust. J. agric. Res. 9:73-122.

(105) Richardson, D.A., Jordan, D.C., and Garrard, E.H. 1957. The influence
of combined nitrogen on nodulation and nitrogen fixation by
Rhizobium meliloti Dangeard. Can. J. Pl. Sci. 37:205-214.

(106) Robson, A.D. 1969. Soil factors affecting the distribution of
annual Medicago species. J. Aust. Inst. agric. Sci. 35:154-67.

(107) Robson, A.D., Edwards, D.G., and Loneragan, J.F. 1970. Calcium
stimulation of phosphate absorption by annual legumes. Aust.
J. agric. Res. 21:601-12.

(108) Robson, A.D. and Loneragan, J.F. 1970a. Sensitivity of annual
Medicago species to manganese toxicity as affected by calcium
and pH. Aust. J. agric. Res. 21:223-32.

(109) _____ and _____ 1970b. Nodulation and growth of Medicago
truncatula on acid soils. I. Effect of calcium carbonate and
inoculation level on the nodulation of Medicago truncatula on
a moderately acid soil. Aust. J. agric. Res. 21:427-34.

(110) _____ and _____ 1970c. ibid. II. Colonization of acid soils
by Rhizobium meliloti. Aust. J. agric. Res. 21:435-45.

(111) _____ and _____ 1970d. Growth of annual Medicago species on an
acid soil. Proc. XIth. Int. Grassl. Congr. 434-8.

(112) Russell, J.S. 1966. Plant growth on a low calcium status solodic
soil in a subtropical environment. I. Legume species, calcium
carbonate, zinc, and other minor element interactions. Aust. J.
agric. Res. 17:673-86.

(113) Shipton, W.A. and Parker, C.A. 1967. Nodulation of lime pelleted
lupins and serradella when inoculated with peat and agar
cultures. Aust. J. exp. Agric. An. Husb. 7:259-62.

(114) Singh, H.G. 1970. Effect of sulphur in preventing the occurrence
of chlorosis in peas. Agron. J. 62:708-11.

52

(115) Siman, A., Cradock, F.W., Nicholls, P.J., and Kirton, H.C. 1971.

Effects of calcium carbonate and ammonium sulphate on manganese

toxicity in an acid soil. Aust. J. agric. Res. 22:201-14.

(116) Souto, S.M. and Dobereiner, J. 1970. Problems in the establishment

of perennial soybean (Glycine javanica L.) in a tropical

region. Proc. 11th. Int. Grassl. Congr. 127-131.

(117) Spencer, D. 1950. The effect of calcium and soil pH on nodulation

of T. subterraneum L. clover on a yellow podsol. Aust. J.

agric. Res. 1:374-381.

(118) Steinberg, R.A. 1938. Applicability of nutrient-solution purificat-

ion to the study of trace-element requirements of Rhizobium and

Azotobacter. J. agric. Res. 57:461-76.

(119) Stephens, D. 1960. Fertilizer experiments with phosphorus, nitrogen,

and sulphur in Ghana. Emp. J. exp. Agric. 28:151-64.

(120) Trumble, H.C., and Shapter, R.E. 1937. The influence of nitrogen

and phosphorus treatment on the yield and chemical composition

of Wimmera ryegrass and subterranean clover, grown separately

and in association. Coun. Sci. Ind. Res. (Aust.) Bull., No.

105, 25-36.

(121) van Schreven, D.A. 1958. Some factors affecting the uptake of

nitrogen by legumes. In Nutrition of the Legumes. (ed.

Hallsworth). pp. 137-163. (Butterworths. London.).

(122) Viets, F.G. 1965. The plant's need for and use of nitrogen. In

Soil Nitrogen. (ed. W.V.Bartholemew and F.E. Clark). Agron.

Series. No. 10. pp. 503-49. Amer. Soc. Agron. : Madison.

(123) Vincent, J.M. 1958. Survival of the root-nodule bacteria. In

Nutrition of the Legumes (ed. Hallsworth). pp. 108-123.

(Butterworths. London.).

(124) Vincent, J.M. 1962a. Australian studies of the root-nodule bacteria

　　　　Proc. Linnean Soc. New South Wales. 87:8-38.

(125) Vincent, J.M. 1962b. Influence of calcium and magnesium on the

　　　　growth of Rhizobium. J. gen. Microbiol. 28:653-663.

(126) Vincent, J.M. 1965. Environmental factors in the fixation of

　　　　nitrogen by legumes. In Soil Nitrogen. (ed. W.V. Bartholemew

　　　　and F.E. Clark). Agronomy series No. 10. pp. 384-435. Amer.

　　　　Soc. Agron. : Madison.

(127) Vincent, J.M., and Crofts, F.C. 1958. Pasture improvement in the

　　　　Pilliga. Univ. Sydney School Agr. Rep. No. 3. pp. 31.

(128) Vincent, J.M., and Waters, Lawrie M. 1954. The root-nodule bacteria

　　　　as factors in clover establishment in the red basaltic soils of

　　　　the Lismore District, New South Wales. 2. Survival and success

　　　　of inocula in laboratory trials. Aust. J. agric. Res. 5:61-76.

(129) Wendt, W.B. 1970. Responses of pasture species in eastern Uganda

　　　　to phosphorus, sulphur, and potassium. E. Afr. agric. for J.

　　　　36:211-9.

(130) White, R.E. 1969. On the measurement of soil pH. J. Aust. Inst.

　　　　agric. Sci. 35:3-14.

(131) Wiersum, L.K. 1951. Water transport in the xylem as related to

　　　　calcium uptake by ground nuts (Arachis hypogaea L.). Pl. Soil

　　　　3:160-9.

(132) Wilson, R.D. 1949. Molybdenum in relation to the scald disease of

　　　　beans. Aust. J. Sci. 11:209-10.

THE INFLUENCE OF PHYSICAL ENVIRONMENTAL FACTORS ON THE ACTIVITY OF RHIZOBIUM IN SOIL AND IN SYMBIOSIS

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Abstract

Nodule bacteria, which are abundant in soil only when associated with their host legumes, are strongly influenced by adverse physical conditions such as heat, drought and acidity. These factors also affect the introduction of nodule bacteria into soil by natural or artificial means. Some stages of root-infection, nodule development and function, are more sensitive to adverse physical conditions than others, and nitrogen fixation seems specially responsive to temperature, light intensity, day-length and their interaction. The differential effects of genetic host/strain factors are discussed.

Radiation (including temperature) and water relations are clearly concerned in the subject under review, and also aeration and the physical effects of the concentration of ions in the soil solution, particularly H. However, purely physical and chemical influences are not easy to separate when mechanisms are incompletely understood. The p O_2 or p N_2, or the total ion concentration in the soil solution, may have purely physical effects or impinge on the chemical reduction of nitrogen, or may directly affect the host's nutrition. Also, it is difficult to discuss pH without considering base exchange or Ca levels, or to cover energy relations without including photochemical effects. Nevertheless I shall interpret my subject restrictively, anticipating that gaps will become filled during discussion.

Before considering these matters, I will first remind you of the characteristics of the Rhizobium cell and of the functioning root nodule - of the structures involved in their different relationships with the external physical environment.

Rhizobium is a rod-shaped Gram-negative organism, (0.5 - 0.9 x 1.2 - 3.0μm). It has a cell wall membrane overlying an intermediate 'rigid' layer of variable thickness, a cytoplasm enclosed in a unit-membrane, a prokaryotic nucleus, ribosomes and sometimes contains poly-hydroxybutyric acid storage granules. Spores or resting bodies are unknown. The cell can be motile by one or more flagella and can produce copious amounts of polysaccharide slime. In culture the optimum temperature ranges from 25 - 30°, and the lethal temperature is low (between 30 and 40°), depending on species and strain. It can live freely in the soil as an aerobic heterotroph, as well as symbiotically in the nodule under conditions that are thought to be almost anaerobic (Kleczkowski, Nutman, Skinner and Vincent 1968).

The nodule is much more complex. The outside coat of loose cortical tissue offers some protection but little impedence to the free diffusion of gases and solutes. In contrast, the interior tissue of the nodule is enclosed in an endodermis (except at the meristematic tip), which, possibly restricts the transfer of material between the nodule and outside.

The nodule's central tissue consists of large isodiametric cells, mostly closely adhering one to the other, except for a network of intercellular air-spaces. Most of the cells of the interior are infected by Rhizobium and the bacteria in them are enclosed in plant-derived membrane. These bacteroids, as they are called, are much larger than the free-living form. Their nuclear material is usually reduced and they seem to have lost their ability for independent

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existence. The host cell, in contrast, retains all its normal organelles: membranes and granules, golgi apparatus and vesticles, plastids and mitochondria. Incidentally most of the plastids and mitrochrondia are to be found next to the intercellular channels, for which there may be a good physical reason. The host cell nucleus is tetraploid or of greater ploidy. The nodule vascular system has its own endodermic sheath and regions of 'transfer cells' the internal wall surfaces of which are very much extended by complex folding; these presumably function in the movement of material to or from the nodule (Allen and Allen 1958, Mosse 1964, Dart and Mercer 1966, Pate, Gunning and Briarty 1969).

This general description fits all nodules, although there is very much variation in detail depending upon host, strain and conditions.

Rhizobium in the Soil

Because Rhizobium species cannot be distinguished unequivocally by cultural means, bio-assay must be used to study their behaviour in soil. Dilutions of soil suspension are inoculated to sterile-grown plants, which then nodulate if Rhizobium of the appropriate species are present. From the dilution, the most probable number (M.P.N.) of Rhizobium present in the original sample can be estimated (Vincent 1970). To distinguish strains other methods must be used such as those based on serology (Vincent 1970) or antibiotic resistance (Obaton 1971). By the M.P.N. method many studies have been made of Rhizobium populations in soil, of their phenomenal build-up under a legume crop, and decline in its absence and on the influence of various conditions on these processes (Brockwell and Dudman 1968, Bell and Nutman 1971).

I will first illustrate, using some of our own results, the importance of soil pH and of drought during fallowing on the four agriculturally important species of Rhizobium in the U.K., namely R.trifolii, R. leguminosarum, R.meliloti and R.lupini, and then go on to consider more adverse environments.

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We counted these rhizobia in soil from a fertilizer trial on meadowland cut for hay (Park Grass), which has been in progress since 1856, in which individual plots are given the same fertilizer dressing each year. During this period very distinct herbage differences have developed on the differently treated plots of this experiment, depending on the amount and form of N applied, on other mineral or organic fertilizers used, and on liming (Nutman and Ross 1970).

Rhizobia of these species were found (with very minor exceptions) only on plots carrying their host plants; species of Trifolium, Vicia, Lathyrus and Lotus. No plot contained any detectable populations of R. meliloti the hosts of which were absent. These differences were correlated with pH, which between different plots ranged from 3.6 - 7.6. The critical pH for survival of each species was in the range 3.8 - 4.2. Population density was also broadly correlated with pH, the largest numbers being $>10^9$ per g. dry rhizosphere soil. Seasonal fluctuations were negligible.

The ability of the populations of clover strains in the plots to fix nitrogen was also examined. Nearly all those isolated were effective in fixing nitrogen; ineffective or partially effective strains of R. trifolii were slightly more frequent only in those plots were pH was marginal for survival. There was no evidence that nitrogen fertilizer per se had any influence on nitrogen fixing effectiveness of the strains. Up to 160 Kg N ha^{-1} (as ammonium sulphate) applied annually for more than a hundred years to this soil did not affect nitrogen fixing activity of the rhizobia found in the soil, provided that lime was also added to prevent acidity. On the other hand the proportion of poorly effective strains of R. trifolii in a permanent pasture at Wye, Kent on a surface water gley soil (pH 6.7) and heavily fertilized with nitrogen, was somewhat larger than in surrounding fields (Escuder 1972).

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A correlation between soil acidity and loss of effectiveness of the indigenous Rhizobium population has been noted in many other studies, and there is some evidence that the availability of certain metals and soil moisture interact with pH in determining the proportion of effective strains; the deleterious effects of pH are much less in some soils than others (Jensen 1942, Holding and King 1963, Munns 1965 a and b, Jones 1966, Döbereiner, Arruda and Penteado 1966, O'Toole and Masterson 1968, Jensen 1969, Jones and Burrows 1969, Holland 1970, Masterson and Sherwood 1970, Robson and Loneragan 1970 a and b, Holding and Lowe 1971.)

These studies indicate that the different species of Rhizobium differ to some extent in their ability to tolerate acidity, and some workers have now obtained strains that can grow at quite low pH; these may have value as inoculants in acid soils (Munns 1965 a and b, Lie 1971 c). Rhizobia are also intolerant of alkaline soils, some species more than others (Jensen 1942, Ibrahim, Kamel and Khada 1970 Wilson 1970, Wilson and Norris 1970). Both drying, through concentrating the soil solution, and increase in soil p CO_2 tends to lower pH. These effects which are somewhat larger in alkaline soils are probably not important for the survival of the rhizobia. In saline soils drying also damages rhizobia by increasing salt concentration in the soil solution.

The nodule bacteria from the tropics, which are mostly strains of R. phaseoli and the cowpea miscellany (R. japonicum), and which form nodules on a vast range of legumes are more tolerant of acid conditions, and this has led to the theory that the ancestral form of Rhizobium developed in warm climates and acid soils, and the much more studied nodule bacteria of the clovers and medics are derivative (Ishizawa 1953, Norris 1964, Norris 1965, Brockwell 1966). Whether or not this is so, it is unwise to apply uncritically to tropical legumes

and conditions all that has been learnt in temperate   agriculture.
The production of acid by rhizobia in pure culture also varies between
species and from strain to strain, but is independent of the pH of
the soil from which the strain was originally isolated (Brockwell 1966).

The comparatively narrow range of soil pH for survival and
multiplication of rhizobia is the reason for the widespread liming of
legume crops.

In the absence of the host the population of nodule bacteria in
the soil declines at a rate depending on conditions. This is illustrated
by the numbers of Rhizobium in two fields at our farms at Rothamsted
and Woburn that were kept permanently bare of vegetation by cultivation.
Over a period of several years the average rate of decline tended to be
exponential, but was quicker in dry years and in the sandier soil of
Woburn than in the heavier soil of Rothamsted. Rhizobium meliloti
disappeared faster than the other kinds of Rhizobium. Although the
decline was at first rapid a small population of some species survived even
after long absence of the host. Possibly bacteria were sporadically
re-introduced from neighbouring fields, for there is evidence that
this can occur (Greenwood 1961, Nutman 1969, Nutman and Ross 1970).
Such reinfestation, on farm implements, on dust and by animals may
or may not be sufficient for good nodulation of the next legume crop;
in the Woburn and Rothamsted fields inoculation was found essential for
lucerne.

The soil itself, possibly its colloid fraction, has some protective
property that aids survival. Certainly, cultures of Rhizobium in
soil allowed to dry slowly in the laboratory retain a small residual
population over many years, some species and strains being more
resistant to drying than others. (Jensen 1961, Marshall 1964, 1970,
Chowdbury, Marshall and Parker 1968, Marshall 1968).

In tropical regions with dry seasons, the rhizobia in the surface layers of soil become drastically diminished in numbers, often to the point of elimination by the end of the dry period (Diatloff 1970). When rains come recolonization can occur from the deeper layers , but this may be too slow to be of practical value, especially in nitrogen-deficient soil where early nodulation is essential. Movement of rhizobia in soil requires free water and does not occur in small moisture tensions adequate for seed germination (Hamdi 1971). The small dressings of fertilizer nitrogen applied at sowing that can aid establishment, may do so by bridging the gap before nodules can form. Mulching seems to help in protecting the soil rhizobia from drought and heat. (Norris 1964, Philpotts 1967, Ratner and Samoilova 1970) Similar considerations with regard to temperature and moisture apply, but with even more force to the survival of Rhizobium in artificial inoculants which are often made up in soil or peat mixtures and where drying and high temperatures are inimical to long life. Some improvements may come from the use of temperature-resistant strains, but because Rhizobium is basically a delicate micro-organism, very easily pasteurized by low heat, the benefits are doubtful or likely to be small.

Like other organisms, rhizobia can be preserved by rapid freeze-drying. Many attempts have been made to produce dry inoculants for commercial use by freezing and using other methods, but without much success, because good inoculants must contain many viable bacteria when added to seed or soil for establishment to succeed in possibly unfavourable conditions. Enough work has been done to advise producers on control methods and standards for manufacture and for the final product, (Date 1970, Roughley 1970b, Vincent 1970), but very poor inoculants continue to be produced.

It should perhaps be mentioned that acidity, heat and drought
are not the only factors with which the soil rhizobia have to contend.
They are eaten by protozoa, attacked and lysed by bacteriophage,
killed by toxins from seeds and micro-organisms, digested by enzymes
and inhibited by antibiotics and bacteriocins - and they have also to
compete with the rest of the soil inhabitants.

EFFECTS ON THE NODULATED LEGUME

Soil Reaction

The process of nodule initiation in clovers and medics is more
sensitive than is the survival of the bacteria, to the pH of the
medium,and root hair infection fails to take place at marginally low
pH (4.4). But once infection-threads have formed nodulation then
usually proceeds normally. (Munns 1957, Munns 1968, Lie 1969b, Munns 1970).
In practice, this can severely restrict the number of nodules formed in
acid soils without apparently affecting crop growth. For example,
nodules may form only in the immediate neighbourhood of a lime-pelleted
seed, but these few nodules become so big that they can provide much or all
of the nitrogen the plant needs.

Within the host the bacteroids are subjected to the pH of the
host's sap, which is little affected by the reaction of the medium in
which it is grown. Further effects of pH are thus indirect, acting
through the legume's nutrition and growth, and response to available
major and minor nutrient elements, which may be strongly affected by
soil reaction. Nodulated legumes require more Mo and Co, than ones
grown on mineral nitrogen and possibly also more B and S. Acid soils
have less available Mo than alkaline ones, but some other elements, for
example Mn, can accumulate to toxic levels in the host (O'Toole and
Masterson 1968, Souto and Döbereiner 1969 a and b, 1970, Mandal and
Tewari 1970, Holding and Lowe 1971, Odu, Fayemi, Ogenwale 1971, Truong,

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Andrew and Wilson 1971). In some soils nodulated plants suffer less
from metal toxicity than those given fertilizer nitrogen.

Liming affects the ionic relationships in the soil and may have
harmful as well as beneficial consequences (Souto and Döbereiner 1969). For
this reason it is sometimes better and more economic to ameliorate the
pH in the immediate neighbourhood of the seed by combining inoculation
with lime-pelleting rather than to lime heavily (Brockwell and Whalley 1970).
Although the rhizobia of the cowpea and phaseolus groups are more
tolerant than others to acidity, generalizations cannot be made about
the tolerances or lime requirements of their hosts.

Water relations and the composition of the soil atmosphere

Fewer nodules form in dry than moist soil, even when the soil
rhizobia populations are similar. This may reflect the requirement
of many legumes for conditions for good root-hair development during
infection. Under dry conditions (or when the foliage is removed by
cutting or heavy grazing) nodules may be sloughed off, and new ones
form when growth is resumed. Even a single wilting can reduce the
nodules'N-fixing efficiency. The effect of unrestricted water supply
depends on the legume and conditions, especially temperature (Souto and
Döbereiner 1968, Sprent 1971). White clover can tolerate conditions
of flood irrigation whereas cowpeas may fix less in soil at its water-
holding capacity than in drier soil (Doku 1970).

Water relations affect the composition of the soil atmosphere, but
except under extreme conditions, such as waterlogging, or mechanical
compaction, the partial pressures of O_2, N_2 or CO_2 usually found in
soil probably have little influence on fixation. Carbon dioxide is
always present in soil and appears to be necessary for N-fixation in
clover, beans and peas especially at lower pH. (Mulder and van Veen
1960). These species are unaffected by as much as 4% CO_2, but the

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nodulation of Phaseolus is reported to be completely inhibited at 3%
CO_2 (Grobbelaar, Clark and Hough 1971). Augmenting the atmosphere
above the soil with CO_2 increases growth and nodulation and can counter-
act the effect of low light intensity (Wilson, Fred and Salmon 1933).
Increased soil respiration can stimulate fixation indirectly by affecting
the host's rooting behaviour but there is no evidence that N_2 can be
limiting or O_2 inhibitory.

The processes of actual fixation in the nodule require near-anaerobic
conditions whereas the nodule itself needs a good supply of O_2; the
first is regulated by nodule respiration and a sequence of reducing
processes in which leghaemoglobin mayhave a controlling function, and
the second mainly by the normal ventilating system of the root
(Bergerson 1962).

Acetylene strongly inhibits N-fixation, becoming reduced to
ethylene in the process (a reaction used as a sensitive test for
fixation), and there is evidence that the small amounts of hydrocarbons
that can form in soil (Ilag and Curtis 1968) may inhibit nitrogen
fixation; ethylene also completely inhibits nodule formation (Gottelaar,
 Clarke and Hough, 1971, Day and Dart 1972). The enzymes for producing
ethylene from methylated thio organic acids are known (Yang 1969).
Whether the production of ethylene in soil has practical significance
on nodulation remains to be seen.

Temperature and light

Since Jones and Tisdale showed in 1921 that nodulation and
nitrogen fixation by lucerne, clover, peas and soybean were differently
affected by soil temperature, a very large literature has accumulated.

Because of observational difficulties the study of how
temperature (and light) influence the infection process has been
neglected and is as yet confined to small - seeded pasture species.

Root-hairs of subterranean clover can be infected over a very wide temperature range ($7°$ - $35°$), but infections are much delayed and fewer in the cold. Infections formed in the cold do not immediately give rise to nodules, unless the plants are transferred to warmer conditions (Gibson 1967,1971 Roughley, Dart, Nutman and Rodriguez-Barrueco 1970). On the other hand, a short period of chilling of the very young seedlings of several clover species stimulates infection of the root-hairs (Nutman, Roughley, Dart and Subba-Rao 1970).

Nodules normally form in the dark recesses of the soil, and not usually on completely etiolated plants, or if so very sparsely and more often at lower than higher temperatures (Lie 1969 a). Root-hairs by contrast can be infected on plants grown in complete darkness and given no carbohydrate; the numbers infected can be quite large (Nutman 1965). Fairly strong light on the root does not much affect root-hair infection although it strongly inhibits nodulation in some hosts (Small, Hough, Clarke and Grobbelaar 1968). Weak light stimulates later nodulation (in the dark) of isolated roots of Phaseolus, when these roots are supplied with nutrients from the cut end. The active radiations are in the blue and far red parts of the spectrum indicating that a high-energy photoreactive system is involved (Grobbelaar, Clarke and Hough 1971 a). With whole pea and bean plants, however, low-energy radiation is active in initiating nodulation and this response is thought possibly to involve phytochrome (Lie 1969, Lie 1971 a, Lie 1971 c). Nodulation of Pisum sativum is reported to be strongly affected by darkness, given either before after inoculation at 10 days. No nodules formed (by 40 days) on plants transferred to darkness after 13 days, or on dark-grown plants transferred to light at later than 25 days; other times of transfer showed that nodulation was diminished more by darkness given early than late (Rudin 1956).

When we come to consider the more general effects of temperature,
light intensity and duration and their interaction on nodulation and
nitrogen fixation, the relations are very complex, especially as they
are often strongly influenced by Rhizobium strain and differ from one
host cultivar to another.

The temperature range for nodulation corresponds generally to
that for root growth, except at the upper limits, where nodulation may be
limited although growth continues (Gukova 1945, Pate 1962, Gibson 1971).
The temperature for optimum nodulation may also be different for main
and lateral roots (Dart and Mercer 1965). In high latitudes, such as
in Alaska or Greenland (Porsild 1930, Allen, Allen and Klebesadel 1963),
and in Alpine regions, legumes and their bacteria have become adapted
to cold growing conditions, and although their efficiency may be less than
that of similar association from more temperate climates these
differences become less when examined in warmer conditions. This
adaptation seems greater nearer the climatic extremes (as for example
between Lapland and South Sweden (Vartiovaara 1937, Ek-Jandér and
Fåhraeus 1971). Over extensive regions of temperate S.E. Australia
physical factors of the environment seems not to influence the distribution
of nodulation, except in-so-far as they affected the actual occurrence
of legumes in the vegetation (Brockwell and Robinson 1970).

An unusual adaptation has been described for the Pisum sativum
cv Iran which does not nodulate with most strains at 20°, at which
temperature other varieties nodulate satisfactorily, but does so at
26°; exceptional strains of rhizobia, however, nodulate this cultivar
at both temperatures (Lie 1971 b). Adaptation to temperature and
cultivar should be taken into account when selecting strains for inocula-
tion.

The influence of sub-optimal temperature (7° - 19°) on the morphological development of the nodule has been extensively studied in a range of cultivars of subterranean clover inoculated with different strains of Rhizobium trifolii (Roughley and Dart 1969 a and b, Roughley 1970 b, Gibson 1971). Nodulation occurred at each temperature but was much delayed by cold. Cold also prolonged the time for the bacteroids to mature and delayed degeneration, to different extents, according to cultivar and strain; only one strain formed bacteroid tissue at 7°. At 19°, nearer the optimum for growth, there was proportionately more degenerate bacteroid tissue. The extended life of the bacteroid tissue in the cold may account for the overwintering of nodules in N. Ireland or sub-alpine regions of Southern Australia (Pate 1958, Bergersen, Helij and Costin 1963).

Under the limited conditions of test-tube culture fewer nodules were formed at temperatures above and below the optimum, but the largest nodule volume per plant was produced at 11°. Cultivar differences were great; those selected to form few or many nodules were true to type at 15° and 19° but not at the lower temperatures (Gibson 1963, Roughley and Dart 1970 b). The cultivar Cranmore was particularly sensitive to temperature: At 7° it did not form nodules but instead produced lateral roots at exactly the positions where at 19° nodules were formed (Roughley and Dart 1970 a). The temperatures for maximum amount of N fixed and for maximum efficiency were different. Nitrogen fixing efficiency per unit mass of nodule was most at a temperature sub optimal for growth (Roughley and Dart 1969 a), and transfer of plants from sub optimal to optimal temperatures can, with some strains, lead to a decline in amounts of nitrogen fixed per gram of nodule and to retention of fixed nitrogen in the nodule (Gibson 1969). At 30° bacteroid development is inhibited, with more bacteroids per cell and per endoplasmic membrane and with hypertrophy of infection threads within the nodule (Gibson 1971),

but the same temperature applied to the shoot only does not have these deleterious effects (Possingham, Moye and Anderson 1965).

The optimum temperature for nitrogen fixation in Lotus corniculatus is between 18 and 24° depending on strain (Kunelius and Clarke 1970), and for Medicago tribuloides and Vicia atropurpurea the optimum temperature for nitrogen fixation is 24°. On these hosts the smaller fixation above 27° can be partly alleviated by lowering night temperature (Pate 1962). There is a sharp temperature optimum (25°C) for the incorporation of ^{15}N into excised nodules of Soybean (Aprison, Magee and Burres 1954).

Most tropical species need a higher temperature for nodule formation and maximum N-fixation than temperate species; the highest recorded temperatures for effective nodule function is 35° for Acacia in the Sudan and 34° (mean daily max.) for Stylosanthes gracilis in Brazil (Habish 1970, Souto and Döbereiner 1970). Tropical species may be more sensitive to diurnal changes (low night temperatures) than are temperate legumes (Mes 1959 a and b), but they are more exposed to damagingly high temperatures. For soybeans, Dolichos lablab and cowpeas grown in the warmer parts of Australia, such harmful effects, either during establishment or afterwards can be ameliorated by deeper sowing and heavier inoculation, or by watering or mulching with straw (Cloonan and Vincent 1967, Philpotts 1967, Brockwell and Philips 1970). As with root-hair infection, nodule formation seems more sensitive to high temperature than nodule growth. Thus Phaseolus roots at 30° will nodulate as well as roots at 25° providing they are first held at 25° for a few days (Barrios, Raggio and Raggio 1963). In fact, actual nodule growth may be stimulated at temperatures above those at which bacteroid tissue remains active. Subterranean clover and lucerne nodules transferred from 25° to 35° grow very large and may become lobed and then produce uninfected rootlets from the nodule lobes (Day and Dart 1971).

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In Trifolium subterraneum temperature affects the amounts of nitrogenase formed but does not influence its activity (as determined by acetylene reduction (Roughley and Dart 1969 b). The nitrogenases from other species of legume have also been examined over a wide temperature range. No activity was observed at 0°, but was present at 3° and the enzyme was inactivated at 45°; between these extremes the optimum temperature for activity differed between hosts. Vicia faba, V. sativa, Trifolium pratense, T. subterraneum, Medicago truncatulata and Glycine max had optima between 20 and 30°. The optimum for Medicago sativa was 35°, and for Vigna sineses it was 40°. (Stalder 1952, Possingham, Moye and Anderson 1965, Small,Hough, Clarke and Grobbelaar 1968, Souto and Döbereiner 1968, Roughley and Dart 1969, Day and Dart 1970, Dart and Day 1971 a and b, Day and Dart 1971, Gibson 1971). These results suggest that the actual chemical reduction of nitrogen is not very sensitive to the normal temperature conditions of cold or hot climates. Rather, it is the formation of the nodule, the synthesis of the nitrogenase and possibly also the transformation and translocation of the fixed nitrogen that is affected.

The photosynthetic and morphogenetic influence of light on nodulation and nitrogen fixation are manifold and the amounts of light that are required for nodule initiation vary from host to host. Trifolium subterraneum needs a 4-hour day of moderate light intensity to initiate nodules but any increase beyond 8 hours has little further effect. Variation in length of day from 12 - 16 h. has no effect on the nodulation of Stizolobium deeringianum (Mes 1959 b, Gibson 1967). T. glomeratum, which is also a short-day plant, forms nodules soonest in short days and these become fewer and larger as day length increases (Subba, Rao, Nutman and George 1969) Vigna unguiculata requires more than 8 hours to form effective nodules (Doku 1970). These

species and <u>Glycine max</u>, <u>Vicia sativa</u> and <u>Phaseolus vulgaris</u> also form more and larger nodules in long days, and the distribution of nodules between the primary and secondary roots is affected by day length. <u>Phaseolus vulgaris</u> nodulates well on lateral roots at low light intensity (15,000 lx) (Day and Dart 1969), but tropical species seem to require brighter light than temperate species for primary root nodula-tion. <u>Medicago truncatula</u>, <u>Medicago sativa</u> and <u>Trifolim pratense</u> are little affected in their nodulation by differences in intensities in the range 15,000 - 30,000 lx (Day and Dart 1970).

Some legumes, for example <u>Glycine max</u> and <u>Vicia atropurpurea</u> are reported to have fewer and poorer nodules in intense light and this is thought to be the cause of "nitrogen hunger" symptoms sometimes seen in young crops, and which can be overcome by shading or applica-tion of nitrogen fertilizer (Dart and Wildon 1970, Davidson, Gibson and Birch 1970). Shading reduces nodule formation in soyabean but increases nodule efficiency (Sampaio and Döbereiner 1968).

The effect of day length on the nitrogenase activity of nodules has been examined using the acetylene reduction method in typical short-day (<u>Glycine max</u>) and long-day (<u>Trifolium subterraneum</u>) plants. Transferring plants from a non-inducing to an inducing day length at first led to a decreased activity, but then fixation increased until flowering, when it declined. This decline was not associated with the shedding of nodules. Activity then increased again as pods filled and this renewed fixation was correlated with the formation of new nodules on the lateral roots (Day and Dart 1971). Other workers have reported no increased fixation during fruit formation (Pate 1958, Howell 1963). Nitrogenase activity is closely related to leaf area. In relation to the amount of nodule tissue formed, nitrogenase activity per g nodule is unrelated to light intensity in <u>Trifolium subterraneum</u> and <u>Medicago</u>

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truncatula but in nodules of Vicia faba, V. hirsuta and V. atropurpurea
is greater in bright than in dim light. At low light intensity (7500 lx)
in short days, nodules of V. atropurpurea fixed less at the end of the
light period, probably because of substrate limitation. In the
experiments on day length, when plants were transferred from darkness
to full light there was a temporary short reduction in nitrogenase
activity, (Day and Dart 1971).

Discussion

The following general conclusions can be made about the effect of
physical factors on the growth and survival of Rhizobium in soil:
soil acidity greater than about pH 3.5 is lethal to Rhizobium and some
strains cannot live in strongly alkaline soils. Rhizobium tolerates
temperatures colder than about 30° but are destroyed at only slightly
higher temperatures. Rhizobium is not resistant to drying, especially
at elevated temperatures, but resistance is strongly affected by the
characteristics of the soil. As facultative aerobes, species of
Rhizobium are not much influenced by differences in the soil's
atmosphere or water content, above about the plant wilting point. The
effects of these factors often interact and species and strains of
Rhizobium differ slightly in their responses to them.

In striking contrast, nearly all aspects of nodule initiation,
growth and function react to the physical environment in a complex
manner. Adding the plant component seems to add more than a dimension
of complexity, the study of which is at an exploratory stage. Only
very general trends can be discussed: nodulation and fixation are
limited to various extents in different host/strain associations by
the physical environment, and to extremes of soil reaction, heat, drought
or regimes of light. Most irregularity and seeming unpredictability is
in respect to temperature and light. This may be because these factors

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have strong formative influences in plant development. The nodulated
legume growing in soil is the epitome of the carbon and nitrogen cycles,
and it may be necessary to know more about its normal controlling
mechanisms before we can interpret effects of the changing environment.

Let us, therefore, look at a legume growing in, say, solution
culture under optimal conditions in a constant environment. It nodulates,
fixes nitrogen and completes its life cycle, and the functional
integration between plant and bacteria is such that the number of
nodules formed, their growth and decay and the quantity of nitrogen
fixed is exactly adjusted to the host's requirements. This degree of
coordination means that the intrinsic mechanisms of control are those
appropriate to normal development; namely, those determining the
balance of top and root increment under the changing physiology of
vegetative and reproductive growth, whether dependent or not on
symbiotically fixed nitrogen. This involves energy transformations,
metabolic and synthetic pathways, assimilation, translocation and
morphogenesis.

Environmental factors undoubtedly influence rate and efficiency
of the nitrogen-fixing process but there is as yet no evidence that
they directly have an important regulatory function, but there is good
evidence that regulation can be through size.

This question has been examined in clover, which like many legumes,
has nodules with apical growing meristems. The size of the entire
fixing system (proportional to aggregate nodule volume) is therefore
an expression of the total amount of meristematic activity in the root.
By comparing cultivars that have equal aggregate nodule volumes but
form very different numbers of nodules, it can be shown that the growing
nodules, through the operation of normal apical dominance, limit the
appearance of new meristems in the root, including those of incipient

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nodules. In this way the first-formed nodules tend to suppress further formation of more nodules, to an extent depending on the nodule's size. This can explain the inverse relationship, almost universal in legumes, between the number of nodules on a plant and their average size, and provides a working hypothesis of a mechanism for controlling the balance between the demand and supply of fixed nitrogen (Nutman 1958, Nutman 1967).

Under adverse conditions, such as high temperature, nodule numbers become restricted and this is not fully compensated for by increased nodule growth or activity.

It remains important to determine the restrictive factors in soil, climate, nutrition and management, to know how to ameliorate them and to try and adapt the bacteria and its host to prevailing conditions, but effort and resources should also be given to the task of manipulating symbiotic nitrogen fixation through a better understanding of its genetics and physiology.

REFERENCES

ALLEN, E.K. and ALLEN, O.N. Biological aspects of symbiotic nitrogen fixation. Handbuch der Pflanzenphysiologie. 8 (1958) 48-118.

ALLEN, E.K., ALLEN, O.N. and KLEBESADEL, L.J. An insight into symbiotic nitrogen-fixing plant associations in Alaska. Proc. 14th Alaskan Science Congress, Anchorage, Alaska (1963) 54-62.

APRISON, M.H., MAGEE, W.E. and BURRIS, R.H. Nitrogen fixation by excised soybean root nodules. J. Biol. Chem. 208 (1954) 29-39.

BARRIOS, S, RAGGIO, N. and RAGGIO, M. Effect of temperature on infection of isolated bean roots by rhizobia. Plant Physiol. 38 (1963) 171-4.

BELL, F. and NUTMAN, P.S. Experiments on nitrogen fixation by nodulated lucerne. Plant and Soil, Special Volume 1971 (1971) 232-64.

BERGERSEN,F.J. The effects of partial pressure of oxygen upon resiration and nitrogen fixation by soybean root nodules. J. gen. Microbiol. 29 (1962) 113-125.

BERGERSEN, F.J., HELY, F.W. and COSTIN, A.B. Overwintering of clover nodules in alpine conditions. Australian J. biol. Sci. 16 (1963) 920-1.

BROCKWELL, J. Acid production by Rhizobia from the genera Trifolium and Lotus. J. Australian Inst. agr. Sci. 32 (1966) 295-7.

BROCKWELL, J. and DUDMAN, W.F. Ecological studies of root-nodule bacteria introduced into field environments. II Initial competition between field environments in the nodulation of Trifolium subterranium L. seedlings. Aust. J. agric. Res. 19 (1968) 749-57.

BROCKWELL, J. and PHILIPS, L.J. Studies on seed pelleting as an aid to legume seed inoculation. 3. Survival of rhizobium applied to seed sown into hot dry soil. Aust. J. exp. Agric. Anim. Husb. 10 (1970) 739-744.

BROCKWELL, J. and ROBINSON, A.C. Observations on the natural distribution of Rhizobium spp. in relation to physical features of the landscape.
Surfers Paradise Australia
Proc. XI Internat. Grasslands Conf./(1970) 438-441.

BROCKWELL, J. and WHALLEY, R.D.B. Studies on seed pelleting as an aid to legume seed inoculation. 2. Survival of Rhizobium melitoti applied to medic seed sown into dry soil. Aust. J. exp. Agr. and Animal Husbandry 10 (1970) 455-459.

CHOWDHURY, M.S., MARSHALL, K.C. and PARKER, C.A. Growth rates of Rhizobium trifolii and Rhizobium lupini in sterilized soils. Aust. J. agr. Res. 19 (1968) 919-25.

CLOOMAN, M.J. and VINCENT, J.M. *The nodulation of annual summer legumes sown on the far north coast of New South Wales. Aust. J. exp. Agr. Animal Husbandry. 7 (1967) 181-9.

DART, P.J. and DAY, J.M. Effects of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules. Plant and Soil, Special Vol. 1971 (1971a) 167-184.

DART, P.J. and DAY, J. Factors affecting nitrogenase activity of legume root nodules. Rothamsted Rep. for 1970 Part I. (1971b) 85-6.

DART, P.J. and MERCER, F.V. The affect of growth temperature, level of ammonium nitrate and light intensity on the growth and nodulation of cowpea (Vigena sinensis Enal. ex Hassk.) Aust. J. Agr. Res. 16 (1965) 321-45.

DART, P.J. and MERCER, F.V. Fine structure of bacteroids in root nodules of Vigena sinensis, Acacia longifolia, Viminaria juncea and Lupinus augustifolius. J. Bacter. 91 (1966) 1314-9.

DART, P.J. and WILDON, D.C. Nodulation and nitrogen fixation by Vigena sinensis and Vicia atropurpurea: the influence of concentration, form and site of application of combined nitrogen. Aust. J. agric. Res. 21 (1970) 45-56.

DATE, R.A. Microbiological problems in the inoculation and nodulation of legumes. Plant and Soil, 32 (1970) 703-25.

DAVIDSON, J.L., GIBSON, A.H. and BIRCH, J.W. Effects of temperature and defoliation on growth and nitrogen fixation in subterranean clover. Proc. XI Internat. Grassland Congress Surfers Paradine Australia (1970) 542-5.

DAY, J. and DART, P.J. Effect of day-length and light intensity on nodulation. Roth. Rept. for 1968 (1969) 87-8.

DAY, J. and DART, P.J. Light intensity, nodulation and nitrogen fixation. Roth. Rept. for 1969 (1970) 105.

DAY, J. and DART, P.J. Factor affecting nitrogenase activity of legume root nodules; root formation by legume nodules. Roth. Rept. 1970 (1971) 85-6.

DAY, J. and DART, P.J. Role of ethylene in legume nodule symbiosis. Roth. Rept. for 1971 (1972) In the press.

DIATLOFF, A. Relationship of soil moisture, temperature and alkalinity to a soybean nodulation failure. Queensland J. agr. Anim. Sci. 27 (1970) 279-93.

DÖBEREINER, J., ARRUDA, N.B. de and PENTEADO A. de F. Problems of soybean inoculation in acid soils. Proc. IX Int. Grassld. Cong. 1965 2 (1966) 1153-7.

DOKU, E.V. Effect of day-length and water on nodulation of cowpea (Vigna unguiculata (L) Walp.) in Ghana. Expl. Agric. 6 (1970) 13-18.

EK-JANDER, J. and FÅHRAEUS, G. Adaptation of Rhizobium to subartic environment in Scandanavia. Plant and Soil, Special Volume 1971 (1971) 129-137.

ESCUDER, A.M.Q. de. A survey of Rhizobium in farm soils at Wye College, Kent. J. appl. Bact. In the press (1972)

GIBSON, A.H. Physical environment and symbiotic nitrogen fixation. 1. The effect of root temperature on recently nodulated Trifolium subterraneum L. plants. Aust. J. biol. Sci. 16 (1963) 28-42.

GIBSON, A.H. Physical environment and symbiotic nitrogen fixation IV Factors affecting the early stages of nodulation. Aust. J. biol. Sci. 20 (1967 1087-1117.

GIBSON, A.H. Physical environment and symbiotic nitrogen fixation VI Nitrogen retention in the nodules of Trifolium subterraneum L. Aust. J. biol. Sci. 22 (1969) 829-38.

GIBSON, A.H. Factors in the physiological and biological environment affecting nodulation and nitrogen fixation by legumes. Plant and Soil Special Volume 1971 (1971) 139-152.

GREENWOOD, R.M. Pasture establishment on a podzolised soil in Northland III Studies on rhizobial populations and effects of inoculation. New Zealand J. Agr. Res. 4 (1961) 375-89.

GROBBELAAR, N., CLARKE, B. and HOUGH, M.C. The nodulation and nitrogen fixation of isolated roots of Phascolus vulgaris L. II The influence of light on nodulation. Plant and Soil, Special Vol. 1971 (1971a) 203-14.

GROBBELAAR, N. CLARKE, B. and HOUGH, M.C. The nodulation and nitrogen

fixation of isolated roots of Phaseolus vulgaris L. III Effects of carbon dioxide and ethylene. Plant and Soil, Special Volume 1971 (1971b) 216-223.

GUKOVA, M.M. The influence of soil temperature on the fixation of nitrogen by nodule bacteria. Trudy seljskohoz Akad. in Timirjazer 30 (1945) 33-43.

HABISH, H.A. Effect of certain soil conditions on nodulation of Acacia spp. Pl. Soil 33 (1970) 1-6.

HAMDI, Y.A. Soil-water-tension and the movement of rhizobia. Soil Biol. Biochem. 3 (1971) 121-6.

HOLDING, A.J. and KING, J. The effectiveness of indigenous populations of Rhizobium trifolii in relation to soil factors. Plant and Soil 18 (1963) 191-8.

HOLDING, A.J. and LOWE, J.F. Some affects of acidity and heavy metals on the Rhizobium—— leguminous plant association. Plant and Soil Special Volume 1971 (1971) 153-165.

HOLLAND, A.A. Competition between soil and seed-borne Rhizobium trifolii in nodulation of introduced Trifolium subterraneum. Plant and Soil 32 (1970) 293-302.

HOWELL, R.W. Physiology of the soybean. In The Soybean. ed. A.G. Norman. Academic Press, New York and London (1963) 75-124.

IBRAHIM, A.N., KAMEL, M. and KHADR, M.S. Effect of some sodium salts on nodule formation and development in legumes. Agrokém Talaft. 19 (1970) 164-172.

ILAG, L. and CURTUS R.W. Production of ethylene by fungi. Science 159 (1968) 1357.

ISHIZAMA, S. Studies on the root-nodule bacteria of leguminous plants. I characters in artificial media Part 3. Physiological characters. J. Sci. Soil and Manure Tokyo 24 (1953) 35-44.

ISLAM R. and DART, P.J. Temperature and symbiosis in grain legumes.
Roth. Rept. for 1971 (1972) In the press.

JENSEN, H.L. Nitrogen fixation in leguminous plants. I. General
characters of root-nodule bacteria isolated from species of Medicago
and Trifolum in Australia. Proc. Linn. Soc. N.S.W. 67 (1942) 98-108.

JENSEN, H.L. Survival of Rhizobium meliloti in soil culture. Nature
192 (1961) 682-3.

JENSEN, H.L. The distribution of lucerne and clover rhizobia in
agricultural soils in Denmark. Saetryk af Tidsskrift for Planteavl 73
(1969) 61-72.

JONES, D.G. The contribution of white clover to a mixed upland sward
II. Factors affecting density and effectiveness of Rhizobium trifolii
Plant and Soil 24 (1966) 250-90.

JONES, D.G. and BURROWS A.C. Acid production and symbiotic effectiveness in
Rhizobium trifolii. Soil Biol. Biochem 1 (1969) 57-61.

JONES, F.R. and TISDALE, W.B. The effect of soil temperature upon the
development of nodules on the roots of certain legumes. J. Agric. Res.
22 (1921) 17-31.

KIECZKOWSKA, J. NUTMAN, P.S., SKINNER, F.A. and VINCENT J.M. Identification
and classification of Rhizobium. Soc. appl. Bact. Identification methods
for microbiologists; B. Technical Series 2, ed. B.M. Gibbs and D.A.
Shapton (1968) 51-65.

KUNELIUS, H.T. and CLARK, K.W. Influence of root temperature on the
early growth of symbiotic nitrogen fixation by nodulated Lotus
corniculatus plants. Canad. J. Plant Sci. 50 (1970) 569-575.

LIE, T.A. Non-photosynthetic effects of red and far-red light on root-
nodule formation by leguminous plants. Plant and Soil 30 (1969a) 391-404.

LIE, T.A. The effect of low pH on different phase of nodule formation in
pea plants. Plant and Soil 31, (1969b) 391-406.

LIE T.A. Nodulation of rooted leaves in leguminous plants. Plant and Soil 34 (1971a) 663-73.

LIE, T.A. Temperature-dependent root-nodule formation in pea cv. Iran. Plant and Soil 34 (1971b) 751-2.

LIE, T.A. Symbiotic nitrogen fixation under stress conditions. Plant and Soil. Special Vol. (1971c) 118-127.

MANDAL, S.C. and TEWARI, S.N. Effect of Mg and K supply in the uptake of Mn and Mo by soybean plants. Indian J. agric. Chem. 3 (1970) 25-27.

MARSHALL, K.C. Survival of root-nodule bacteria in dry soils exposed to high temperatures. Aust. J. agr. Res. 15 (1964) 273-81.

MARSHALL, K.C. The nature of bacterium-clay interactions and its significance in survival of Rhizobium under arid conditions. Proc. IX Int. Congress of Soil Sci. Adelaide 2 (1968) 275-280.

MARSHALL, K.C. Methods of study and ecological significance of rhizobium clay interactions. In"Methods of Study in Soil Ecology"(Phillipson, J., ed) Proc. UNESCO/IBP Symp. Paris 1967 (1970) 107-110.

MASTERSON, C.L. and SHERWOOD, M.T. White clover/Rhizobium symbiosis. Proc. Symp. White Clover Res. British Grassland Soc. Hurley. (1970) 11-39.

MES, M.G. Influence of temperature on the symbiotic nitrogen fixation of legumes. Nature 184 (1959a) 2032-33.

MES, M.G. The influence of night temperature and day length on the growth, nodulation, nitrogen assimilation and flowering of Stizalobium deeringianum (velvet bean) South Afr. J. Sci. 55 (1959b) 35-9.

MOSSE, B. Electron microscope studies of nodule development in some clover species. J. gen. Microbiol 36 (1964) 49-66.

MULDER, E.G. and VAN VEEN, W.L. The influence of carbon dioxide on symbiotic nitrogen fixation. Plant and Soil 13 (1960) 265-278.

MUNNS, D.N. Soil acidity and growth of legumes. I. Interactions of lime with nitrogen and phosphate on growth of Medicago sativa and Trifolii

subterraneum L. Aust. J. Agr. Res. 16 (1965a) 733-41.

MUNNS, D.N. Soil acidity and growth of legumes III. Interactions of lime and phosphate on growth of Medicago sativa in relation to Al toxicity and phosphate fixation. Aust. J. agric. Res. 16 (1965b) 743-58.

MUNNS, D.N. Nodulation of Medicago sativa in solution culture. 1. Acid-sensitive steps. Plant and Soil 28 (1968) 129-146.

MUNNS, D.N. Nodulation of Medicago sativa in solution culture V. Calcium and pH requirements during infection. Plant and Soil 32 (1970) 90-102.

NORRIS, D.O. Legume bacteriology. In "Some concepts and methods in subtropical pasture research". C.A.B. Bulletin 47 (1964) 102-117.

NORRIS, D.O. Acid production by Rhizobium. A unifying concept. Plant and Soil 22 (1965) 143-66.

NUTMAN, P.S. Physiology of nodule formation. In "Nutrition of the Legumes" Proc. Univ. Nottingham 5th Easter Sch. of agr. Sci. (1958) 87-107 Butterworths Sci. Pub. Lond.

NUTMAN, P.S. Varietal differences in the nodulation of subterranean clover. Aust. J. agric. Res. 18 (1967) 381-425.

NUTMAN, P.S. Microbiology of Broadbalk soils; Legume nodule bacteria. Roth. Rep. for 1968, Part 2. (1969) 179-81.

NUTMAN, P.S. and ROSS, G.J.S. Rhizobium in the soils of Rothamsted and Woburn farms. Roth. Rep. for 1969, Part 2 (1970) 148-167.

NUTMAN, P.S., ROUGHLEY, R.J., DART, P.J. and SUBBA RAO,N.S. Effect of low temperature pre-treatment on infection of clover root-hairs by Rhizobium. Plant and Soil 33 (1970) 257-9.

OBATON, M. Use of antibiotic-resistant spontaneous mutants for studying the ecology of Rhizobium. C. v. hebd. Séanc. Acad. Sci. Paris. 272D (1971) 2630-3.

ODUN, C.T.I., FAYEMI, A.A. and OGUNWALE, J.A. Effect of pH on the growth, nodulation and nitrogen fixation of Centrosema pubescens and Stylosanthes gracilis. J. Sci. Food Agr. 22 (1971) 57-9.

O'TOOLE, M.A. and MASTERSON, C.L. The interaction of calcium and

hydrogen and concentration on nodulation of white clover on peat. Irish

J. agric. Res. 7 (1968) 129-31.

PATE, J.S. Nodulation studies in legumes. II. The influence of various

environmental factors on symbiotic expression in the vetch (Vicia sativa L.)

and other legumes. Aust. J. biol. Sci. 11 (1958) 496-515.

PATE, J.S. Nodulation studies in legumes. V. The effect of temperature

on symbiotic performances of bacterial associations of Medicago tribuloides Desm.

and Vicia atropurpuria Desf. Phyton. 18 (1962) 65-74.

PATE, J.S., GUNNING, B.E.S. and BRIARTY, L.G. Ultrastructive and function-

ing of the transport system of the leguminous root nodule. Planta 85 (1969)

1-34.

PHILPOTTS, R. The affect of soil temperatures on nodulation of cowpeas

(Vigna sinensis). Aust. J. exp. Agr. and Anim. Husb. 7 (1967) 372-6.

PORSILD, M.P. "Giebt es Knöllchenbakterien auf Disko in Grönland?"

Dansk Botan. Ark. 6 (1930) 1-7.

POSSINGHAM, J.V., MOYE, D.V. and ANDERSON, A.J. Influence of elevated

shoot and root temperature on nitrogen fixation. Plant Physiol. 39

(1965) 561-3.

RATNER, E.I. and SAMOILOVA, S.A. Effect of mineral nitrogen and temperature

of the medium on growth, development and nitrogen fixation by soya bean

in relation to phosphorus metabolism in its nodules. Agrokhimiya. 8

(1970) 3-7.

ROBSON, A.D. and LONERAGAN, J.F. Nodulation and growth of Medicago

truncatula on acid soils. I. Effect of calcium carbonate and inoculation

level on the nodulation of Medicago truncatula on a moderately acid soil.

Aust. J. agr. Res. 21, (1970a) 427-34.

ROBSON, A.D. and LONERAGAN, J.F. Nodulation and growth of Medicago

truncatula on acid soils II. Colonization of acid soils by Rhizobium

mililoti. Aust. J. agr. Res. 21 (1970b) 435-45.

81

ROUGHLEY, R.J. The preparation and use of legume seed inoculants. Plant and soil. 32 (1970a) 675-701.

ROUGHLEY, R.J. The influence of root temperature, Rhizobium strain and host selection on the structure and nitrogen fixing efficiency of the root nodules of Trifolium subterraneum. Ann. Bot. 34 (1970b) 631-46.

ROUGHLEY, R.J. and DART, P.J. The effect of root temperature on nodulation of subterranean clover. Roth. Rep. for 1968 (1969a) 88-9.

ROUGHLEY, R.J. and DART, P.J. Reductions of acetylene by nodules of Trifolium subterraneum as affected by root temperature, Rhizobium strain and host cultivar. Arch. Mikrobiol. 69 (1969b) 171-179.

ROUGHLEY, R.J. and DART, P.J. Root temperature and root-hair infection of Trifolium subterraneum L. cv. Cranmore. Plant and Soil 32 (1970a) 518-20.

ROUGHLEY, R.J. and DART, P.J. Growth of Trifolium subterraneum L. selected for sparse and abundant nodulation as affected by root temperature and Rhizobium strain. J. exp. Bot. 21 (1970b) 776-86.

ROUGHLEY, R.J., DART, P.J., NUTMAN, P.S. and RODRIGUEZ-BARRUECO, C. The influence of root temperature on root-hair infection of Trifolii subterraneum by Rhizobium trifolii. Proc. XI Internat. Grassld. Cong. Surfers Paradise Aust. (1970).

RUDIN, P.E. Versuche zur Physiologie der Knöllchenbildung bei Pisum Sativum. Phytopathol. Z. 28 (1956) 57-80.

SAMPAIO, I.B.M. and DOBEREINER, J. Effect of shading and lime treatment on nitrogen fixation rates and on the efficiency of soya bean nodules (Glycine max). Pesq. agropec. bras. 3 (1968) 255-262.

SMALL, J.C.G., HOUGH, M.C. and CLARKE, B. and GROBBELAAR, N. The effect of temperature on the nodulation of whole plants and isolated roots of Phaseolus vulgaris L. South Afr. J. Sci. 64 (1968) 218-24.

SOUTO, S.M. and DOBEREINER, J. Efeito do fósforo, temperatura e

imridade do solo na nodulaçaõ e no desenvolvimento de duas variedades
de sojo pirenne (Glycine javanica L.) Pesq. agropec. bras. 3 (1968) 215-21).

SOUTO, S.M. and DOBEREINER, J. Fixaçào de nitrogênis e estabelecimento
de duas variedadas de soya perene (Glycine javanica L.) com toxidez de
manganês. Pesq. agropee. bras. 4 (1969a) 59-66.

SOUTO, S.M. and DOBEREINER, J. Toxidiz di manganês em leguminosas
forragivas tropicais. Pesq. agropec. bras. 4, (1969b) 129-38.

SOUTO, S.M. and DOBEREINER, J. Soil temperature effects on nitrogen
fixation and growth of Stybosanthes gracilis and Pueraria javanica.
Pesq. agropec. bras. 5 (1970) 365-71.

SPRENT, J.I. Effects of water stress on nitrogen fixation in root
nodules. Plant and Soil Special Volume 1971 (1971) 225-8.

STALDER, L. Uber dispositions verschiebungen bei der Bildung von
Wurzelknöllchem. Phytopathol Z. 18 (1952) 376.

SUBBA RAO, N.S., NUTMAN, P.S. and GEORGE, S. Effect of day length on
nodulation of Trifolium glomeratium. Roth. Rept. for 1968 (1969) 88.

TRUONG, N.V., ANDREW, C.S. and WILSON, G.L. Manganese toxicity in
pasture legumes. II. Effects of pH and molybdenum levels in the substrate.
Plant and Soil 34 (1971) 547-60.

VARTIOVAARA, U. Investigation on the root-nodule bacteria of leguminous
plants XXI.. The growth of the root nodule organisms and inoculated
peas at low temperature. J. Agr. Sci. 27 (1937) 626-37.

VINCENT, J.M. A manual for the practical study of root-nodule bacteria.
IBP Handbook No. 15. Blackwell Scientific Publications Oxf. and Edin. (1970).

WILSON, J.R. Response to salinity in Glycine VI. Some affects of a range
of short term salt stresses on the growth, nodulation and nitrogen
fixation of Glycine wightii (formerly javanica). Aust. J. Agric. Res. 21
(1970) 571-82.

WILSON, P.W., FRED, E.B. and SALMON, M.R. Relation between carbondioxide and elemental nitrogen assimilation in leguminous plants. Soil Sci. 35 (1933) 145-65.

WILSON, J.R. and NORRIS, D.O. Some effects of salinity on Glycine javanica and its Rhizobium symbiosis. Proc. XI Internat. Grassld. Cong. Surfers Paradise, Queensland, Aust. 455-458 (1970)

YANG, S.F. Further studies on ethylene formation from ∝ kebo-ɣ- methylthiobutyric acid or β-methylthiopropionaldehyde by peroxidase in the presence of sulphite and oxygen. J. biol. Chem. 244 (1969) 4360.

ASSAY OF NITROGENASE ACTIVITY BY ACETYLENE REDUCTION*

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ABSTRACT

The acetylene reduction assay for nitrogenase activity of plant material, soil cores and soil-plant systems is described in detail and comments made on the technique.

INTRODUCTION

Nitrogenase (N_2ase) from different organisms has very similar enzymatic properties. The two protein components, one containing Mo and Fe with a MW. $\underline{c}$ 200,000 and the other only Fe (MW. $\underline{c}$ 50,000), even when isolated from different organisms, can often be recombined to yield active preparations [1-8]. N_2ase is a versatile enzyme reducing N_2 to NH_3, CH_2 to CH_4 and various other substrates (e.g. N_2O, N_3^-, H-CN, R-NC, R-CCH). The N_2ase system apparently functions in a similar fashion for each substrate reduced. ATP and reductant are required and NH_3 and C_2H_4 do not inhibit the reduction of N_2 or C_2H_2 [1 - 5]. The assay is based on the similarity in the rate of electron activation and transfer to either N_2 or C_2H_2 as substrate assuming that $N_2 + 6H^+ \xrightarrow{6e} 2NH_3$

and C_2H_2 + $2H^+$ $\xrightarrow{2e}$ C_2H_4 and C_2H_2 + $2H^+$ $\xrightarrow{2e}$ C_2H_4 giving a theoretical ratio of $1N_2 \equiv 3C_2H_2$. Acetylene and ethylene can be readily measured by gas chromatography, with an increase in sensitivity over ^{15}N methods of some 1000 times.

METHODS

Sample Preparation

Assays should be made immediately after sampling [9]. Nodules are best assayed while still attached to the roots [10]. Removing the tops usually has no measurable effect on short-term assays.

Some N_2ase systems are apparently cold labile [4, 12] and samples should not be stored cold before assay. N_2ase activity of nodulated roots may vary with the light intensity and temperature throughout the day [9, 10, 11, 13] , and activity of plants grown in artificial cabinets oscillates for up to 2h after the lights turn on at the beginning of the day. For comparisons, sampling and assays should take place at the same time each day.

Activity is diminished when the surface film of H_2O remaining after washing nodulated roots is not carefully removed [14]. Drying of nodules also diminishes activity [15]. When possible, nodulated roots should be shaken free from soil and assayed without washing. Samples are assayed in conveniently sized containers, e.g. glass bottles, with a rubber septum in the stopper to allow it to be pierced by a hypodermic needle. N_2ase is sensitive to oxygen tension [9, 11, 16]. The

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activity of nodules decreases as the pO_2 falls below 0.20

atm.   For rhizosphere, non-symbiotic, organisms, a pO_2

about 0.04 atm. is often optimal [17].   Assay vessels

should be large enough to minimise changes in pO_2.

Liquid samples should be well shaken during assay.   It

is preferable to assay soil as intact cores.   Samples

containing blue-green algae should be illuminated at

light intensities corresponding to those in situ in the

field.   Small samples of nodules can be conveniently

assayed in wide-mouthed McCartney vials (c. 28 ml capacity)

stoppered with serum caps or Suba-seal rubber stoppers [18].

For assays of soil-plant systems, soil cores can be

placed in an airtight plastic container (such as a plastic

plant pot with a clear plastic bag or rigid, clear plastic

cover [19]) connected to the base by a water or 2% agar

seal (Fig. 1).   The containers should be incubated in

the light as the activity of the rhizosphere-associated,

nitrogen-fixing bacteria is influenced by photosynthesis

[17, 20].   Alternatively, a metal tube can be driven into

the soil and the top covered for in situ assays [21].

A known amount of propane to give c. 100 vpm is injected

and used as an internal calibration standard to measure

the volume of gas in the incubation chamber and to monitor

leaks and other losses of C_2H_4 (soil absorption?).

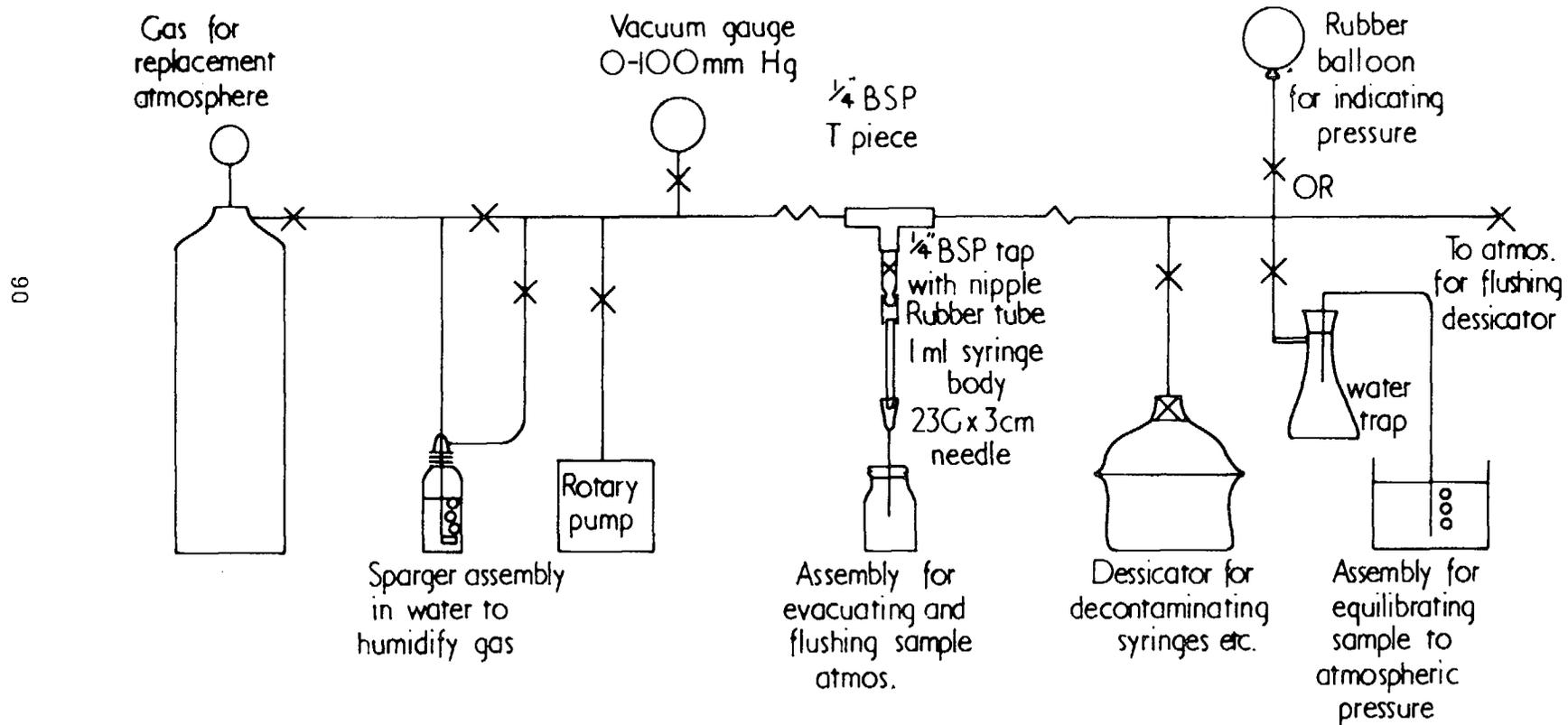Fig.1. C_2H_2 incubation assembly for soil-plant cores

Suba-seal

Metal ring araldited to plastic

Clear plastic or glass dome (or plastic bag)

H_2O or 2% AGAR as seal for dome

Soil core in plastic pot

Hole with rubber plug to drain excess H_2O
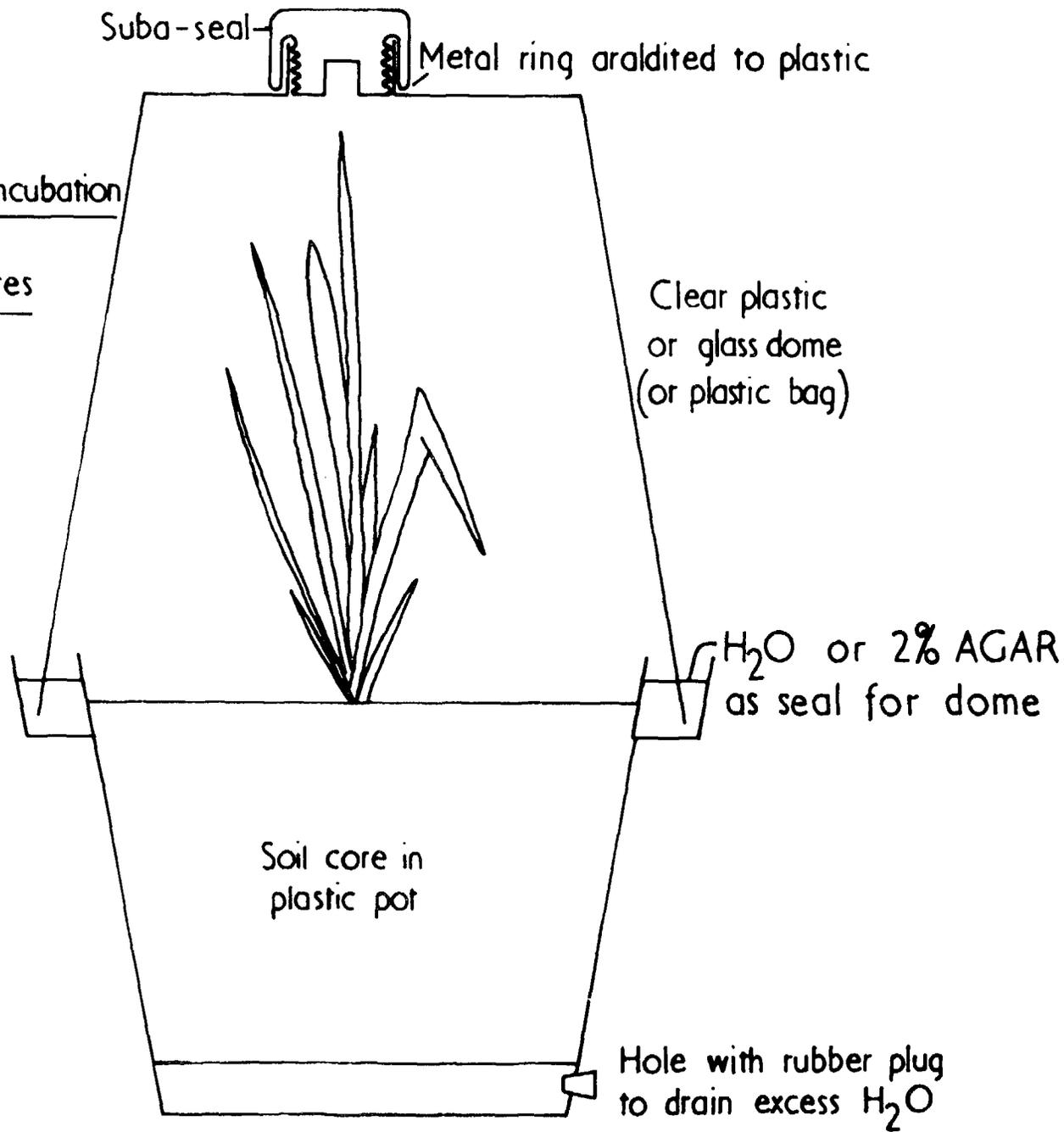
Assay Procedure

For active systems - e.g. nodules or blue-green algae [22] - assays can be made in air, provided c. 10% C_2H_2 is used. The affinity of N_2ase for C_2H_2 much exceeds that for N_2; for a wide range of organisms, the Km of C_2H_2 reduction by N_2ase, is in the range 0.004 - 0.01. atm. both in vivo and in vitro [1 - 4]. The atmosphere in the vials can be altered by alternately evacuating to c. 30 mm Hg and flushing with an appropriate gas mixture (usually Ar/O_2) two or three times. We have built a suitable gas line from commercial metal gas fittings (Fig. 2). If gases premixed in cylinders are not available, they can be conveniently mixed by displacing water in suitable glass vessels (e.g. aspirator) with volumes marked.

Some gas is withdrawn from the sample container by syringe and replaced with acetylene to give a final concentration c. 10% or acetylene may be premixed with the gas phase introduced into the sample container. Samples without C_2H_2 added should be assayed for C_2H_4 produced endogenously [23-25] . Temperature affects N_2ase activity [9] so that samples are best incubated at either temperatures which are constant, or related to the in vivo temperature.

After a suitable time (30' for legume root nodules, up to 24h for soil), gas samples (0. 5-1 ml) are taken with a disposable plastic syringe. For periods up to about 3h, the samples can be stored by stabbing the syringe needle into a rubber bung. For storing samples over

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Fig. 2. Gas line

Gas for
replacement
atmosphere

Vacuum gauge
0-100mm Hg

¼ BSP
T piece

Rubber
balloon
for indicating
pressure

OR

To atmos.
for flushing
dessicator

¼ BSP tap
with nipple
Rubber tube
I ml syringe
body
23G x 3cm
needle

water
trap

Rotary
pump

Sparger assembly
in water to
humidify gas

Assembly for
evacuating and
flushing sample
atmos.

Dessicator for
decontaminating
syringes etc.

Assembly for
equilibrating
sample to
atmospheric
pressure

90

long periods, gas samples can be transferred to pre-evacuated containers (e.g. B-D Vacutainer) [26, 27].

The gas volume of the assay vessel is measured by displacement with water (using a burette) after assay; this is not necessary with propane used as an internal standard.

Gas Chromatography

Gas mixtures can be separated by gas chromatography and then quantitated by flame ionisation or thermal conductivity detectors. For acetylene, ehtylene and propane, various column packing materials can be used; a convenient system is 80 -100 mesh Poropak N or T in a 2m x 0.003m diam. stainless steel column at $100^{\circ}C$, with a nitrogen carrier gas flow rate of 25 ml/min, using a hydrogen/air flame ionisation detector. Most detectors can routinely detect 0.1 vpm C_2H_4 in a 0.5 ml gas sample. For Poropak N and T, CH_4 has the shortest retention time followed by C_2H_4, C_2H_2 and C_3H_8 (Fig. 4). At most concentrations encountered in the assay, peak heights can be taken as linearly related to concentration without any great inaccuracy. Durapak$^{(R)}$ phenyl isocyanate on Porasil C, 80-100 mesh in 2m x 0.006m diam. glass (or metal) columns at 35° is convenient for assays which include propane.

Calculation

A suitable calculation for the C_2H_4 produced, in μM C_2H_4/h, is as follows:

$$C_2H_4 \text{ sample C.U. X } \frac{\text{vol. gas in sample container}}{\text{vol. injected into G.L.C.}} \text{ X assay time (h) X K}$$

$$\text{minus } C_2H_4 \text{ blank C.U. X } \frac{\text{vol. gas in blank container}}{\text{vol. injected into G.L.C.}} \text{ X K}$$

Where C.U. = chart units used to measure peak height.

The blank is a sample container with added C_2H_2 only.

K = conversion factor obtained using a standard

mixture to calibrate the chromatograph.

e.g. for 100 vpm C_2H_4 standard, K is derived as follows:-

1 ml of 100 vpm C_2H_4 contains 100×10^{-6} ml C_2H_4 and = x C.U.

22.4 1 C_2H_4 at S.T.P. = 1 Mole C_2H_4

$$1 \text{ ml of } 100 \text{ vpm } C_2H_4 = \frac{100 \times 10^{-6}}{22.4 \times 10^3} \text{ moles } C_2H_4$$

$$= 0.00446 \text{ } \mu\text{moles } C_2H_4 = \text{ x C.U.}$$

then K (or 1 C.U.) $= \dfrac{0.00446}{x} \text{ } \mu\text{moles } C_2H_4$

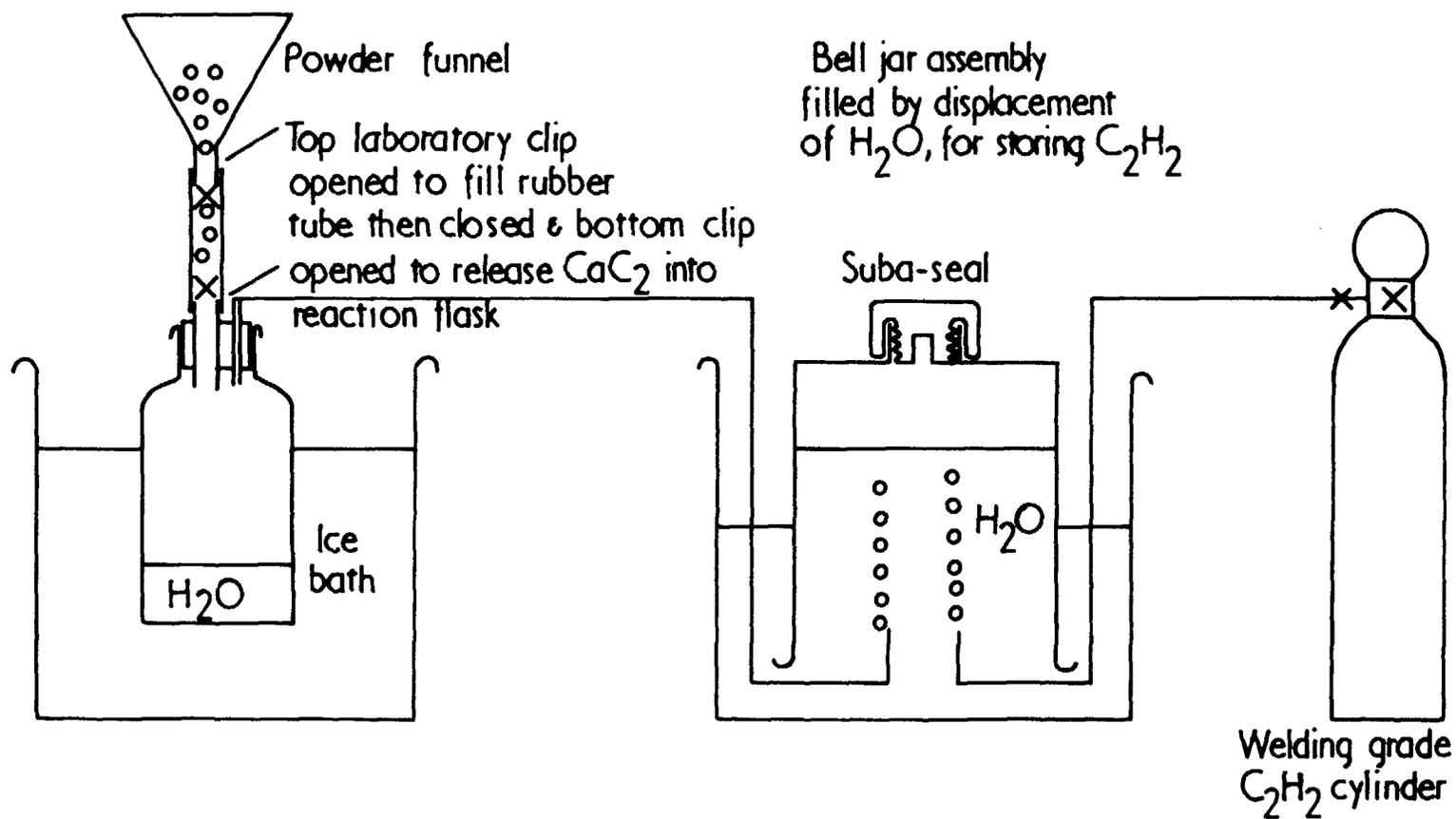C_2H_2 reduction methodology is also presented in other accounts [11, 13, 21, 22, 28-30].

PRECAUTIONS AND COMMENTS

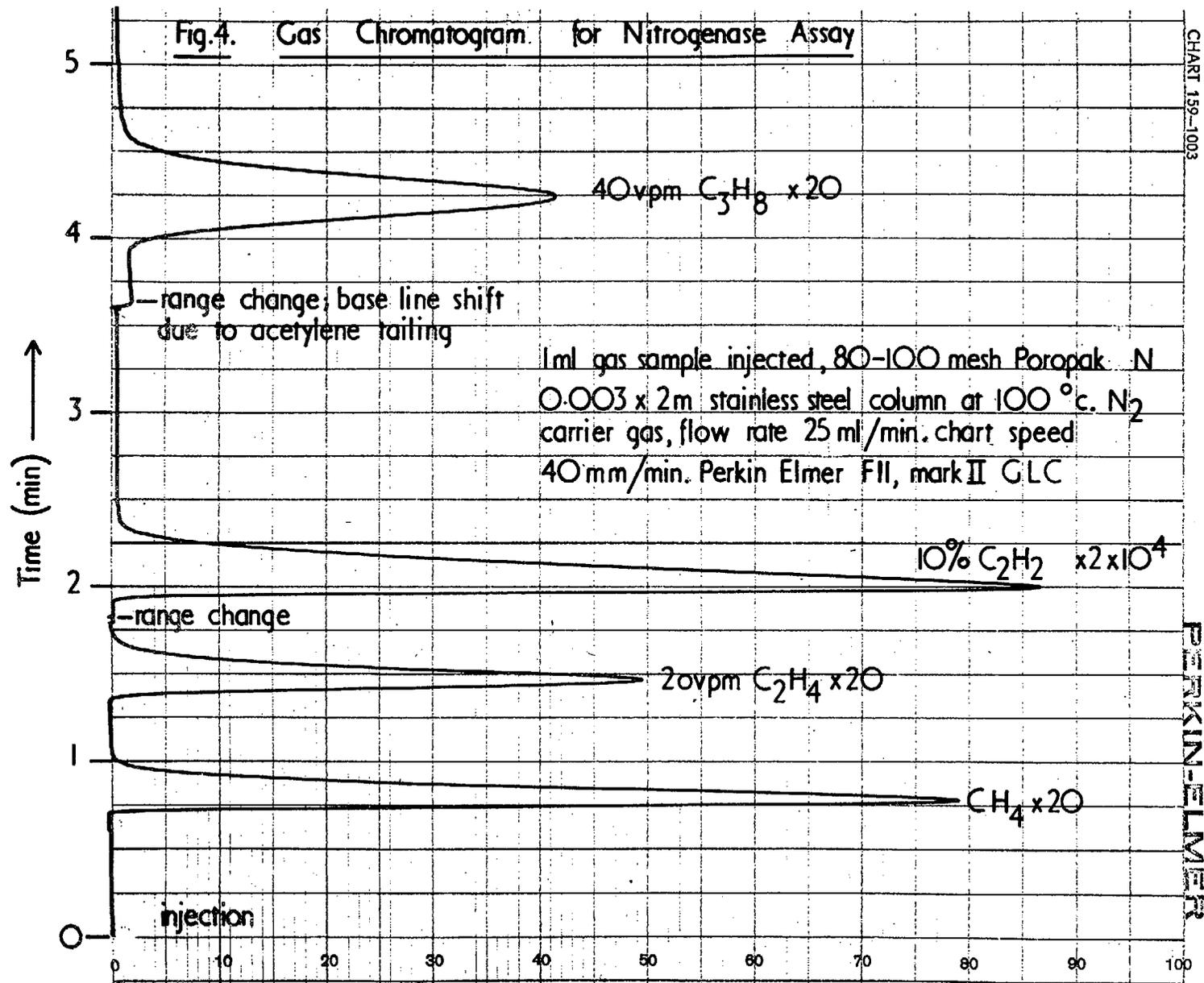1. C_2H_4 is soluble in silicone rubber [23, 31] and produces autoclaving/C_2H_4 from rubber such as Suba-Seals [32].
C_2H_4 contamination in rubber can be decreased by steaming with water for 10 - 15 mins. and leaving to stand 24 h before further use.

2. Disposable plastic syringes and 25 G needles ($\underline{c}$. 0.5 mm O.D.) are suitable for samples and gas transfers [27]. Contaminating C_2H_4 can be decreased by evacuating and flushing the syringes with air about 6 times.

3. C_2H_2 can be bought in cylinders (welding grade is suitable) or it can be produced by reacting calcium carbide with water (Fig. 3); C_2H_2 from either source contains a variable amount of contaminating C_2H_4 (in addition to CH_4 and phosphine) and suitable control blank samples should be taken to monitor this.

4. C_2H_2 can be used as an internal standard in the assay to monitor sampling and injection errors. Propane can also be used in the assay atmosphere as a standard. Most F.I.D. detectors respond linearly to increasing amounts of C_2H_4 over the range of concentrations likely to be encountered, but it is important to work within the linear range of the detector.

5. The chromatography of each series of assays should be calibrated; a standard, premixed cylinder of C_2H_4 ($\underline{c}$. 100 vpm) in Ar or N_2 is useful for this.

6. C_2H_4 is a plant growth hormone, and most responses are saturated by 2 ppm C_2H_4 [33]. Plant membrane integrity is affected by C_2H_4 and/or C_2H_2.

7. Because C_2H_2 is more soluble than N_2 in water, the theoretical conversion factor of 3 may not apply and the actual ratio should be found by using $15N_2$ or Kjeldahl methods [10, 11, 13, 28, 34, 35].

Fig.3. C_2H_2 production

Powder funnel

Top laboratory clip
opened to fill rubber
tube then closed & bottom clip
opened to release CaC_2 into
reaction flask

Bell jar assembly
filled by displacement
of H_2O, for storing C_2H_2

Suba-seal

Ice
bath

H_2O

H_2O

Welding grade
C_2H_2 cylinder

CHART 159—1003

Fig.4. Gas Chromatogram for Nitrogenase Assay

5 —

$40 \text{vpm } C_3H_8 \times 20$

4 —

—range change; base line shift
due to acetylene tailing

Iml gas sample injected, 80–100 mesh Poropak N
$0.003 \times 2m$ stainless steel column at 100 °c. N_2
carrier gas, flow rate 25 ml/min. chart speed
40 mm/min. Perkin Elmer FII, mark II GLC

3 —

$10\% \ C_2H_2 \quad \times 2 \times 10^4$

Time (min)

2 —

—range change

$20 \text{vpm } C_2H_4 \times 20$

1 —

$CH_4 \times 20$

O —

injection

0   10   20   30   40   50   60   70   80   90   100

[1]   HARDY, R.W.F., BURNS, R.C., Biological nitrogen
       fixation, Ann. Rev. Biochem. 37 (1968) 331.

[2]   HARDY, R.W.F., KNIGHT, E., The biochemistry and
       postulated mechanisms of nitrogen fixation,
       in Progress in Phytochemistry. John Wiley
       and Sons, Ltd., Sussex, England 1 (1967)
       387.

[3]   HARDY, R.W.F., BURNS, R.C., PARSHALL, G.W. ,
       "Bioinorganic chemistry of dinitrogen (1)
       fixation" in Inorganic Biochemistry,
       EICHHORN, G., Ed., Elsevier Co., The Nether-
       lands, In press.

[4]   POSTGATE, J.R., (Ed.) "The Chemistry and Bio-
       chemistry of Nitrogen Fixation", Plenum
       Publishing Co. Ltd., London (1971).

[5]   BERGERSEN, F.J., Biochemistry of symbiotic
       nitrogen fixation in legumes, Ann. Rev.
       Plant Physiol. 22 (1971) 121.

[6]   DETROY, R.W., WITZ, D.E., PAREJKO, R.A.,
       WILSON, P.W., Reduction of N_2 by comple-
       mentary functioning of two components from
       nitrogen-fixing bacteria, Proc. Natl.
       Acad. Sci. U.S., 61 (1968) 537.

[7]   KELLY, M., Comparisons and cross reactions of
       nitrogenase from Klebsiella pneumoniae,
       Azotobacter chroococcum and Bacillus
       polymyxa, Biochim. Biophys. Acta 191 (1969) 527.

[8] SMITH, R.V., TELFER, A., EVANS, M.C.W., Comple-
mentary functioning of nitrogenase components
from a blue-green alga and a photosynthetic
bacterium, J. Bacteriol. 107 (1971) 574.

[9] DART, P.J., DAY, J.M., Effects of incubation
temperature and oxygen tension on nitrogen-
ase activity of legume root nodules, Plant
and Soil Special Volume (1971) 167-184.

[10] BERGERSEN, F.J., The quantitative relationship
between nitrogen fixation and the acetylene-
reduction assay, Aust, J. Biol. Sci. 33
(1970) 1015.

[11] MAGUE, T.H., BURRIS, R.H., Reduction of acetylene
and nitrogen by field-grown soybeans.
New Phytol. 71 (1972) 275.

[12] MOUSTAFA, E., Cold lability of the nitrogen
fixation system in excised leguminous
nodules, Phytochem. 8 (1969) 993.

[13] HARDY, R.W.F., HOLSTEN, R.D., JACKSON, E.K.,
BURNS, R.C., The acetylene-ethylene assay
for N_2 fixation: laboratory and field
evaluation, Plant Physiol. 43 (1968) 1185.

[14] SCHWINGHAMER, E.A., EVANS, H.J., DAWSON, M.D.,
Evaluation of effectiveness in mutant strains
of Rhizobium by acetylene reduction relative
to other criteria of N_2 fixation, Plant
and Soil 33 (1970) 192.

[15]   SPRENT, J.I.,   The effects of water stress on
          nitrogen fixing root nodules.   1. Effects
          on the physiology of detached soybean nodules,
          New Phytol. 70 (1971) 9.

[16]   BERGERSEN, F.J.,   The effects of partial pressure
          of oxygen upon respiration and nitrogen
          fixation by sobean root nodules,   J. gen.
          Microbiol. 29 (1962) 113.

[17]   DÖBEREINER, J., DAY, J.M., DART, P.J.,   Nitrogen-
          ase activity and oxygen sensitivity of the
          Paspalum notatum-Azotobacter paspali asso-
          ciation,   J. gen. Microbiol. 71 (1972) 103.

[18]   SUBA-SEAL Closures, William Freeman and Co. Ltd.,
          Suba-Seal Works, Staincross, Barnsley, England.

[19]   We have found "propagating domes and tubes" obtained
          from Stewart Plastics Ltd., Purley Way,
          Croydon, CR9 4HS, England, to be suitable.

[20]   BALANDREAU, Y., WEINHARD, P.,RINAUDO, G.
          DOMMERGUES, Y.,   Influence de l'intensité
          de l'éclairement de la plante sur la fixation
          non-symbiotique de l'azote dans la rhizosphere,
          Oecologia Plantarum 6 (1971) 341.

[21]   BALANDREAU, J., DOMMERGUES, Y.,   Mesure in situ
          de l'activité nitrogénasique,   C.R. Acad.
          Sci. 273 (1971) 2020.

[22]   STEWART, W.D.P., MAGUE, T., FITZGERALD, G.P.,
          BURRIS, R.H.,   Nitrogenase activity in
          Wisconsin Lakes of differing degrees of
          eutrophication,   New Phytol. 70 (1971) 497.

[23] SKINNER, F.A., Hydrocarbons from waterlogged
soils, Rep. Rothamsted Exp. Sta. for 1969
Part 1, (1970) 99.

[24] SMITH, K.A., SCOTT-RUSSELL, R., Occurrence of
ethylene, and its significance in anaerobic
soil, Nature (London) 222 (1969) 769.

[25] ILAG, L., CURTIS, R.W., Production of ethylene
by fungi, Science 159 (1968) 1357.

[26] SCHELL, P.M., ALEXANDER, V., Improved incubation
and gas sampling techniques for nitrogen fix-
ation studies, Limnol. Oceanogr. 15 (1970)
961.

[27] We HAVE FOUND suitable Becton Dickinson Plastipak
syringes. York House, Empire Way, Wembley,
Middlesex, England.

[28] STEWART, W.D.P., FITZGERALD, G.P., BURRIS, R.H.,
In situ studies on N_2 fixation using the
aceytlene reduction technique. Proc. Natl.
Acad. Sci. (U.S.) 58 (1967) 2071.

[29] POSTGATE, J.R., The acetylene reduction test
for nitrogen fixation in 'Methods in Micro-
biology', (J.R. Norris and D. Ribbons, eds.)
Vol. 6B, London. Acad. Press (1971) 342.

[30] A SPECIAL VOLUME of PLANT AND SOIL contains papers
presented at the IBP Technical Meeting
"Biological Nitrogen Fixation in Natural
and Agricultural Habitats", Wageningen,
September 1970 published 1971; many of
these discuss the acetylene reduction assay
for measuring nitrogen fixation.

[31] KAVANAGH, E.P., POSTGATE, J.R., Absorption and
release of hydrocarbons by rubber closures:
a source of error in some biological assays,
Lab. Practice 19 (1970) 159.

[32] JACOBSON, J.V., McGLASSON, W.B., Ethylene produc-
tion by autoclaved rubber injection caps
used in biological systems, Plant Physiol.
45 (1970) 631.

[33] PRATT, H.K., GOESCHL, J.D., Physiological roles
of ethylene in plants, Ann. Rev. Plant
Physiol. 20 (1969) 541.

[34] RICE, W.A., PAUL, E.A., The acetylene reduction
assay for measuring nitrogen fixation in
water logged soil, Can. J. Microbiol.
17 (1971) 1049.

[35] RINAUDO, G., DOMMERGUES, Y., Validité de
l'estimation de la fixation biologique de
l'azote dans la rhizosphere par la méthode
de l'acetylene, Ann. Inst. Past. 121
(1971) 93.

FERTILIZING THE SOYBEAN CROP [Glycine max [L.] Merrill] *)
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ABSTRACT

        Soybeans, the world's most extensively produced grain legume crop, has
significant human nutrition implications by reason of the high quality
protein flour produced from the residue after oil and fat extraction. Al-
though the crop is high in mineral nutrient content it is not especially
responsive to direct fertilizer application. This paper elaborates studies
on fertilizer response of soybeans and considers measures which might assist
in breaking current yield barriers. Lime responses are reasonably assured
where the crop is grown on soils of pH less than 6.2. Because N fixation
accomplished symbiotically in nodulating lines is inadequate for producing
maximum possible yields, yield increases from fertilizer N are commonly
achieved. Straight-forward K responses are obtained on soils of low
exchangeable K supply. Conventional rates of fertilizer P rarely influence
yields unless soil P level is very low but heavy P rates, even at modest
soil P levels, increase nodulation and, depending on variety, may increase
yields due to enhanced N nutrition. Tracer methods afford an invaluable
tool for sorting out the interactions involved.

Introduction

        Although the soybean crop has one of the oldest histories of cultivation
among modern crops, it is largely produced in only two countries, the United
States and China. The crop's world-wide significance is apparent from the
fact that the 30 million tons total production approximately doubles that of
peanuts or dry peas and triples that of dry edible beans, the next most
extensively produced grain legume crops. Beyond its value for the oils and
fats extracted, the soybean flour produced from the remaining cake has very
significant human nutrition implications. Protein of this meal contains
moderate amounts of sulfur-containing amino acids which are deficient in such
grains as corn and wheat. Consequently, soybean meal is being used increas-
ingly as an additive to other flours for improving their nutritional value.

        As with most Leguminosae, mineral nutrient contents of soybeans are
notably higher than occurs in cereals. This would seem to portend exception-
ally good response to applied fertilizer nutrients. Consensus of most
agronomists who have worked with soybeans, however, is that the crop is not
especially responsive to fertilizers and that nutritional needs are better
served by a high level of residual soil fertility. At the same time, soy-
beans are more dependent than other field crops on various other practices
involved in effective soil and crop management for economic yield. Most
troublesome is the fact that average soybean yield was 62 percent of that of
corn in the U.S. in 1939 and only 37 percent in 1970, a period during which
fertilizer consumption more than quadrupled.

It will be the objective of this paper to review fertilizer response studies with soybeans in the U.S. and to consider measures which might assist in breaking current yield barriers. Emphasis of this Panel being on fertilizer utilization for protein production, coverage will be restricted to matters dealing with soil fertility and plant nutrition.

Rooting Habit

Character of the root system of the soybean crop undoubtedly has substantial bearing on the uptake of required nutrients from the soil and, correspondingly, the most effective manner and depth of fertilizer application. Root studies of Borst and Thatcher [1] suggest that the taproot system of the crop has potential, depending on soil type, for rooting to 150 cm depth, but with most of the system concentrated in the upper 60 cm. Lateral root spread was noted to be limited to a circle of approximately 45 cm diameter, contrasted with the 180 cm lateral spread of maize. Also differing from maize was an observed dominant primary root contrasted with the completely fibrous system in maize with all roots of secondary origin. Most of the soybean root nodules were found concentrated around the primary root in the surface 15 or 20 cm of soil.

Mitchell and Russell [2] found 90 percent or more of the total root weight in the upper 15 cm of soil in the latter portion of the growing season. During the earlier stages of root development, four verticle rows of laterals developed off the upper 10-15 cm of the radicle at 90° angles around its circumference which reached 35-40 cm length before turning downward. The 5-6 major laterals among these reached depths of 122-183 cm, exceeding the depth of taproot penetration.

Soybeans appear to actively absorb nutrients from greater depth than most cereals, presumably due to the taproot system. Tracer studies with ^{85}Sr by Menzel et al. [2a] showed uptake at 50 cm depth to be only slightly less than that from the soil surface. Additionally, substantially more P is taken up by the crop late in the season than by corn [3]. The combination suggests that optimum fertilizer placement might be different than for Maize.

Why Do Soybeans Respond Poorly to Fertilizers?

The data of Table I acquired with the use of ^{32}P tagged fertilizer give evidence of the strong feeding capacity for soil P exhibited by soybeans compared with other common crops [4]. In this study soybeans utilized soil P approximately 70 percent more effectively than other legumes and 30-50 percent better than the non-legume crops investigated. The greater fertilizer utilization associated with banding compared with mixed placement may be partially attributed to the influence of contiguous N in promoting fertilizer P uptake. Along with lower utilization of fertilizer P by soybeans noted, other treatments not presented in the table [P only vs NP] indicated significantly larger positive effects of N on P uptake with all other crops than with soybeans.

It has been suggested that the primary reason why soybeans give limited response to applied nutrients is that the crop evolved on quite infertile soils such that natural selection enhanced its capacity to produce reasonably well even on soils very poor in nutrients. Under these circumstances the genetic components for high yield under more favorable nutrient environment did not develop. Furthermore, with 20th century acceptance of the crop in

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the U.S. the primary use was for hay. Yields of hay were quite readily increased by fertilizer treatments, not so for seed. It has only been since the early 1940's that seed production exceeded production for hay and the interval thereafter for varietal improvement through breeding has been short compared with the time during which cereal breeding has gone on in the U.S.

Contributing to the varietal deficiency suggested above is the fact that storage of energy in oil and protein [21 and 40 percent average composition, respectively] of the bean requires twice as much energy as for starch storage in the grain of maize [5]. Also, no hybrids are available in soybeans by reason of self pollination, whereas corn hybrids are known to be more fertilizer responsive than open pollinated lines.

Liming Results

Limited information exists in the literature on the subject of liming the soybean crop. Optimum pH reported by different investigations varies rather widely from 6.0 to 6.9. Thatcher et al. [6], for example, growing the

Table I. Utilization of fertilizer P by soybeans compared with that of other common crops grown at the same time in greenhouse pots [4].

| Crop | Utilization of applied P [80 + 50* + 0],% from fertilizer a/ | | |
	Mixed	Banded	Mean
	%	%	%
Soybeans	30.8	42.4	36.6
Other legumes			
Alfalfa	52.6	62.1	57.3
Red Clover	56.6	67.7	62.1
Sweetclover	54.2	73.0	63.6
Nonlegumes			
Maize	46.6	48.0	47.3
Wheat	38.9	56.7	47.8
Oats	43.3	60.4	51.8
Bromegrass	52.4	53.1	52.7
Sorghum	63.1	44.8	53.9

a/ Tagged fertilizers were either 'mixed' with all of the soil in the pot or were 'banded' 2.5 cm to the side of the seed row for nonlegumes, and 1 cm to side and 1 cm below legume seeds. Harvest and assay were effected at or near flowering for each crop. Data represent mean values for three soils -- Crofton [Calcareous], Moody [neutral], and Nemaha [acid].

crop on soils varying in pH from 4.6 to 7.7 obtained maximum yields at pH 6.8. Soil acidity is probably a larger factor in Rhizobium survival and effective nodulation than is Ca availability [7]. Adequacy of Ca may be the major determinant of crop yield response, however, since Ca requirement of the plant itself for growth is much greater than that of the Rhizobia.

Data from the Field Laboratory of the University of Nebraska at Mead on Sharpsburg silty clay loam are perhaps representative of yield results from liming soils of medium acidity [8]:

Treatment	Yield kg/ha
Control	3091
Lime only	3293
P + K	3494
Lime + P + K	3763

Soil pH without liming was 6.0 and 6.8 after applying 3 tons lime/acre. The unanswered question here, of course, is in how far a small rate of fertilizer N might have afforded the same effects.

It seems most probable that the first requirement for effecting economic soybean yields on many humid tropical region soils will be provision of an adequate calcium supply for normal nutrition of the crop. Certainly the majority of soils in these regions possess inadequate calcium release potentials for the introduction and successful growth of the soybean.

Pertinent to liming results reported in the literature for acid soils is the recognized differential aluminum tolerance of different varieties [9]. Deficiency of Ca observed in many cases may well be Al induced, as Al appears to interfere with uptake and utilization of Ca. Foy et al. suggest that plant breeders should consider developing varieties that can root more effectively in acid, Al-toxic subsoils.

An additional malady of soybeans noted on acid soils of southeastern U.S. is that of 'crinkle leaf' due to Mn toxicity. It is accentuated by the acidifying action of high rates of fertilization and alleviated by liming [10]. Equating the Latosolic soils of this portion of the U.S. with similar soils in the developing world would portend a strong likelihood of Mn toxicity problems there as well.

Nitrogen Fertilizer Results

A number of field experiments as summarized by Ohlrogge [11] have indicated that N fixation by R. japonicum on nodulating soybean lines is inadequate for producing maximum possible yields. Importance of N for maximum yield is confirmed by work of Lathwell and Evans [12] in which yield was shown to be determined by number of pods retained by the plant, and that number in turn by the N available during the bloom period. One of the early reports of N response of soybeans was by Eastin [13] with irrigation culture in Nebraska evidencing small but economic responses from application of low rates of fertilizer N.

Use of ^{15}N as a tracer has facilitated studies on the effects of combined nitrogen on the fixation process. By tagging the applied N, Norman and Krampitz [14] were able to confirm that nitrogen fixation decreased with

increasing amounts of fertilizer N applied even though total N per plant increased. Atmospheric origin of N in the plant varied from 100 percent with no fertilizer N applied to a low of 30 percent with a large rate of fertilizer N added to the soil. Weber [15] reported a high correlation between nodule mass and symbiotic N fixation, further that nodule weight, number and size were inversely related to applied N.

A recent study by Beard and Hoover [16] including rates and times of N application compounded with straw residue treatments found essentially no differences in yield from the treatments. A linear inverse relation was found between nodule number per plant and rate of N applied. Nodule number was reduced by 56 kg/ha or more of N applied at planting time, but little reduction accompanied twice that amount when applied at flowering. Thus, timing of applied N would appear to assume major significance for assuring maximum N utilization without penalizing the symbiotic relationship. Burning off the straw residue also resulted in greater reduction in nodule number with increasing N rate than occurred without burning.

It seems quite clear that the key question concerning N fertilization for soybeans revolves around the amount of available N that can be counted on from symbiotic fixation plus the amounts residually present in the soil rooting zone and that likely to be mineralized from the soil organic matter during the growing season. Procedures for balancing these three sources against the amount of fertilizer N to be applied will be required if the producer is to capitalize on all possible 'free' nitrogen and simultaneously limit the chances of mineral N escaping the root zone and contributing to environmental pollution.

Phosphate Fertilizer Results

The majority of fertility investigations with soybeans in midwestern U.S. have shown little or no response to applied phosphate unless the sites involved were extremely low in available soil P. Thus, Walker and Long [17] reported a strong relation between soil P tests and yield even though applied P did not influence yield in their studies. Their maximum predicted yield came with pH of 6.92, soil P level of '105 pounds per acre' and soil K of '264 pounds'. Kamprath and Miller [18] similarly concluded from a North Carolina survey that yields throughout the state were highly related to soil P test and pH.

Investigations with tagged phosphate have shown that little difficulty is experienced in getting P into the soybean plant [19, 20], so inhibited uptake of P cannot be given as cause for limited response. The ^{32}P tag has also demonstrated, however, that gross absorption of P continues later into the season with beans than with corn [20]. Studies of Bureau et al. [3] detected that 83 percent of the recovered P was taken up in the 39 days between late bloom stage and just prior to leaf fall. These observations would suggest that soybeans are more capable than most annual crops of effectively utilizing the deep subsoil available P supplies commonly found in zonal soils of the midwestern U.S., as depicted in the loessial soil profiles of Nebraska of Figure 1 [21]. They may further partially explain the limited fertilizer P responses noted by most investigators since the late root arrival and activity in this zone would correspond with the substantial late uptake of total P by the crop.

There are indications that adequate P for the plant's nutrition may not be the full resolution of the plant's needs and rather additional P may be needed for other functions. Thus, deMooy and Pesek [22] have found that maximum nodule weight and activity occurred only at very high levels of applied P and K salts. By applying P at rates of 400-500 pp2m and K at 600-800 pp2m nodule weight at flowering was tripled, and maximum nodule activity required even higher levels of applied P. The amount of N in the plant leaves also peaked at the point where nodulation was at its maximum. Weber [23] confirmed that increased N fixation was directly related to nodule number and size.

Evidence has been provided by Lathwell and Evans [12] and by Erdman and Means [24] that N supply of the soybean plant is highly related to yield. Such being the case, there would seem to be good probability that increased nodule formation and activity from P application should increase the plant's N supply with potential yield effects.

Potash Fertilizer Results

Yield responses of soybeans from direct application of potassium fertilizers have been more common and striking than reported for phosphate. Thus, Nelson et al. [25] report striking seed yield responses to applied K for three different varieties on a medium textured soil of North Carolina. A larger number of pods, a higher degree of pod filling and greater seed weight were associated with the increase. Such responses are common in the humid southeastern U.S. They are much less common Mollisols [Brunizem and Chernozem] in the midwestern U.S., and there primarily with the sandier soils, the thoroughly leached Alfisols [Gray Wooded and Gray-Brown Podzolic] on fine textured till, and some of the organic types. Prediction of likely response to K fertilizer through soil testing has been less difficult than for N and P.

Soluble-salt damage to germination is likely with any fertilizer material applied in contact with the seed as soybeans are very sensitive to such injury. Potash salts are notably more damaging than phosphates. As with P, there is no certainty that optimum placement of K is known for the crop.

Other Secondary and Trace Element Results

Magnesium deficiency of soybeans has been rarely observed in the corn belt of the U.S., a region of temperate subhumid climate and soils of the Mollisol order. In the warmer, more humid southeast, however, responses to applied Mg are fairly common, exemplified by data of Nelson et al. [25] on a moderately sandy Ultisol of North Carolina. A general consensus exists that a Mg response is likely when exchangeable Mg is less than 10 percent of the cation exchange capacity. Certainly many soils of the warm and humid regions of the developing world fit this description suggesting a good likelihood of extensive Mg needs if soybeans are to be grown.

Sulfur responses of soybeans are rarely observed in the U.S. Explanation perhaps lies in the fact that the crop is not commonly grown on sandy soils of the midwest known to be S deficient, while in the southeastern production area fallout S and residuals from low analysis S-containing fertilizers of past years have restricted responses to date. In consideration of the known

effect of S on oil production, however, there is good reason to anticipate response to the element in soils of low organic matter content of tropical regions as reported by Venema [26] and as observed for the groundnut in northern Nigeria [27].

Problems of Fe deficiency have frequently been observed where soybeans were produced on calcareous soils. Inactivation of absorbed Fe in the roots in the calcareous soil medium is apparently due to combined effects of pH, Ca and P [28]. Two or more foliar applications of ferrous sulfate have been found effective for control [29]. Ratio of Fe:Mn is critical, with some authors contending that Fe deficiency and Mn toxicity are synonomous [30]. Large differences have been found between varieties. The better growth of efficient varieties was controlled by a single gene which causes lower pH of the expressed cell sap and thereby less inactivation of Fe [31].

Deficiency of Zn is also observed on occasion where soybeans are grown on calcareous soil, especially where land grading has exposed a horizon of low organic matter content and compacted the soil in the process. Deficiency appears most severe on oldest leaves [32], and is readily corrected by foliar spray and by soil treatment with soluble Zn carriers or even ZnO if finely divided. It is believed now that many of the early reports of yield reduction from applied P [33, 34, 35, 36] may have been the result of P induced Zn deficiency. It is suggested by data of Paulsen and Rotimi [37] for soybeans and reported in maize by Stukenholtz et al. [38] that Zn may be readily absorbed but its translocation from roots to tops is inhibited by increased P availability to and absorption by the root system.

Seed treatment with Mo solution has afforded yield response in some acid soil locations. Lavy and Barber [39] concluded that a concentration of 1.6 ppm Mo or less in seed of untreated plots assured response to applied Mo. Seed treatment would seem to be good insurance for regions of highly leached ancient soils, acid in reaction, as has proved desirable for maize production in the high veldt of Swaziland [40]. Neither sources nor rates of Mo seed treatment within practical means followed by extended seed storage have caused germination loss of significance [41].

Few field data exist on yield response of soybeans to Cu and Mn except on organic soils. Neither is there much evidence of B deficiency of the crop with soils on which it is commonly grown in the U.S.

Variety-Fertility Interactions

Significant varietal differences in yield response were reported over a wide range of P treatments by Howell [34]. Later, Howell and Bernard [36] classified 44 varieties according to sensitivity to high rates of fertilizer P. Certain plant introduction lines were reported by Dunphy et al. [42] to be outstanding in their response to P and K fertilizers, which characteristic was consistent from year to year. Highest yielding lines were also the most responsive to fertilizer. Hughes [42a] grew 18 varieties and 2 experimental lines for two years on a productive soil with two fertility levels provided, viz., none and 108 + 47 + 65 kg/ha annually. Significant differences between treatments and between genotypes were found for seed yield and for uptake and accumulation of N, P, K, Ca, Mg, Cu and Fe. Close relation existed between yield and leaf N, leaf N and leaf P, and leaf N and pods per plant. It seems quite apparent, therefore, that yield response to nutrient investigations must take into account the genetic materials employed as these may determine results and recommendations.

Peterson [43] carried out a detailed field study for identifying soybean lines more responsive to P fertilization than those commercially grown and for evaluating chemical composition and nodulation properties of the responsive lines. Four promising experimental lines were selected from a preliminary study involving two P levels of 0 and 560 kg/ha broadcast. Fertilizer P was placed in a 15 x 15 cm zone of the seed row for the four promising lines and two standard varieties at rates of 0, 150, 300, 600 and 1200 pp2m P. Seed yield increases were quite consistent up to 600 pp2m P. Response was due to seed set rather than seed size increases and occurred at rates where concentration of P in leaves exceeded recognized optimum levels. He concluded that improved N supply for the plants and not improved P nutrition per se was responsible for the yield increases at the highest P rates. Increased nodulation due to P was not solely responsible for the altered N supply, since even those varieties without seed yield response had increased nodulation from P fertilization. An interesting adjunct to this study would have been a nominal fertilizer N application to determine if it would have similar yield effects.

A philosophic attitude has developed on the part of some who have worked with soybeans that if the nodules were removed the new genotypes might act like other crops in their response to N and thereby allow higher yields. Evidence to date with non-nodulating lines, however, has not been especially promising in this respect. For example, in no case did Weber [23] working with a pair of nodulating and non-nodulating soybean isolines obtain seed yield of the latter exceeding the nodulating line, irrespective of rate of applied N. With moisture limiting, 56 kg N/ha was needed to approximately equalize yields of the two lines, but with favorable moisture 169 kg N/ha was not quite sufficient to bring the non-nod line to equivalence. Where severe N deficiency was induced by addition of ground maize cobs, 675 kg N/ha was required to bring the non-nod line up to the approximate yield level of the nod line. At the 675 kg rate the nodule mass of the nod line was reduced to about 1/5 that where no N was applied.

Influence on Subsequent Crops

In the central U.S., soybeans most commonly are planted after a year or more of maize in anticipation of the crop having access to significant amounts of residual fertility from that applied for the maize. Previous discussion has emphasized the limited response of the beans to fertilizer directly applied but also the benefit derived from a good overall nutrient supply in the soil. It has been well recognized, too, that maize following soybeans commonly yields better than when following maize or other nonlegume crops. This has been attributed to higher levels of available nitrogen and a better physical condition of the soil. Thus, Weber [15] reported the residual benefit in short-term rotation studies of a nodulating soybean crop to be some 37 kg/ha. Prior to the advent of extensive fertilizer N use on the maize crop, i.e., before the mid 1950's the greater residual soil N supply was presumably the dominant factor. Even with N compensated in recent years, however, benefits are still noted following beans which must be in large part related to soil physical properties.

Farmers readily observe that soils on which soybeans have grown are more friable than following other crops. Experimental data have confirmed increased soil aggregation and this has been attributed to the protective canopy of the crop, desiccation of soil due to high root concentration, and increased aggregation from decomposition of roots, nodules and tops [44]. It

is believed that wetting and drying was the dominant aggregating agent. Soils under soybeans have been noted to be notably drier in the surface 30-40 cm than under corn, explained by the proportionately greater root concentration in surface layers and by the greater water requirement per unit of dry weight.

The looser and better aggregated soil, especially when very fine textured, has also been responsible for better water penetration from rain or irrigation [45]. The consequent reduction in runoff loss, too, may have been a factor in the increased yields noted after soybeans in cases where moisture supply was critical.

LITERATURE CITED

[1] Borst, H. L. and L. E. Thatcher. 1931. Ohio Agr. Exp. Sta. Res. Bull. 494.
[2] Mitchell, R. L., and W. J. Russell. 1971. Agron. J. 63:313-316.
[2a] Menzel, R. G., H. V. Eck, and D. F. Champion. 1967. Effect of placement depth and root-inhibiting chemicals on uptake of strontium-85 by field crops. Agron. J. 59:70-72.
[3] Bureau, M. F., H. J. Mederski, and C. E. Evans. 1953. Agron. J. 45:150-154.
[4] Green, Richard E. 1957. M. S. Thesis, Univ. of Nebraska.
[5] Oldham, M. G. 1966. Soybean fertilization. Fert. Solu. 10[2]:74-75.
[6] Thatcher, L. E., C. J. Willard, and R. D. Lewis. 1937. Ohio Agr. Expt. Sta. Bull. 588.
[7] Vincent, J. M. 1965. Environmental factors in the fixation of nitrogen by the legume. Soil Nitrogen, Agron. Monograph 10:387-435.
[8] Stryker, R. B. 1967. M. S. Thesis, Univ. of Nebraska.
[9] Foy, C. D., A. L. Fleming, and W. H. Armiger. 1969. Agron. J. 61: 505-511.
[10] Parker, M. B., H. B. Harris, H. D. Morris, and H. F. Perkins. 1969. Agron. J. 61:515-518.
[11] Ohlrogge, A. J. 1963. The Soybean. Academic Press, New York.
[12] Lathwell, D. J. and C. E. Evans. 1951. Agron. J. 43:264-270.
[13] Eastin, J. D. 1955. M.S. Thesis, Univ. of Nebraska.
[14] Norman, A. G. and L. O. Krampitz. 1946. Soil Sci. Soc. Amer. Proc. 10:191-196.
[15] Weber, C. R. 1966. Agron. J. 58:46-49.
[16] Beard, Benjamin H. and Richard M. Hoover. 1971. Agron. J. 63:815-816.
[17] Walker, W. M. and O. H. Long. 1966. Agron. J. 58:403-405.
[18] Kamprath, E. J. and E. V. Miller. 1958. Soil Sci. Soc. Amer. Proc. 22:317-319.
[19] Krantz, B. A., W. L. Nelson, C. D. Welch, and N. S. Hall. 1949. Soil Sci. 68:171-177.
[20] Welch, C. D., N. S. Hall, and W. L. Nelson. 1949. Soil Sci. Soc. Amer. Proc. 14:231-235.
[21] Dreier, A. F., D. Heuerman, and R. A. Olson. 1967. Nebr. Agr. Exp. Sta. Quart. [Summer].
[22] deMooy, C. J. and J. Pesek. 1966. Agron. J. 58:275-280.
[23] Weber, C. R. 1966. Agron. J. 58:43-46.
[24] Erdman, L. W. and U. M. Means. 1952. Soil Sci. 73:231.
[25] Nelson, W. L., L. Burkhart, and W. E. Colwell. 1946. Soil Sci. Soc. Amer. Proc. 10:224-229.

[26] Venema, K. C. W. 1962. Potash Trop. Agr. 5.

[27] Greenwood, M. 1951. Emp. J. Exp. Agr. 19:225-241.

[28] Brown, J. C., L. O. Tiffin, R. S. Holmes, A. W. Specht, and J. W. Resnicky. 1959. Soil Sci. 87:89-94.

[29] Nelson, L. B. 1948. Ia. Agr. Exp. Sta.

[30] Somers, I. I. and J. W. Shive. 1942. Plant Physiol. 17:582-602.

[31] Weiss, M. G. 1943. Genetics 28:253-268.

[32] Viets, F. G., Jr., L. C. Boawn, and C. L. Crawford. 1954. Soil Sci. 78:305-316.

[33] Pierre, W. H. 1944. Soybean Digest 4[6]:12-13.

[34] Howell, R. W. 1954. Plant Physiol. 29:477-483.

[35] Fletcher, H. F. 1961. Ph.D. Thesis, Univ. of Ill.

[36] Howell, R. W. and R. L. Bernard. 1961. Crop Science 1:311-313.

[37] Paulsen, Gary M. and Olusegun A. Rotimi. 1968. Soil Sci. Soc. Amer. Proc. 32:73-76.

[38] Stukenholtz, D. D., R. J. Olsen, Gerald Gogan, and R. A. Olson. 1966. Soil Sci. Soc. Amer. Proc. 30:759-762.

[39] Lavy, T. L. and S. A. Barber. 1963. Agron. J. 55:154-155.

[40] Jackson, P., A. Venn, and J. I'Ons. 1965. OAU/STRC Pub. No. 98, Khartoum.

[41] Johnson, B. J., H. B. Harris, M. B. Parker, and E. E. Winstead. 1966. Agron. J. 58:517-518.

[42] Dunphy, E. J., L. T. Kurtz, and R. W. Howell. 1966. Soil Sci. Soc. Amer. Proc. 30:233-236.

[42a] Hughes, J. L. 1971. Ph.D. Thesis, Univ. of Nebraska.

[43] Peterson, Gary A. 1967. Ph.D. Thesis, Ia. St. Univ.

[44] Browning, G. M., M. B. Russell, and J. R. Johnston. 1943. Soil Sci. Soc. Amer. Proc. 7:108-113.

[45] Van Doren, C. A. and R. S. Stauffer. 1944. Soil Sci. Soc. Amer. Proc. 8:97-101.
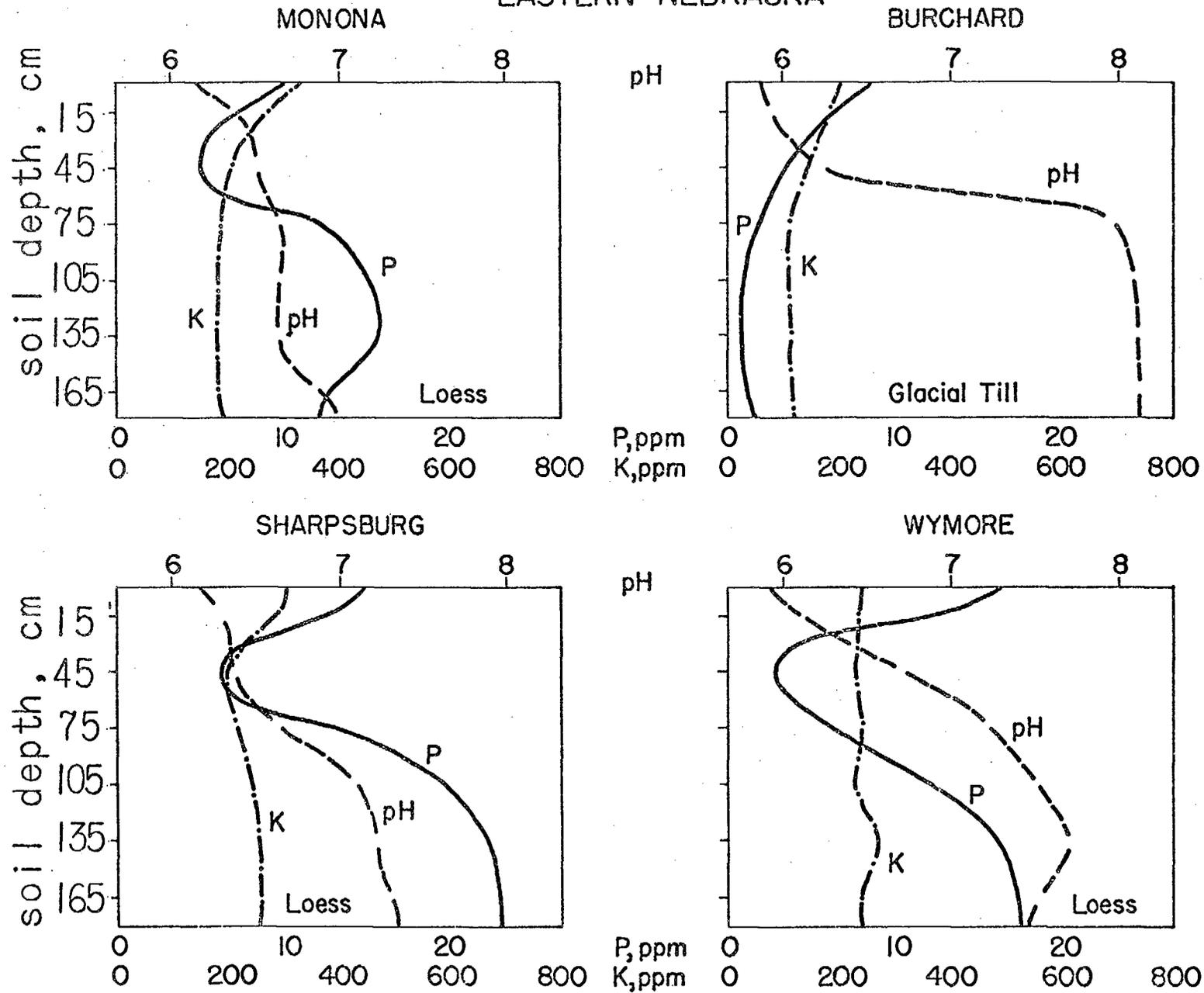
Figure 1. Soil phosphorus (Bray and Kurtz no. 1), exchangeable potassium and pH levels of the 180-cm profile of major soils in eastern Nebraska.

Fertility Studies On Some Legume Crops In Egypt

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ABSTRACT

The present report describes and discusses the results that were obtained from several series of field and greenhouse experiments to study the following topics :

I - Response of bean plant (Vicia Faba) to N, P & K .

II - The comparative effect of the soil and foliar application of P. on bean plant.

III- The effect of salinity on nodule formation, yield, N and P uptake by bean plant and cowpeas.

The main target of these studies is to increase the production of such crops through the efficient use of fertilizer and soil reclamation.

I- The First Topic .

Response of bean plant (Vicia Faba) to N, P, & K.

A- Introduction :-

(Vicia Faba), the bean plant is one of the most important winter leguminous crops raised for seeds in Egypt. It owes its importance cheifly to its high protein content as it contains

about 24 %.   It is also well supplied with P and Ca.   In
addition, it is relatively not expensive crop to produce and it
promices high return when properly grown.

According to the available statistics (1) an area of
about 316.722 acres was cultivated by this crop in 1970.   The
total production of which was 277.434 metric tons of grains with
an average yield of 876 Kgs / acre.   Such an average is much
higher than any of those of Italy, Spain, Mexico & Brazil;   but
it is still lower than those of West Germany and Britain.

It is beleived that under local conditions, the average
yield of this crop could be raised by planting the high yield
potential varieties and improving the cultural practices.
Among these practices, is the adoption of proper fertilization.

B - Objectives :-
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The objectives of this topic could be summarized in the
following .

1-   Studying the response of bean plant to N, P & K and determin-
     ing the optimum economical rate of fertilizer recommendation.
2-   Studying the relationship between the soil test values and
     the response of bean plant to various nutrient elements.

C- Experimental Work :-
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For the perpose, two series of field experiments were
conducted.   The main feature of these experiments was that
they were carried out yearly in different locations for conside-
rable length of times.   Thus valuable accumulation of experi-
mental results were secured with great number of replications.

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The first series of experiments was initiated in 1959/60.
During a four year period (1959 / 60 - 62 / 63) fifteen simple
field trials were conducted in Lower, Middle & Upper Egypt as
a pilot work to explore the major nutrients which are lacking
in Egyptian Soils for bean production.   Sites were chosen to
tupify major bean cultivation regions.

Each experiment consisted of 5 manurial treatments as shown
in table (1).   The experimental set up was designed in randoe
mized complete blocks in four replications.

In the light of the experimental data obtained from the 1st
series, the manurial treatments of the second series were planned
to have more information about the response of this particular
crop to different rates of nitrogen and phosphorus and the inter-
action between these two nutrient elements when they are applied
together in different combinations.   In addition; the relation-
ship between the soil test values and the response of bean plant
to N & P was also examined.

A series of 25 field trials - over a five year period
(1956/67 - 70/71) - was conducted throughout the country rep-
resenting the major area of bean plantation in Egypt.

Each experiment consisted of nine fertilizer treatments
including three levels of each of N & P and the different combina-
tions of the two elements.   The levels of nitrogen were 0, 18 &
36 Kg N/ha and three levels of P were 0, 36 & 72 Kg P_2O_5/ha.

The experimental set up was designed as a factorial, experi-
ment in randomized complete blocks having five replications.

Composite soil samples were taken from the experimental sites to the depth of 25 c.m.   The soil samples were airdried, ground to pass through 1 m.m.

Available N. which include the total ammonium and nitrate nitrogen soluble in 1 % K_2SO_4 solution was determined according to Koing method (1930).

Available P was determined by two methods namely;   Olsen et al. (1954) & Hibbard (1931).

Other chemical constituents were determined according to the Official chemical methods (Jackson 1958).

D - Results & Discussion :-

1. The First Series :-

Data obtained from this series are presented in table (1). It is clearly demonstrated throughout the results that bean plant did respond significantly to N and P when each of them was applied alone or when N was combined with P.

The % increase in yield due to N application at the rate of 18 Kg N/ha was 12.4 over that obtained from the check treatment. Increase in yield due to P application at the rate of 54 Kg P_2O_5/ha amounted to 14.3 % .   When N & P were applied together at the same rates the yield was more pronounced than that obtained from either N or P alone.   % increase in yield over that obtained from the control was 19.3.

On the other hand yield increase due to K application was neither significant nor economical and could be neglected.

Table (1)

Response of bean plant (Vicia Faba) to N, P & K

(Average results of 15 field expts).

Manurial Treats. in kg / ha			Yield in	Increase over control Kg/ha	% Increase
N	P_2O_5	K_2O	Kg/ha		
–	–	–	3118	–	–
18	–	–	3506	388	12.4
–	54	–	3565	447	14.3
18	54	–	3720	602	19.3
18	54	35	3761	643	20.6
L.S.D.	(.01)		284		
	(.05)		214		

Therefore, such pilot work has revealed that N & P are of significant importance for acheiving high yield of bean plant. As for potash, experimental data proved that Egyptian soils have enough available K to meet the nutritional requirements of this particular crop . Such result was in close agreement with the previous findings of several workers (Shalaby 1950) Serry & Banna 1962 & Hamissa et al. 1969) who pointed out the adequate amounts of available potassium in Egyptian soils.

2. The Second Series :-

The data obtained from this series are presented and dis-
cussed under the following headings.

a. Response of bean plant to N.

b. Response of bean plant to P.

c. Response of bean plant to N & P interactions.

d. Yield production of N & P unit.

e. Soil description and the relationship between soil test
   values and the response of bean plant to N & P.

a- Response of bean plant to N.

Average results of 25 experiments are reported in table
(2).   It is clear out of these data that bean plant responded
significantly to N when applied alone.   In this series of
experiments, the increase in yield due to N application ranged
between 10 to 11 % over that obtained from the check treatment.
However, it has been shown that no significant effect had been
obtained when the rate of N was increased from 18 to 36 Kg N/ha.
indicating that if nitrogen has to be applied alone high rate is
not preferable.   It has been also reported (Waksman 1932 &
Erdman 1953) that in fertile soil well supplied with available
N there may be little fixation as the legume plants seem to use
this available N rather than encourage the bacteria to fix more
of it.

(Table 2)

Response of bean plant to N at different rates

(Mean results of 25 field expts.)

Kg N/ha.	Yield in Kg/ha.	Increase over cont. Kg/ha.	% Increase
—	2687	—	—
18	2956	269	10
36	2982	295	11
L.S.D. 1 %	188		
5 %	140		

b- Response of bean plant to P

Table (3) illustrates the effect of P in different rates on the yield of bean plant. The results show that the yield was consistently and significantly increased as a result of P application. % increase in yield over that obtained from the check treatment due to P application amounted to 11.7 & 19.8 for 36 & 72 Kg P_2O_5/ha. respectively. Further work in this respect (8) indicated that no significant increase in yield was obtained beyond these rates.

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Table (3)- Response of bean plant to P at different rates.

(Mean results of 25 field expts.)

Kg P_2O_5/ha.	Yield in Kg/ha.	Increase over cont. Kg/ha.	% Increase
—	2687	—	—
36	3000	313	11.7
72	3218	531	19.8
L.S.D. .01	251		
.05	185		

c- Effect of N & P in different combinations.

Data reported in table (4) indicate that the effect of the interaction between N & P when they are applied in different combinations .

As it is shown that the maximum increase in yield was attained by the use of 36 kg N & 72 Kg P_2O_5/ ha. At this rate the increase in yield over that obtained from the control amounted to about 30 %.

In general; such results were in close agreement with the results obtained from the 1st series confirming the necessity of the two major nutrient elements (N & P) for acheiving high production of this particular crop.

The data also support the previous findings of Cracie & Khalil (1948) and Taha, Zaki & Hammad (1968).

Table (4)- Response of bean plant to N & P in different
combinations.

(Mean of 25 field expts.)

Kg /ha		Yield in	Increase over cont.	%
N	P_2O_5	Kg /ha.	Kg/ha.	Increase
---	---	2687	---	---
18	36	3166	479	17.9
36	36	3218	531	19.8
18	72	3351	664	24.7
36	72	3484	697	29.7
L.S.D.	.01	273		
	.05	207		

d- Yield Production of N & P unit.

The over all average increase in yield due to the applica-
tion of 1 kg of each of N & P_2O_5 when they were combined together
in the most effective treatment - (72 kg P_2O_5 & 36 Kg N/ha).
was calculated and presented in tables (5 & 6).

Table (5)- Average increase in yield in Kg. bean seeds
per one Kg N/ha.

(Average results of 25 field expts.)

Fertilizer rate (Kg/ha)		Yield in	Increase in yield due to	Av. increase due to
P_2O_5	N	Kg/ha.	N. (Kg /ha.)	1 Kg N Kg /ha.
72	-	3218	---	---
72	36	3484	266	7.4

As indicated in table (5) average increase in yield per one
Kg N was 7.4 Kgs. bean seeds /ha.

Almost the same result was obtained as far as P is concerned. Data reported in table (6) indicate an average increase in yield per one Kg P_2O_5 was about 7 Kgs. bean grain /ha. which was competable with that obtained from one Kg. of N. Taking the economical side in consideration and according to the current prices of the fertilizers N & P and the crop produced, such increases in yield secure a very good return. Another point could be detected from the data obtained concerning the net return resulting from each of the two nutrient elements. As the P unit is much cheaper than the N unit, therefore, it could be stated according to the results at hand, that P application is more profitable than N application for bean production. However, to obtain the highest yield both nutrient elements should be applied.

Table (6)- Average increase in Yield [x]in Kg bean
seeds per one Kg P_2O_5/ha

Fertilizer rate (Kg/ha)		Yield	Yield	Av. increase due
N	P_2O_5	Kg/ha	increase Kg /ha.	to one Kg P_2O_5
36	—	2982	—	—
36	72	3484	502	7.0

[x] Mean results of 25 field experiments.

e- Description of the soil samples used in the study:-
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As mentioned before, soil samples were taken from 25 experimental sites for description and chemical analyses. The samples covered eleven main governorates and represent to a fair extent

the type of soils under bean plant cultivation.   In general most
of these soils are classified according to the world soil map as
Fluvisol soil.   The chemical analyses of these samples are pre-
sented in table (7).   It is clear from the data reported in the
table that there is a wide range in the different chemical consti-
tuents of the various soils.   However, average results indicates
that pH values of the soil tested fell in the range of the alkaline
reaction.   Soils are generally rich in calcium carbonate and
poor in organic matter.   Average determinations of the total
soluble salts are low and under the level of toxicity.   As far as
the N, P & K are concerned, in general most of the soil samples are
poor in nitrogen, moderate in P but fairly rich in K.

Table (7)- Chemical analyses of soil samples taken

from the different sites of the

experimental fields.

Determination		Range of results of the different samples	Mean Values
pH		7.2 - 8.7	8.1
Calcium Carbonate	%	2.3 - 10.4	3.7
Organic matter	%	1.29- 2.38	1.63
Total Sol. Salts	%	0.08- 0.20	0.13
Total N content	%	0.08- 0.18	0.11
Soluble N in 1 % K_2SO_4 ppm		21.0 — 94.0	52.0
Nitrate N	ppm	3.0 - 36.0	15.0
Available - P in Soil :			
Olsen Method	ppm	4.0 - 28.0	16.0
Hibbard Method	ppm	0.1 - 4.4	1.8
Available - K	ppm	175 - 800	423

(Amm. Acetate extr.)

Relationship between soil test values and the response of bean

to N & P :-

Correlation coefficients were used to study the relation-
ship between the soil test values and the field results.   The
total soluble nitrogen content in the soil was correlated with
the N- index, which indicates average yield response of bean to
N fertilizer treatments.   Amounts of available P in soil samples
were also correlated with P-index which indicates average yield
response to phosphorus manured treatments.   In other words, %
yield was taken as an index for evaluating the validity of soil
test values.   The % yield was calculated according to the follow-
ing equation :

$$\% \text{ Yield} = \frac{\text{Yield without the tested nutrient}}{\text{Yield with tested nutrient.}} \times 100 \ .$$

The obtained statistical correlations show the followings :

1)- Nitrogen correlations :-

As indicated from the results presented in table (8) the %
yield values showed that bean plant in 20 out of the 25 field
experiments were positively responsive to N fertilization.
However no significant correlation was obtained between N-index
and the soil test values for soluble N extracted by 1 % K_2SO_4
solution.   Thus, other chemical methods for estimating the availa-
ble N in soil should be tried in this respect.

2)- Phosphorus correlations :-

The available phosphorus content in the soil samples as
determined by Olsen's et al. (1954) and Hibbard's (1931) methods-
was correlated with P- index.   It is obvious from the results

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Table (8)- Correlation between response of horse-bean
to N & P fertilization and Soil testing values.

No.	Locality	N-index	Available N ppm	P-index	Available P-Olsen ppm p	Available P Hibbard ppm p
1	Behera	63	25	52	4	3.4
2	Kafr ElSheik	95	33	97	20	1.1
3	Garbia	85	63	88	20	0.8
4	Garbia	85	63	93	13	0.8
5	Garbia	76	22	66	27	1.7
6	Garbia	96	33	101	21	0.4
7	Garbia	82	—	81	—	—
8	Garbia	82	60	93	16	2.7
9	Kalubia	97	75	97	13	4.4
10	Kalubia	99	—	101	—	—
11	Monofia	88	—	71	—	—
12	Monofia	98	34	92	6	1.5
13	Monofia	84	75	88	18	2.5
14	Sharkia	89	—	90	5	1.2
15	Giza	84	65	71	27	3.9
16	Giza	101	—	99	—	—
17	Beni-Suef	75	94	60	28	2.7
18	Fayoum	105	40	94	7	0.7
19	Fayoum	94	28	34	25	3.1
20	Minia	77	57	86	14	0.1
21	Minia	103	49	115	7	0.9
22	Minia	100	65	72	13	1.5
23	Minia	109	33	102	28	0.9
24	Souhage	91	70	105	14	2.4
25	Souhage	79	—	74	—	—
d.f.			17		18	17
r			- 0.15		- 0.30	- 0.39

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presented in table (8), that about 80 % of the soils gave positive response to P fertilization while 20 % was not responsive. The correlation coefficients between P-index and amounts of available phosphorus determined by the two chemical methods were also found to be insignificant indicating a real need for a concentrated research effort on soil testing methods.

E- Summary and Conclusion :-
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A long term study was carried out through out the country to check upon the response of bean plant (Vicia Faba) to N, P & K .

Two series of experiments were conducted. The first series was a preliminary study to explore the major nutrient elements which are lacking in Egyptian soils for bean production. It included 15 field trials accomplished through the period (1959/60 - 1962/63).

In the light of the data obtained from the first series, the manurial treatments of the second series were planned to have more information about the response of this particular crop to different rates of N & P and the interaction between the two nutrient elements when they are applied in different combinations. In addition , the relationship between the soil test values and the response of bean plant to N & P was examined. 25 field trials were conducted over a five year period (1966/67 - 1970/71). Composite soil samples were brought from each experimental site for soil description and chemical analyses to asses their fertility status. Available nitrogen was extracted by 1 % K_2SO_4 solution and available phosphorus was determined by Olsen's and Hibbard's methods. Correlation coefficients were used to study the relation-ship between the soil test values and the % yields.

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The main findings of the two series could be summarized in the following :-

1. Bean plant (Vicia Faba) did respond significantly to N application in different rates. Increase in yield due to N application ranged between 10 - 12 % over that obtained from the check treatment. However, no significant difference was detected between the application of 18 and 36 Kg N/ha.

2. The general magnitude of P response by bean plant was rather high. Yield increased gradually and significantly as the rate of P application increased up to 72 Kg P_2O_5/ha. Increase in yield due to P application ranged between 11.7 and 19.8 % over that obtained from the control for 36 and 72 Kg P_2O_5 /ha. respectively.

3. The maximum yield was obtained from the combination of N & P at the rate of 36 Kg N and 72 Kg P_2O_5 per hectar. Increase in yield due to the application of one kg of N under such treatment was estimated on the average by 7.4 Kgs bean seeds. For one kg of P_2O_5, the increase in yield was 7.0 Kgs of bean seeds. Such increases in yield secure a very good return.

4. No significant effect was detected due to K, indicating that bean plant does not require any application of potash.

5. N-and P- index studies revealed that about 80 % of the field trials did respond positively to N and P. The rest did not give any response .

6. The responses of bean plant to N & P applications were not well correlated with the soil test values indicating a real need for a concentrated research effort on the soil testing methods.

R e f e r e n c e s

1. Department of Agricultural Economy and Statistic   1971
      Per Sonal Contact Min. Of. Agric. Cairo

2. Erdman, L. W.   1953
      Legume inoculation; what it is, what it
      does.
      U.S.D.A. Bull. 2003.

3. Gracie, D. S. and Khalil   1948
      The total and available phosphoric acid
      in Egyptian soils and the effect of super-
      phosphate on the main Agric. Crops. Min.
      Agric. Bull no. 251 Cairo.

4. Hamissa, M. R. and Eid, M. T.   1969
      Maximaum Fertilizer Limits For Cotton ,
      Maize, wheat and Rice. Abs. J.El-Felaha, 49,
      U.A .R.   (In arabic).

5. Hibbard, P. L.·   1931
      Chemical methods for estimating the availa-
      bility of soil phosphorus. Soil (Sci., 31.
          : 437 - 467 .

6. Jackson, M. L.   1958
      Soil Chemical analysis; Prentice
      Hall, Inc. Englewood Cliffs, N. J.

7. Koeing, J.   1930
      Die Frmittlung des Dunger bedurfnisses
      des Bodens, P. Parey, Berlin. (Afler Allam).

8. Plant Nutrition Section   (1971)
      Experimental Results
      Data partially unpublished, Min. Agric.
      Cairo.

9. Olsen, S. R. et al.   1954
      Estimation of available phosphorus in soils by
      extraction with sodium bicarbonate, U.S. Dept.
      Agric. Circ. 939 .

128

10. Serry, A and El Banna, E.   1962
         A study of correlation between rapid soil
         tests and response of ground nut to K
         application of some Sharkia sandy soils in

         pot experiments. Agric. Res. Rev. Min. of
         Agric. Cairo, 4 no. 4 (40 - 48).

11. Shalaby, A.   1950
         Scientific Research results as means for
         improving agricultural production in Egypt
         Egyptian chemical Soc. 2 nd annual meeting
         Cairo.

12. Taha. S. M., Zaki, and Mahmoud, S.   1968
         Inoculation of Leguminous crops with
         rhizobia.
         El-Felaha, 5, 34 Cairo (In Arabic).

13. Waksman, S. A. (1932) Principles of Soil Microbiology
     Williams and Wilkins, Baltemore, Md.

II- The Second Topic

The Comparative Effect of The Soil And

Foliar Application of P. on Bean Plant (Vicia Faba)

A- Introduction :-

During the recent years, there has been a surge of
interest in the spray application of fertilizer to the foliage
of plants as a means of feeding plants the major elements ,
particularly phosphorus.

Much research has been done throughout the world on
this subject, but very little studies were carried out under
local conditions.

As it is Known that P when applied to the soil undergoes
several reactions and may become bound to the soil complex
resulting in reducing its availability for the plant. Thus,
it is thought that foliar application may provide more effici-
ent utilization of this particular element. On this concept
the investigation was based . Research on this subject is
still under way tackling several angles, such as the utiliza-
tion percentage of the P applied to the plant, the time of
spraying and the concentrations of the solution to be used on
different crops.

The present work will deal only with the increase in
yield due to foliar application in comparison with soil
application.

B- Experimental Work :-

Three field trials were conducted in different location to compare the foliar application of fertilizer phosphorus with soil application.

As shown in table (4) each experiment was consisted of seven treatments including P in two rates namely 18 and 36 Kg P_2O_5 /ha. Each rate was subjected to different methods of application. The experiment was also included a control treatment to check upon the response to P. in general .

P was applied in the form of monocalcium phosphate. Soil application of P was applied preplanting, while the foliar application was splitted into three equal doses to be sprayed three times namely; after one, $1\frac{1}{2}$ and two months after planting. The concentration of the phosphatic solution was 2 % .

The experimental set up was designed in randomized complete blocks in four replications. A representative surface soil sample to the depth of one foot was taken from each experimental site for chemical analysis and soil description.

C- Results and Discussion :-

1. Soil Description :-

Average data of the chemical identifications of the soil samples used in the study are presented in table (1). In general, it could be noted from the background data of the soil analysis that the soils tested are slightly alkaline in reaction and contain ample amounts of calcium carbonate. Soil organic matter content is rather poor. A fairly low concentration of the water-soluble salts prevailed in the

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soil samples. Available nitrogen - extracted by 1 % K_2SO_4
solution is 55 ppm. Available P. determined by Olsen method
is 3.4 ppm.

Table (1)

Background data on soil analysis (Depth: 0-25 cm)

pH	O.M	$CaCO_3$	E.C	Total	Available N K P in ppm		
	%	%	M.M	N %	N T.Soluble $1\% K_2SO_4$	No 3	P Olsen
8.0	1.6	3.2	0.40	0.088	55	10.5	3.4

2. Yield Data :-

The experimental data are summarized and grouped under the
following headings .

a. Response of bean plant to P.

b. Comparison between soil and foliar application of

fertilizer P.

c. The various combinations of the fertilizer treatments.

a. Response of bean plant to P.

Yields obtained from the control treatment, 18 and 36 kg
P_2O_5 / ha. were grouped - regardless the methods used for appli-
cation - and presented in table (2). As demonestrated from
the data reported, bean plant responded markedly to P applied
in different rates. Average yield in the check treatment was
2827 Kg /ha; with 18 Kg P_2O_5/ha. it was 3387; and with 36 Kg
P_2O_5/ha. it was 3711. Increase in yield due to P. application

amounted to 19.8 % and 31.3 % when 18 & 36 Kg P_2O_5 /ha. were applied respectively. This fact showed that the results are valuable to discuss the effect of P application on Yield when applied by different methods.

Again the data obtained, in general, coincided with the results of the series of experiments previously discussed under the first topic.

Table (2)

Response of bean plant to P (average of 3 field trials)

Kgs P_2O_5/ha.	Yield Kg / ha	Increase over control Kg /ha.	% Increase
0	2827	—	—
18	3387	560	19.8
36	3711	884	31.3

b- Comparison between soil and foliar application of fertilizer P.

Data reported in table (3) represent the average yields obtained from the different methods used for P application - regardless the rates of fertilizer application used. Considering the general average yield of the soil application as loo, the relative value when half of the amount of P was applied as foliar and the rest as soil application was 109. The yield was more pronounced when the whole amount of P was applied as

foliar spray, as the relative value in this case was found to
be 117 indicating a higher efficiency of the phosphorus when
applied as a foliar application to bean plant. Such result
was in close agreement with the findings of (Egorov (1957) ,
Wittwer (1957), Last & Nour (1961), Kabish (1965) & Abdou (1968)
who pointed out the superiority of the foliar spray of fertilizer
P to the soil application.

Table (3)

Comparison between soil and foliar application of
fertilizer P (Average results of 3 expts.).

Treatment	Yield Kg/ha.	Relative yield
Soil application	3268	100
½ Soil + ½ foliar	3568	109
Foliar application.	3810	117

c- The various combinations of the fertilizer treatments:-

Data reported in table (4) indicate the average yields
of various combinations of the rates of P when applied by
different methods.   The data show conclusively that under
each rate of P application, the foliar method over-yielded the
soil method. When the amount of P was devided into two halves
to be equally applied as soil and foliar application, thes yield
in this case was in between those obtained from the two methods.

The most interresting result which could be detected from
the data given in table (4) is that 18 Kg P_2O_5/ha. applied   as

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foliar application did produce the same yield, but little more than that obtained from 36 kg P_2O_5/ha. added as soil application. In other words, foliar application had increased the relative effectiveness of the P unit.

Table (4)

Various combinations of rates and method of P application

(Average results of 3 expts.).

Method of application	P_2O_5 Kg/ha.	Yield Kg/ha.	Increase over control Kg/ha.	% Increase
0	0	2827	---	---
Soil	18	3099	272	9.6
Application	36	3435	608	21.5
½ Soil +	18	3391	564	20.7
½ Foliar	36	3746	919	32.5
Foliar	18	3666	839	29.7
Application	36	3954	1127	39.9

D- Summary and Conclusion :-

Three field trials were conducted in different locations to compare the foliar application of fertilizer P with soil application. Each experiment was consisted of seven treatments including P in two rates namely; 18 and 36 Kg P$_2$O$_5$ /ha. Each rate was subjected to different methods of applications. The experiment was also included a control treatment to check upon the response to P in general.

P was applied in the form of monocalcium phosphate. Soil application of P was applied preplanting, while the foliar application was splitted into three equal doses to be sprayed three times namely; after one, 1½ and two months after planting. The concentration of the phosphatic solution was 2 % .

The experimental set up was designed in randomized complete blocks in four replications. A representative surface soil sample to the depth of one foot was taken from each experiment for chemical analysis and soil description.

The following are the most important results :

1. Bean plant did respond markedly to P application. The yield increased gradually as the rate increased. % increase in yield over over that obtained from the check treatment amounted to 19.8 & 31.3 when 18 and 36 Kg P$_2$O$_5$ / ha. were applied respectively.

2. Foliar application of P to bean plant was markedly superior to the soil application. 18 Kg P$_2$O$_5$/ha. applied as foliar application yielded little more than 36 KgP$_2$O$_5$/ha. did when applied as soil application.

R e f e r e n c e s : -

Abdou, E. K.   1968

   Comparison between normal and foliar application
   of fertilizers using P^{32} as a tracer technique.
   M.Sc. Thesis, Faculty of Agric., Cairo Univ.

Egorov, A. V.   1957

   Foliar absorption of phosphorus by plants.
   Chemical Abstracts (1959), 53 : 632 .

Kabesh, M. O.   1965

   Effect of phosphorus fertilization by soil or
   foliar application on the yield and chemical
   composition of broadbean.
   M.Sc. Thesis, Faculty of Agric., Cairo. Univ.

Last, T. F., and Nour, M. A. 1961

   Cultivation of Vicia faba in Northern Sudan.
   The Empire J. of Experimental Agric., 29 : 60

Wittwer , S. H... 1957

   Nutrient uptake with special reference to foliar
   absorption.
   "Atomic Energy & Agric." McGraw- Hill Book Co.,
   36 N.Y. USA.

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III - The Third Topic[*]

Effect of Salinity on Nodule Formation ,Yield,

N & P Uptake by Some Legume Crops.

A- Introduction :-

The need to provide additional land for farming, in order to feed ever increasing populations in Egypt has intensified the interest of the agriculturists and the goverment planning authorities in various types of unproductive land. Among these types are the saline soils which comprise vast areas in the Nile Valley particularly in the northern part of the Delta. Such areas are soils on which most crops cannot make normal growth owing to the presence of soluble salts in the soil solution in high concentration.

It was the target of this investigation to study the effect of reclamation of saline soils on nodule formation, yield, nitrogen and phosphorus uptake by two Leguminous plants namely, bean plant and cowpeas.

B- Materials & Methods :-

The effect of salinity and soil reclamation on nodule formation, yield, N and P content of two Leguminous crops namely: bean plant (Vicia Faba) and cow peas (Vigna Sinensis), was studied in a greenhouse pot experiment.

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[*] This topic was done with collaboration of Dr. M. Kamel & Dr. M.N. Ibrahim of the Fac. of Agric. El-Azhar Univ. and M.S. Kadr plant Nutrition Section Min. of Agriculture.

The physical & Chemical properties of the soil used were determined using the official methods reported by (Jackson 1958).

The toxic salts were Leached by tap water. Leaching was done by flooding the soil each week after which, it was left to natural drainage.

Soil was divided to five parts where the first part was left without leaching, the second part was Leached once, the third, the 4th and the 5th parts were Leached twice, three & 4 times respectively.

Earthenware pots measured 25 cm in diameter were used, each pot received 5 kgs of soil and replicated for 4 times.

Bean plant and cow peas seeds were planted as legume test plants. Inoculation was carried out at planting time. Two weeks later, seedlings were thinned to 3 plants / pot.

Fresh, dry yield, N & P contents of the plants were estimated using the analytical methods reported by piper 1947 & Jackson 1958).

C- Results & Discussion :-
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1. Definition of the soil sample :-
_____

The physical and chemical composition of the soil used for the investigation are presented in table (1) . The data indicate that the soil is a clay soil having 50 % & 13.5 % clay and silt particles respectively. It is poor in O.M. but very rich in exchangeable K. The concentration of available N & P was rather high. As for the total soluble salts,

5 % could be considered as a high toxic level of salt content under which no or very poor growth of plants should be expected.

Table (1)

Analyses of the soil sample used in the study

Analyses	Contents	Analyses	Contents
Texture		pH value	7.8
Coarse sand	0.73 %	Total N	0.072 %
Fine sand	20.90 %	Total P	.124 %
Silt	18.5 %	Soluble N (1% K_2SO_4)	100 ppm.
Clay	50.0 %	Available P (Olsen)	33.9 ppm.
Calcium carbonate	2.81 %	Exchangeable	920 ppm.
Organic Matter	1.17	K (Amm. acetate)	
Total Soluble Salts	5.20		

2- Effect of Leaching on salt content & pH value of the soil :-

Data reported in table (2) indicate that Leaching of the soil markedly decreased the amounts of toxic salts. In both experiments, the maximum amount of salt were removed after the first Leaching; There-after a gradual decrease in the salt content was noticed. However in the bean plant experiment , the drop in the salt content as a result of Ieaching was greater than that in the cow peas experiment. On the other hand pH values showed a gradual increase as a result of Ieaching of the toxic salts. Similar results were recorded by Taha, Mahmoud & Ibrahim (1967),.

Table (2)

Effect of leaching on salt content and pH value

Number of	Bean plant Expt.		Cowpeas Expt.	
Leachings	T.S.S.[x] %	pH	T.S.S. %	pH
—	5.2	7.8	5.2	7.8
I	0.98	8.0	2.13	7.95
II	0.60	8.0	1.98	8.00
III	0.23	8.15	1.46	8.00
IV	0.20	8.35	1.04	8.00

[x] T.S.S. = Total soluble salts.

3- Effect of Leaching on the number of nodules of bean plant and cowpeas plants :-

As indicated in table (3) number of nodules was significantly affected by Leaching. The more the number of Leachings the higher counts of nodules in both crops. It is also noted that the number of nodules on the bean plant roots was higher than that formed on the cowpeas which showed no nodules when the soil was not treated.

Table (3)- Effect of Leaching on the number of nodules of bean plant & cowpeas plant

Number of leachings	Bean plant		Cowpeas	
	T.S.S. %	Number of nodules	T.S.S. %	Number of nodules
—	5.2	26	5.2	0
I	.98	133	2.13	12
II	.60	133	1.98	39
III	.23	176	1.46	72
IV	.20	151	1.04	93
L.S.D. 5 %		81		36.3

Table (4)

Effect of leaching on the dry matter of the broad beans

& Cow peas in g. / pot.

Number of Leachings	Bean Plant					Cow Peas				
	Seeds	Seed Coats	Leaves & Stems	Roots	Total	Seeds	Seed Coats	Leaves & Stems	Roots	Total
—	13.7	2.91	5.58	1.66	23.85	—	—	—	—	—
I	36.9	8.86	12.83	4.78	63.36	1.6	0.3	4.2	1.1	7.2
II	36.7	9.38	13.43	6.97	65.05	3.7	1.4	10.6	3.1	18.8
III	41.6	9.98	15.14	6.79	73.48	4.1	1.1	11.3	3.0	19.5
IV	42.1	9.69	12.65	5.97	70.35	8.7	2.4	10.0	2.8	23.9
L.S.D. 5 %					28.27	2.4	0.69	5.55	1.69	9.04

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4. **Effect of Leaching on the yields of bean plant and cow-**
    peas.

The effect of leaching of the toxic salts on the dry
matter yield of bean plant and cow peas is presented in table
(4).   Leaching of toxic salts significantly increased the
yield of the beans and cowpeas.

For beans the average dry weight of seeds were found
to be 13.70 g./pot in the saline soil and increased up to
36.9, 36.70, 41.60 and 42.70 g./pot as a result of 1, 2, 3 &
4 leachings respectively.   The same trend was recorded for
the different plant parts.

Similar trend was also noticed in cow peas results.
However, the data clearly show that bean plant was more tolerant
to the toxic salts than cowpeas which failed to grow under
original salt content of the soil.   Such results were in close
agreement ^with^ the findings of Bernstein (1958) & Ayers and Eberhard
(1960).

It was also noticed that one leaching for the bean plant
and two leachings for the cowpeas had almost the same effect
of more leachings without any significant difference.

5. **Effect of leaching on the N & P contents of bean plant**
    & cow peas :-

Results of N & P contents in bean plants show & cow-
peas plants which are recorded in table (5) show the same
trend of yield results.   Leaching the toxic salts significan-
tly increased the N & P content in the plants.   For N., of

coarse, no doubt that a part of the utilized N came from the symbiotic N fixation by the increasing number of nodules as a result of leaching the toxic salts.

It is also noticed that the amount of nitrogen utilized by bean plant plants were much higher than that utilized by cow peas plants denoting the great feeding power and the higher nutritional requirements of the former crop.

Table (5) - Effect of leaching on the N & P contents
of bean plant & Cow peas.

Number of leaching	M.g. N & P / pot.			
	bean plant		cow peas	
	N	P	N	P
---	712.3	73.8	-	-
I	1567.4	185.4	158.8	22.6
II	1600.1	181.7	362.2	59.1
III	1948.5	169.0	402.6	57.9
IV	1780.9	156.65	528.6	59.9
L.S.D. 5 %	807.3		180.3	23.2

D- Summary & Conclusion :-

The effect of reclamation of saline soil on the nodule formation, yield, N & P content of two leguminous crops namely, bean plant (Vicia Faba) and cow peas (Vigna Sinensis) was studied in a green house pot expt.

The physical and chemical composition of the saline soil used were estimated.

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The toxic salts were leached by tap water.   Soil sample
was divided to five parts, where a part was left without leach-
ing, leached once, Twice, three and four times respectively.
Bean plant and cow peas were planted in the soil, where inocu-
lation was carried out with effective rhizobial cultures.
Results could be summarized in the following.

1. Reclamation of the saline soil significantly decreased the
   total soluble salts.   This however was accompanied by an
   increase in pH values.

2. Number of nodules showed a significant increase as a result
   of leaching of the toxic salts.

3. Yields of bean plant & cow peas were significantly increased
   by leaching the toxic salts.

   However, bean plant has shown to be more salt tolerant
   than cow peas.

4. N & P contents of bean plant & col peas plants were signifi-
   cantly increased as a result of leaching the toxic salts in
   the soil.

5. Amounts of N & P utilized by bean plant were much higher
   than those utilized by cow peas.

R e f e r e n c e s :-

Ayers, A .D.; Eberhard, D. L.   1960
     Response of ediable broad bean to several levels
     of salinity.
     Agron. J., 52 : 110.

Bernstein, L.   1958
        Salt tolerance of grasses and forage legumes.
        Washington U.S. Gov. Print. Off. Agric. Inform.
        Bull. 194 .

Jackson, M.L.   1958
        Soil chemical analysis.
        Prentice-Hall, Inc. Englewood Cliffs, N.J.

Piper, C.S.   1947
        Soil and plant analysis.
        The university of Adelaide, Australia.

Taha, S. M.; Mahmoud, S.A. Z.;   Ibrahim, A. N.   1967
        Chemical and microbiological effects of the recl-
        mation of a saline soil in the U.A.R. g : 1/2*
        J. Microbiol. U.A.R. 2: 151.

BEAN FERTILIZATION IN CENTRAL AND SOUTH AMERICA

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Abstract

Beans and other pulses are very important as cheap and high quality protein sources for over 80 % of the population of Latin America. If adequate varieties were used, in most instances, fertilizers containing N and P have increased appropriately and economically the production in volume but not in quality. Many problems remain however, to be solved, the solution of which could contribute and to a better nutrition as to a better economy of the rural population of a significant part of the region.

General considerations

The main grain legume crop of South and Central America is beans (Phaseolus vulgaris L.). In accordance to archeological evidence, this crop is used as food for many millenia (8). It's widely diffuse consume has various explanations, as the antiquity of its cultivation, its great adaptability to variable climatic and soil conditions and the fact that it is the cheapest high quality protein which quite adequately complements the main vegetable proteins derived from rice for about one half of the population of Latin America and the proteins from corn, used by about one third of the population.

As a result, large areas are cultivated to beans. Other pulses as broad beans (Vicia faba L.) and peas (Pisum sativum L.) are also of some importance in parts of the area. The extension of the land used

for these crops in some countries forms an important percentage of the total cultivated area, as for ex. in Perú where approximately 7,5% of the agriculturally used land is planted to these crops. Colombia also has an area exceeding half a million hectares in beans.

Soybeans (Glycine max L.) and peanuts (Arachis hypogaca L.) are also growing in importance in Latin America are important crops.

In spite that many areas have the adequate ecological conditions, the average production figures for beans are low, as shown in Table 1, which presents some representative average results.

It is considered that these low productivities, shown in Table 1 are generally caused by low yielding varieties, not responsive to fertilizer application (17), the damages caused by diseases and pests and by low soil fertility. Many experiments have shown that an adequate control of one of these limiting factors can result in increases of production in order of 50 to 100% and that a control of all main limiting conditions allows yield increases up to 300 to 400% (6, 11, 20).

It is also reported, that in spite that in many conditions it is economically advisable to use fertilizers, even in areas of rather progressive agriculture, only a fraction of the bean growers use fertilizers. The Cauca valley of Colombia can be cited as an example, where in accordance to recent studies only one third of the bean produced receives fertilizers.

The present paper presents a selection of experiments, hoping to illustrate the problems of the region. This is not a complete literature review for which the Bean Bibliography, compiled in the library of the Inter-American Institute of Agricultural Sciences should be consulted (15).

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As most of the literature is concerned with responses to N, P and K, these elements will receive special attention while the problems of liming and application of minor elements will only be considered briefly.

Fertilization with phosphorus

As P deficiency is widespread in Central and in South America, responses of beans and other grain legums to this element are common (1, 2, 3, 5, 6, 7, 10, 12, 13, 15, 19, 20). In some instances, these responses where small, a fact which various authors attribute to the inability of the used varieties to respond well to fertilizers (3, 13, 17).

It is believed also, that a lack of more complete information, obtainable by more sofisticated investigations, as for example tracer experiments on fertilizer placement or fertilizer form evaluation, could shed light on many poorly understood problems, permiting a more adequate use of fertilizers and of the natural resources present.

In some experiments, the lack of response to P is due to the residual effect of the fertilizers applied previously. This was reported for many experiments in Central America by Lizarraga (10) who noticed a favorable response of beans to P applied to the previous corn crop. As this rotation is rather common, it contributes appreciably to a better use of fertilizer P. The effect of P application on the protein content and protein quality of beans was investigated by Bressani (5) who found little influence of the fertilization on these characteristics. Only a few authors have evaluated economically their results (6, 10). In an interesting paper, Conagin has shown the economic soundness to apply variable P amounts to all studied soils of São Paulo (6). The economic optima indicated varied from 46 to 138 kg P_2O_5 per ha in

accordance to soil type (1). Evaluating 103 experiments with 45 or 90 kg/ha of P_2O_5 with or without other nutrients, Lizarraga obtained the results shown on Table 2. These data resumed from the FAO fertilizer experiments show that for Costa Rica and Guatemala the results for P alone or as part of a complete fertilizer are encouraging and economically sound, allowing substantial gains, particularly in the case of the use of NPK. The margin for El Salvador is quite narrow and the results for Honduras were not included.

It is considered, that better understanding of the conditions in Honduras and El Salvador could have indicated ways for an economical use of P, as many of the soils of these countries are known to responde to it (2). The results from Perú indicate economical optima between 40 and 80 kg of P_2O_5/ha for most of the studied areas, in accordance of the P availability of the corresponding soils.

In Colombia on volcanic soils up to 90 kg/ha of P_2O_5 is reported as adequate (11).

While in most experiments triple superphosphate is suggested the peruvian results indicate simple superphosphate as most adequate. The high S content of the material might be the explanation.

Generally, it is estimated that most soils where beans and other pulses are grown in the area respond well to P and that the correct use of the element represents an appreciable economic gain for the growers under most conditions.

Fertilization with potassium

Responses of beans to K in Latin America are less common than to N or P, in accordance to Vieira (20). Lack of response to applied K is quite commonly reported (3, 13, 15, 19).

The positive responses usually occure in case of higher P applications as shown by the work of Lizarraga (10) and Conagin (6). The positive responses are more common or the strongly acid soils developed in the wet areas (7) and these soils, usually respond to complete fertilizers as in São Paulo (6).

Experiments in Peru indicate the necessity of K application in about one third of the studied locations, even on some soils with pH above 7. It is believed that this is particularly important if the amounts of N applied are rather high.

Considering the important crop losses due to diseases and the known effect of K on improving disease resistance, this aspect of bean fertilization would deserve a larger attention.

Generally, if N and P are applied, K is used also, in part as a preventive practice, in part due to the general availability of NPK containing fertilizers. It is believed, that with the intensification of agriculture K needs will become more common and should receive attention accordingly.

There are also reports indicating a harmful effect of K application (15).

Fertilization with nitrogen

In spite that beans as N fixers can contribut! positively to the N balance of soils, favorable responses to this element are very common in Latin America (20).* Vieira estimates an average need of 40 kg/ha for the region and explains it as caused by problems in N fixation. The positive responses reported (6, 7, 9, 10, 11, 12, 14, 15, 20) are

*Virgin soils represent an important group well provided with initial N, which do not respond to N (3).

widespread and when no responses are reported, many authors consider that this is caused by varieties unresponsive to fertilizers.

By far, too little is known on the microbiological aspects but a few authors report no responses to inoculation (5, 20) which clearly indicates the need of additional work.

Studying the amounts of N to which positive responses are obtained, one can observe that these amounts are quite high, far above the quantities considered or "starter nitrogen," recommended by many to overcome a potential low N status of the soil, prior to the contribution of the N fixed by the bean Rhyzobia. The only area where the N recommendations are low, of the starter type, is São Paulo State, where Conagin suggests 9, 12 and 35 kg/ha of N as economic optima for different soils (6). On the contrary, the amounts to which positive results where noticed in Central America were of the order of 45 to 90 kg/ha (15). For Colombia similar amounts were suggested (11, 14), and the data from Peru agree with those of Central America. There is little work on different N sources but no differences were reported so far.

As it is well known that nitrogen fixation decreases with increasing amounts of fertilizer N applied, one should not overdue the application of N as this represents a loss of valuable fixable material. However, as little is known on nitrogen fixation under the variable conditions of Latin America, more work is required in the area to secure the optimum use of this capacity of the plant.

Due to the high price of N in fertilizers the value/cost ratios for N alone are generally lower than for P_2O_5 as shown in Table 2, except for Guatemala where the low available N status of the soils makes N application very profitable.

It is believed, that at least moderate N amounts will be needed for successful bean production even if it will be possible to increase to efficiency of fixation by better understanding of it in Latin America conditions.

Liming and minor elements

For nodule formation adequate calcium levels are needed and some Rhizobium strains are adversely affected by already medium soil acidity while others are quite tollerant. As liming also influences the availability of most nutrients, it was considered in various experiments in the area. In Honduras, at the Panamerican School of Agriculture, Awan reported over 50% bean yield increase by liming, attributing the favorable response mainly to an increase in P availability (2). For the volcanic soil area of Colombia, Parra (13) reported positive results for low levels of liming (2 - t/ha) but negative ones for medium and high levels (over 4 - t/ha) so for field beans as for peanuts. Peanut production was also economically increased on "campo cerrado" soils of Central Brazil, particularly if dolomitic limestonewas applied with complete fertilizer, as reported by Mikkelsen et al (12).

It is believed that quite a lot more information is needed on liming, considering it from the point of view of pH as affecting Rhizobium development of the effect of the practice on Ca and eventually Mg levels, and considering the influence of it on the general availability of the nutrients present.

Magnesium deficiency was reported and successfully corrected by soil application (4) of the element. The same paper presents evidence showing the interactions between different minor elements indicating the necessity of rather complete studies if an understanding of the phenomena observed is desired.

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In the Zn deficient "Bajio" region of Mexico, Schmidt (18) obtained excellent results when $ZnSO_4$ was applied to the soils or Zn chelates was applied as sprays. It was observed, that response to mayor nutrient application was almost nil unless the Zn deficiency was also corrected. This observation might be extrapolated concluding that possibly many variable responses, as found for example in Peru to applied mayor elements, could be explained by variable levels of minor elements present, which limit the use of the other nutrients. The need of minor elements including Zn, was shown also for the "campos cerrados" area of Brazil (12) also and for the bean area in São Paulo, in the came county (20).

Table 1. Average bean production for different areas of Latin America

Area	Average production kg/ha	Authors	Period
São Paulo Brazil	408	Conagin	1957 - 62
Colombia	379 - 600	Lopera & Hildebrandt	
Peru	967	Estad.Agric.	1969
South America	Aprox. 500	Vieira	

Table 2. Net gains (in US$) and value/cost (v/c) ratios from bean fertilizer experiments (10).

Country	Treatment N		P₂O₅		N P K	
	v/c	gain	v/c	gain	v/c	gain
Costa Rica	1.7	30.6	2.6	83.6	2.5	102.7
El Salvador	1.8	32.3	1.3	40.9	1.4	59.0
Guatemala	3.4	61.8	2.3	71.5	2.7	108.0
Honduras	1.2	39.2	-	-	-	-

REFERENCE

(1) ACUÑA, E.J., SANCHEZ, P.C. Fertilité no. 35, Nov.-Dec. 1969, 3.

(2) AWAN, A.B. Soil Sci. Soc. Amer. Proc. 28 (1964) 672.

(3) BARRIOS, A., RODRIGUEZ B., A., ORTEGA, S., PEREZ S.,S. Agron. Trop. (Venezuela) 20 (1970) 355.

(4) BERRIOS, L., BERGMAN, E.I. Proc. Trop. Reg. Amer. Soc. Hort. Sci. 15th, Boquete, Panamá 1967. 151.

(5) BRESSANI, R. Reun. An. Progr. Coop. Centroam. Mejoramiento de Cultivos Alimen. 13a, S. José, Costa Rica, 1967. 42.

(6) CONAGIN, A. En: Investigación Económica y Experimentación Agrícola, Montevideo, IICA-Zona Sur 1967, 143.

(7) FASSBENDER, H. W. Turrialba 17 (1967) 46.

(8) KAPLAN, L. Econ. Bot. 19 (1965) 358.

(9) LAWTON, K., PATIÑO, E. Soil Sci. Soc. Amer. Proc. 24 (1960) 202.

(10) LIZARRAGA, H. Reun. An. Progr. Coop. Centroam. para Mejoramiento de Cultivos Alimen. 12° Managua, Nicaragua, 1966, 51.

(11) LOPERA, P., HILDEBRANDT, P.E. La Brecha en la Productividad Agrícola en Colombia. ICA, Dept. Economía, Bol. Dept. No. 3, Agrícola, 1969, 22.

(12) MIKKELSEN, D.S., DE FREITAS, L.M.M., McKLUNG, A.C. IRI Res. Inst. Bol. 29. 1963, 40.

(13) MIRANDA, H., MASAYA, P. Reun. An. Progr. Coop. Centroam. para Mejoramiento de Cultivos Alimen. 15° San Salvador, El Salvador, 1969 44.

(14) PARRA, J. Cenicafé (Colombia) 20 (1969) 91.

(15) PAZ DE ERICKSON, A.M. Bibliografía Phaseolus spp. frijol Inst. Interam. Cien. Agric. (Turrialba, Costa Rica) 4 (1965) 347.

(16) RODRIGUEZ M., M., RODRIGUEZ M., L. Reun An. Progr. Coop. Centroam. para Mejoramiento de Cultivos Alimen. 13° San José, Costa Rica 1967, 47.

(17) SALAZAR, J.R. Reun. An. Progr. Coop. Centram. para Mejoramiento de Cultivos Alimen. 16° Antigua, Guatemala 1970, 42.

(18) SCHMIDT, W.A. Proc. Trop. Reg. Amer. Soc. Hort. Sci. 14h. México, México, 1963. 17.

(19) TAPIA, H. Reun. An. Progr. Coop. Centroam. para Mejoramiento de Cultivos Alimen. 11° Panamá, Rep. Panamaá, 1965, 91.

(20) VIEIRA, C. Act Reunión Latinom. de Fitotec. 6°, Lima, Perú, 1964, Vol. I. 229.

RESPONSE AND CURRENT FERTILIZATION PRACTICE OF
GRAIN LEGUME CROPS IN ROMANIA

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ABSTRACT

The increased importance of grain legume crops in Romania may be
asigned both to their high nutritive value and to the fact that the le-
gumes are good previous crops for small grains. Among the grain legumes
soybean is preponderent, followed by beans and peas.

Owing to specific biological traits of these plants, the experimen-
tal results obtained with classical methods in the field of fertiliza-
tion are contradictory and limited.

Field experiments were carried out in order to study some aspects
concerning the judicious use of N and P fertilizers. Labelled fertili-
zers with ^{15}N and ^{32}P were applied to soybeans – the main legume crop in
Romania – on a medium leached chernozeme, in the Fundulea Research
Institute for Cereals and Technical Plants.

First results obtained during 1970 emphasized the advantage of the
band and mixed applications of N and P fertilizers. This method increased
the efficiency of fertilizers, especially in case of P. N – fertilizers
proved to be most efficient when side-dressed during the growing season.
Application of N – fertilizers increased the protein content in soybean
grains.

The results obtained during 1971 are under study.

INTRODUCTION.

Estimated at world range,approximately one third of
the population of the developing countries is presently insuffi.
ciently provided with proteins[1][2].

Extension of legume crops and increase of their yield/ha
could widely contribute to meet world requirements in proteins.

Among legumes cropped on wider areas soybeans, peas
and beans should be mentioned.Particularly soybeans constitute
the main source of protein forage and also form the raw matter
for industrial products.Their use in human food - especially
by some peoples of Asia - should also be mentioned.

Soybean exceptional economic value incited the agricul-
turally advanced countries to confer great importance to these
crops so as to meet protein requiriments.

The fact that legumes symbiotically fix atmospheric
nitrogen,contributes to the raising of soil fertility,and en-
riches it with organic nitrogen[2][3][4][5][6][7].

Investigation are now under way in the great cropping
countries in order to raise production per hectare,by impro-
ving seed genetic potential and by improving cultural practices
[8]. Special attention should be conferred to fertilization
method. In this field results obtained in different countries
often are contradictory[9][10][11][12]. Some authors consider
that legumes do not require nitrogen fertilizers as thy can
meet their requirements through symbiosis with bacteria(Rhizobium)
Other researchers assert that legumes should be fertilized with
small nitrogen rates,acting as a " starter "[10][11][13][14].

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Use of high fertilizer rates,by fractioned application or by different incorporation methods (at various depths,on band,by side dressing at 5 cm from the seeds) [10] [11] [15] has been lately used.

Bacterial seed treatment with Rhizobium is compulsory when legumes are grown for the first time on a soil,or when the crop is again grown on a land after a long time [12] [16] [17] [18] [19] [20].

On poorly fertile (acid and cold) soils,fertilization with high nitrogen rates is often used alongside bacterial seed treatment.

DISCUSSION AND RESULTS.

In Romaniam the legumes most spread in crops are peas, beans and soybeans. Formerly peas and beans ware most frequently growen,but lately,soybean planted areas increased due to the importance of this crop.

In Europe,Romania hold the first place in soybean cropping [1] [14].

Fig.1 . shows the development of soybean cropped areas between 1930 and 1971.

Prospectively,the areas planted with legumes for grains is shown in table n°.1.

Simultaneously with cropped area increase,mean yield per ha also increased as a consequence of technology improvement in soybean crops from one year tb the other (Fig.2).

As compared to peas and beans,soybeans are more exacting regarding soil and climate.Soybean water consumption for the

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whole growth period is in the south of the country (Fundulea) of about 340 mm in non-irrigated crops and of about 650 mm in the irrigated crops.Highest consumption is recorded in July,at flower-budding - anthesis - grain formation lhenologic stages, i.e. of 4.0 mm/day in noh-irrigated and 6.7 mm/day in irrigated crops (Fig.no.3).

Water amount from rainfall is much lower than plant consumption during this period and this is why high soybean yields can be obtained only in on irrigation regime under these conditions.When analysing the origin of water used by soybeans and its effect on yield,it appears that 19 % of the water comes from soil supply,41 % from rainfall and 40 % from irrigation (Fig.4).

Considering the rather high environment requirements, three ecologic zones were differentiated in Romania in soybeans crops.In the highly favourable zone,in which yields of over 2000 kg/ha may be obtained most of the years,fertile soils are encountered and climate conditions are particularly favourable especially at fruiting period.Water supply of plants is provided by rainfall,water table or irrigation.These conditions are encountered in Moldavia,Transylvania and Banat and especially on meadow soils.

The favourable soil includes most of the Moldavia and of the Romanian Plain as well as zones close to the highly favourable zones of Transylvania, Banat and Crişana.In this zone it is necessary to compensate the limitative factors,e.g.

water (by irrigation) in case of the Bărăgan plain or soil fertility (by fertilization) - in case of the Sub-Carpathian zone.

Under non-irrigation soybean is cropped in a corn - wheat - soybean rotation,and under irrigation a 3 year rotation with 4 crops (soybean - wheat - stubble crop - corn), or a 4 year rotation with 5 crops,adding sugar beet to it.

The soil improving qualities of the plant are thus turned to profit,emphasizing its good features as previous plant, especially for wheat.

Unlike soybeans,peas and beans are grown in Romania in nearly all regions,giving exceptionally good results in the west of the country and poorer results in the pre-mountain region,in the north of Dobrogea and on sands. In many regions suitability is shown either by climate or by soil conditions. In rotation, these plants always precede winter wheat crops.

In order to increase yield per hectare in these crops, a vast research programme is carried out in Roumania under different pedoclimatic conditions,both concerning development of new varieties and improvement of cropping technology.

With respect to fertilizer use,the investigations emphasized [21] that legumes are generally little effected by both N and P fertilizers,particularly on fertile soils (table 2,3,4).

The experiments carried out in soybean crops under natural non-irrigated conditions,proved that in this crop fertilizers have a weak effect especially in zones with unfavourable rainfall regime. Under such conditions, yield is reduced as compared to the zones in wich natural moisture regime is better.

Thus for instance, at the Albota Experiment Station (Table no.5) mean yields in the Chipewa variety werw 18.9 q/ha and in the Violeta variety 21.2 q/ha,while at the Secuieni. Experiment Station,with a deficient rainfall regime,the same varieties avaraged a 14.3 q/ha(Chippewa) and a 12.9 q/ha (Violeta)yield respectively(Sarpe and co - workers 1971). In the same experiments yield gains rose to 5.4 q/ha in the Chippewa variety and to 7.9 q/ha in the Violeta variety at the Albota Station and to only 2.8 and o.6 q/ha in the 2 varieties at the Secuieni Station.

Favourable results emergend at the Tg. Mureş Experiment Station, sited in the flood plain of the Mureş River, in a zone mote favourable with respect to moisture regime and with a lower temperature in summer (table no.6). Under such conditions,yields ranged from 13.2 q/ha to 2o.o q/ha presenting thus a 6.8 q/ha rate.

Optimum nitrogen rates are of about 3o - 5o kg N active ingredient/ha. Rate exces may some times lead to harvest decrease, a phenomenon recorded in a series of experiments and proved by the results obtained at the Turda and Secuieni Experiment Station(Table no.5) The same was stressed in the south of the country, on a reddish - brown forest soil (table no.7), where fertilizer rates of $N_{96}P_{128}$ kg/ha avaraged a mean yield varying from 2o.1 q/ha,i.e that obtained in the $N_{48}P_{64}$ kg/ha tratment to 16.9 q/ha,this yield being close to control (16.4 q/ha).

Under irrigation,yields in soybean are much higher, reaching frequently 25 - 3o q/ha or even more.Thus in the experiments undertaken by Dorneanu and co-workers(197o) on a reddishbrown forest soil in the outskirts of Bucharest, the

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yields ranged according to variety and soil from 14.3 q/ha and 22.2 q/ha in non-irrigated crops and from 25.o q/ha and 33.5 q/ha in irrigated crops (Table no.7).Mean results (by varieties and years) indicate substantial gains as to the non-irrigated treatment both in the non-fertilized (26.7 q/ha as to 16.4 q/ha) and in the fertilized treatment (32.3 as to 2o.6 q/ha).

In experiments undertaken by Hulpoi and co-workers (1971) under varions domestic pedoclimatic conditions on irrigated soils mean yields by years and stations rose up to 23.7 q/haiin the steppe none and to 25.7 q/ha in the forest steppe (table no.8).Nitrogen fertilizers increased mean yield by 6.o q/ha,highest yieds (average by station)achieved being 8.1 q/ha.

From the analysed investigations as well as from other studies it apears that in the steppes,with high sumesr temperatures and a deficient rainfall regime,high and economic yield gains in soybean crops may be obtained only under irrigation.

Referring to fertilizer rates,we consider that they must not exceed 3o - 51 kg/ha when no irrigation is applied and 4o - 7o kg/ha under irrigation; phosphorus rate must be in good agreement with nitrogen,i.e.:1:1 or o,7:1 N:P in non-irrigated crops and 1:o,7 or 1:1 N:P under irrigation.

It is generally not recommended to use excessive nitrogen rates,especially when biological fertilization by bacterial seed treatment is used.(Table no.9).

The investigations undertaken by Bălan and co-workors with dofferent Rhizobium strains proved that in this way high yields simultaneously with yield gains coult be obtained(Fig.5), (table no.1o).

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From data shewn in table 1o it appears that certain
types of Rhizobium (SO-75) gave yield gains,as compared to those
with no bacterial seed treatment ranging from 5.o to 9.o q/ha.

However,from investigations started these last years
it appears that in order to exceed a certain yield level,
bacterial seed treatment alone is not sufficient and nitrogen
fertilizer should also be intrbduced.

From this viewpoint it appears that a ceatain advan-
tage would be presented by top dressing after bacterial symbio-
sis was estabilished.

A series of aspects connected to soybean fertilization,
such as: estabilishment of utilization coefficient of fertili-
zers according to application rate and method,interaction
between fertilizing elements and soil elements,etc.,cannot be
elucidated by help of classical methods.This is why , begining
with 197o,a series of trials were conducted at the Fundulea
Institute and it is tried to solve these problems by help of
fertilizers labelled with the ^{15}N and ^{32}P izotopes (table no.11).

Thus,it was found that different incorporation methods
affect the way in which soybean plants use fertilizers particu-
larlyduring early developmsnt stages (table no.12).During this
stage,application of nitrogen fertilizer broadcast proved
inferior to band application.Simultaneously with progress in
vegetation,the differenoes disappeared.

As distinguished from nitrogen uptake,phosphorus uptake
from fertilizers is fawoured fertilizers(table no.13).

In 1971,a new trial was conducted so as to investigate
interaction between fertilization and bacterial seed treatment.
The results are being prosessed (table no.14).

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Future investigations should be channeled so as to solve certain matters correlated to fertilizer efficiency under intensive cropping.A basic field experimental programme to study the effects and interactions of innoculant,nitrogen and phospherus fertilizer should be taken into consideration.The most important problems which can be studied in a coordonated programme by using labelled fertilizers with stable and radioactive isotopes are rates,time,method of application and source of nitrogen and phosphomus under irrigated and non-irrigated conditions.
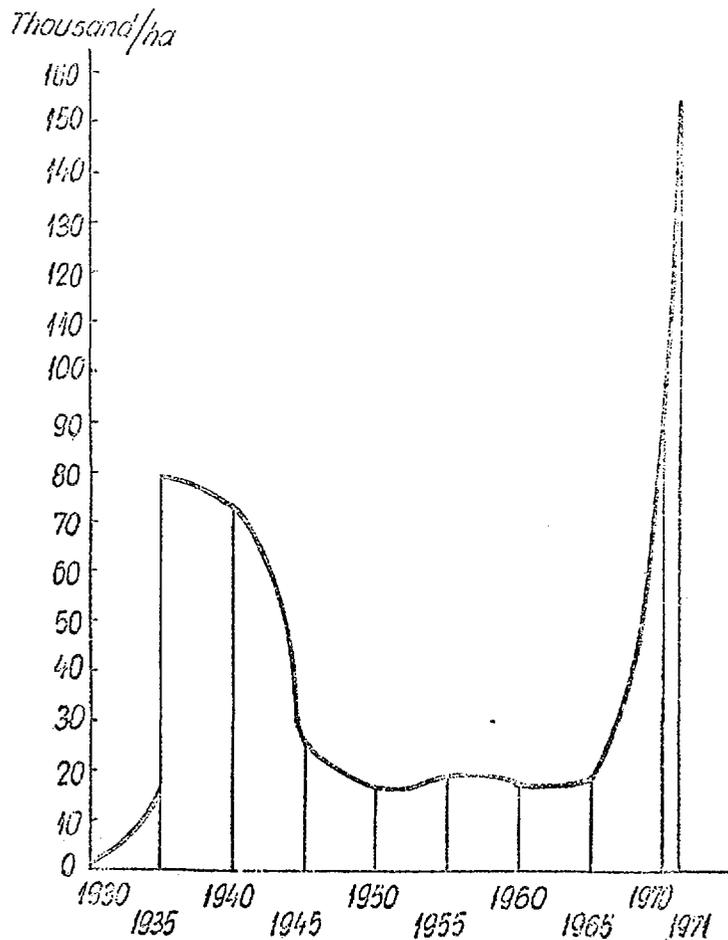
Fig.1 Development of soybean cropped areas in
Romania between 1930-1971

Areas (in thousand ha) in the 1971 - 1975 period

Plant	Year				
	1971	1972	1973	1974	1975
Soybeans	161,5	186,5	231,5	271,5	3o1,5
Peas	87,5	9o,5	9o,5	89,5	73,5
Beans[x]	72	75	78	81	84

[x] To these areas 13oo ha underseeded corn crop has to be added.

Thousand/ha

yield in Kg/ha
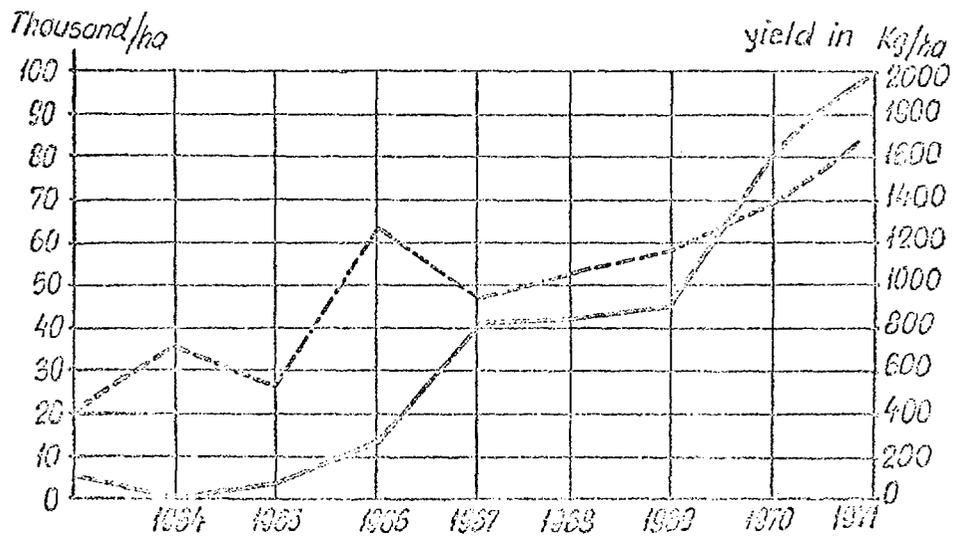
Fig. 2 Soybean cropped area and average annual yield obtained
between 1964–1970
in State Agriculture Farm

Legend:
.... Average yield
.... Area

Fig. 3 Evapotranspiration in soybean crops in the
Southern parts of Romania (Fundulea)
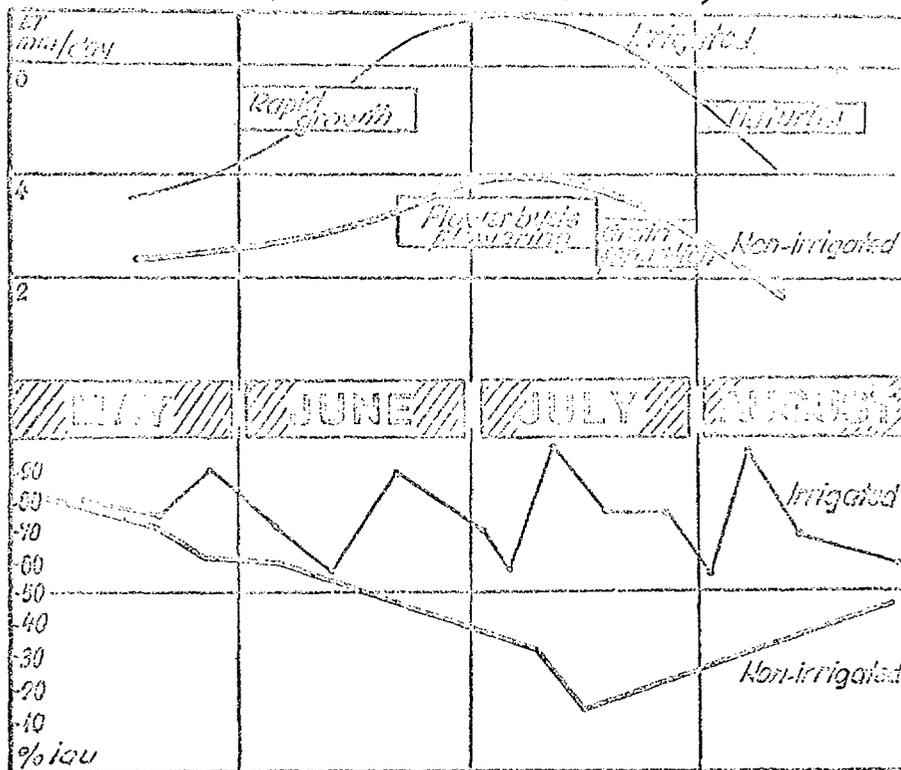
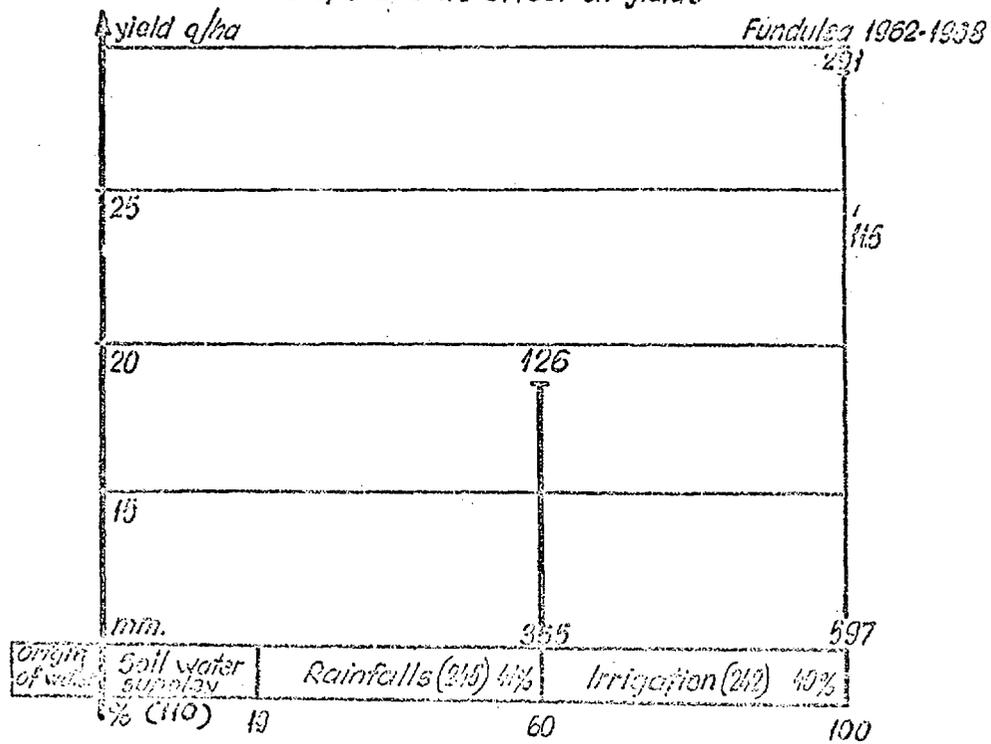Fig. 4 Origin of water used by soybean crops and it's effect on yields

Fundulea 1962-1968

Table no. 2

Effect of fertilizers on bean yields

Fundulea

Treatment	Yield q/ha	Difference q/ha
Control	17,7	-
N_{50} P_{25}	19,0	1,3
N_{50} P_{50}	18,3	0,6
N_{50} P_{100}	16,2	-1,5
N_{50} P_{100} K_{100}	18,4	0,7
LD 5 %		0,9

Effect of fertilizers on bean yields obtained at
the Experiment Station Podu Iloaie - 1971

Treatment	Yield q/ha	Difference q/ha
N_0 (P_{60})	12,6	-
$N_{30}(P_{60})$	14,8	2,2
$N_{60}(P_{60})$	15,6	3,0
$N_{90}(P_{60})$	15,5	2,9
LD 5 %		1,2

Table no.4

Effect of fertilizers on peas yields at the
Experiment Station Podu-Iloaie - 1971

Treatment	Yield q/ha	Difference q/ha
N_0 (P_{60})	15,5	-
N_{30} (P_{60})	17,7	2,2
N_{60} (P_{60})	19,8	4,3
N_{90} (P_{60})	19,1	3,6
LD 5 %		1,4

Effect of N-fertilizers on yields of Chippewa and

Violeta varieties of soybean - 197o

Variety	Experiment Stations	N_oP_{6o}	$N_{3o}P_{6o}$	$N_{6o}P_{6o}$	$N_{9o}P_{6o}$	Average
Chippewa	Turda	11,5	14,2	13,1	14,1	13,2
	Albota	15,3	2o,o	19,4	2o,7	18,9
	Secuieni	13,o	13,6	15,8	11,1	13,4
	Average	13,2	15,9	16,1	15,3	-
Violeta	Turda	14,4	2o,3	15,7	13,6	16,o
	Albota	16,2	21,9	22,6	24,o	21,2
	Secuieni	13,7	14,3	12,9	1o,6	12,9
	Average	14,7	18,8	17,1	16,1	-
A v e r a g e		13,9	17,3	16,6	15,7	-

Table no. 6

Effect of N and P fertilizers on soybean yields at

the Experiment Station Tg. Mureş (Average

1969 - 197o)

P_2O_5	N - kg/ha				
kg/ha	0	5o	1oo	15o	Average
0	13,2	16,8	18,2	19,o	16,8
40	14,6	16,9	18,o	2o,o	17,4
Average	13,9	16,8	18,1	19,5	-

Effect of fertilizers on three soybean varieties on a
redish brown forest soil in the South-Romania
(q/ha)

Fertilizers	Chippewa 1966-1968		Merit 1966 - 1968		Mandarin 1966-1967		Average	
	non irrigated	irrigated	non irrigated	irrigated	non irrigated	irrigated	non irrigated	irrigated
Control	17,5	28,7	14,3	25,0	17,5	26,4	16,4	26,7
N_{64}	19,0	30,2	14,8	27,4	19,0	28,8	17,6	28,8
P_{64}	18,4	29,0	14,5	26,2	18,5	28,6	17,1	27,9
$N_{64}P_{64}$	21,1	32,8	16,5	28,9	21,3	31,2	19,6	30,9
$N_{64}P_{96}$	19,6	32,0	16,2	28,7	20,8	30,9	18,9	30,5
$N_{48}P_{64}$	21,5	31,2	17,1	27,4	21,7	28,9	20,1	29,2
$N_{48}P_{64}K_{60}$	22,2	31,9	17,7	28,4	21,8	31,1	20,6	30,4
$N_{96}P_{64}$	17,3	33,5	15,4	30,4	19,3	33,0	17,3	32,3
$N_{96}P_{128}$	16,2	32,3	15,1	29,9	19,3	32,4	16,9	31,5
Average	19,2	31,3	15,7	28,0	19,9	31,3	18,3	30,2
Difference	–	12,1	–	12,3	–	11,4	–	11,9
LD 5 %	1,0	1,8	0,7	1,4	0,9	1,3		

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Effects of N P K fertilization on average yield of

irrigated soybean (q/ha)

| Rate of N kg/ha | Steppe zone | | | | | Forest-steppe zone | | | | | Average |
| | PK - rate | | | | | PK - rate | | | | | |
	P_0K_0	$P_{40}K_0$	$P_{80}K_0$	$P_{80}K_{80}$	Average	P_0K_0	$P_{40}K_0$	$P_{80}K_0$	$P_{80}K_{80}$	Average	
0	15,9	15,9	15,6	15,8	15,8	21,2	21,1	20,8	20,6	20,9	18,4
40	19,0	18,9	18,3	18,9	18,8	22,9	23,1	23,2	23,0	23,1	21,0
80	20,5	20,8	20,3	20,8	20,6	24,4	24,3	24,5	24,0	24,3	22,5
120	22,3	22,6	21,6	21,9	22,1	24,8	25,1	25,2	24,7	25,0	23,6
160	23,0	23,7	23,3	22,9	23,2	25,3	25,6	25,7	25,7	25,6	24,4
Average	20,2	20,4	19,8	20,1	-	23,7	23,8	23,9	23,6	-	-

Table no. 9

Effect of soybean seed treatment with

nodulating bacteria on yields, under irrigation

(q/ha) (average of 5 locations)

Fertilizer treatments	Seed treatment	
	No bacterial treatment	Bacterial seed treatment
N_0P_{50}	19,8	24,8
$N_{30}P_{50}$	22,0	25,4
$N_{60}P_{50}$	23,3	25,1
$N_{90}P_{50}$	23,8	24,7
Average q/ha	22,2	25,0
%	100,0	112,6

Fig.5 Influence of seed treatment with Nitragin on irrigated soybean yields. (average on 3 years in 7 locations)
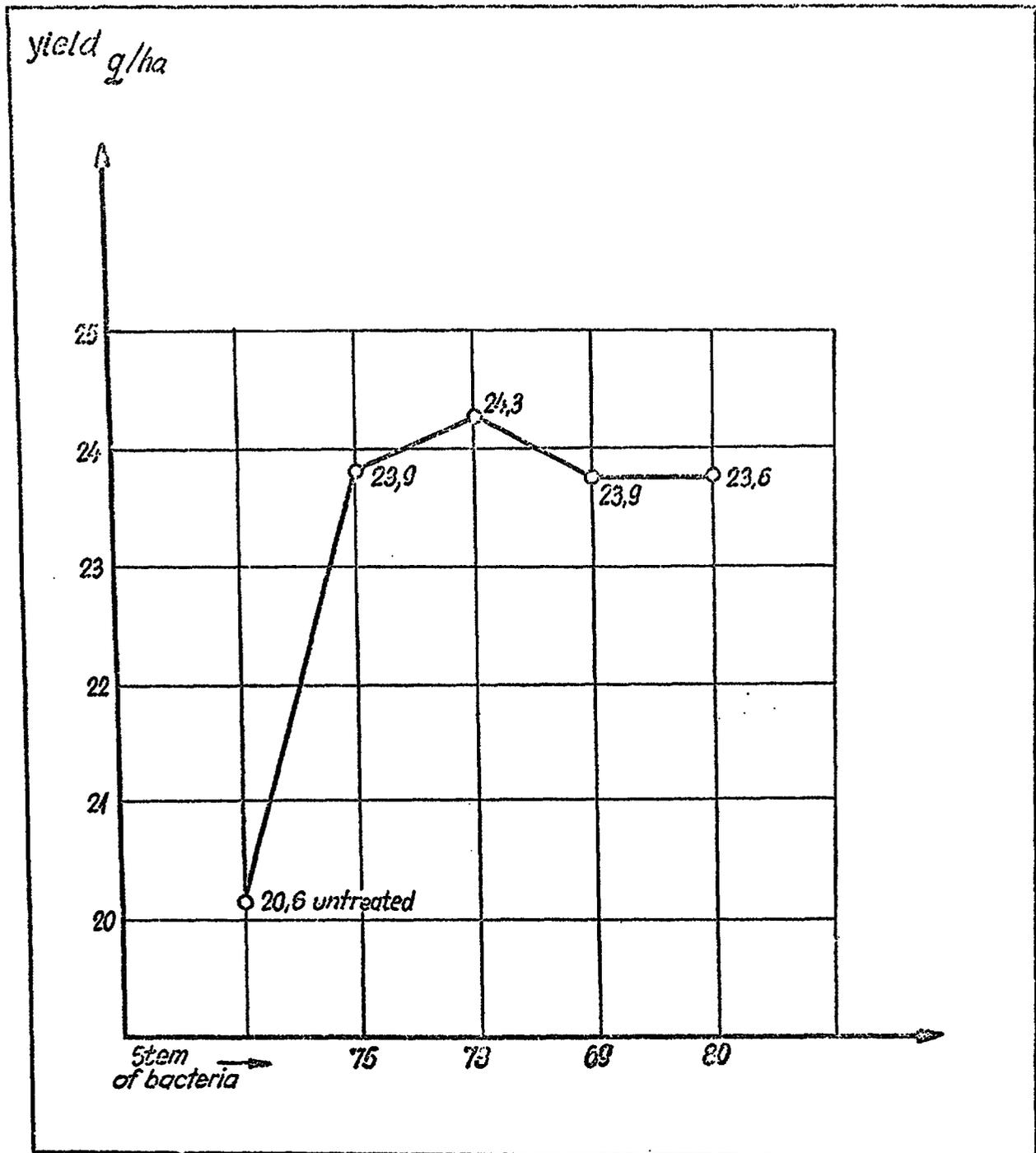
Effect of soybean seed treatment with different
strains of Rhizobium japonicum on yields,
in different locations (on a 4o kg/ha
P_2O_5 backgraund) - 197o

Treatment	Dohangia Alluvial soil - irrigated -		Oltenița Alluvial soil - irrigated -		Dâlga Brown chernozem soil - un irrigated -	
	Yield q/ha	Dif.	Yield q/ha	Dif.	Yield q/ha	Dif.
No bacterial seed treatment - N_o	24,3	-	18,5	-	13,o	-
No bacterial seed treatment + N_{6o}	25,5	1,2	21,o	2,5	17,8	4,8
SO - 69	27,2	2,9	23,3	4,8	17,5	4,5
SO - 75	29,3	5,o	28,4	-9,9	2o,o	7,o
SO - 97	29,5	5,2	22,6	4,1	19,8	6,8
SO - 146	3o,5	6,2	25,7	-7,2	18,5	5,5
LD 5 %	2,1		2,2		2,o	

1970 EXPERIMENT WITH ^{15}N and ^{32}P

Rate, time and method of application of fertilizers

At seeding time		During the vegetation periode (N)	Method of application
N	P_2O_5		
o	o	—	
30^X	60^X	—	Broadcasted
30^X	60^X	—	N and P, mixed in band
30^X	60^X	—	N and P Separated in band
o	60^X	30^X	In band
60^X	60^X	—	N and P mixed in band
90^X	60^X	—	N and P mixed in band
120^X	60^X	—	N and P mixed in band

x) labelled N and P fertilizers - ^{15}N, ^{32}P

Effect of N and P fertilizers application method on N-uptake from fertilizers

Method of application (3o kg N + 6o kg P_2O_5)	First yield - 15.VI.-		Second yield - 1o.VIII.-		Final yield - Grains -	
	% N in plants	of which % N from fertilizer	% N in plants	of which % N from fertilizer	% N in grains	of Which % N from fertilizer
Broadcasting	3,14	14,6	2,oo	13,3	4,66	1o,7
Mixed bands	3,13	28,9	2,1o	12,3	4,95	8,1
N and P in separated - bands	3,36	26,o	2,11	12,4	4,75	7,8
N applied in period of vegetation	2,98	-	2,o3	21,2	4,82	12,4

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Effect of N and P fertilizers application method on the P

uptake from fertilizer

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Method of application (3o kg N + 6o kg P_2O_5	First yield – 15.VI. –		Second yield 1o.VIII.–		Final yield – Grains –	
	% P_2O_5 in plants	of which % P_2O_5 from fertilizer	% P_2O_5 in plants	of which % P_2O_5 from fertilizer	% P_2O_5 in grains	of which % P_2O_5 from fertilizer
Broad casting	o,746	1,5	o,660	4,4	1,67	3,9
Mixed bands	o,75o	27,o	o,682	15,4	1,6o	9,o
N and P in separated bands	o,75o	13,o	o,671	7,7	1,61	6,5
N applied in period of vegetation	o,752	14,1	o,69o	9,5	1,6o	6,3

1971 EXPERIMENT WITH ^{15}N and ^{32}P

with bacterial treatment

At seeding time		During the vegetation period (N)	Method of application
N	P_2O_5		
30^x	80^x	–	N and P broadcasted
60^x	80^x	–	N and P broadcasted
90^x	80^x	–	N and P broadcasted
30^x	80^x	–	N and P mixed in band
60^x	80^x	–	N and P mixed in band
90^x	80^x	–	N and P mixed in band
30^x	80^x	30	30 N and 80 P broadcasted 30 N in band
30^x	80^x	30^x	30 N and 80 P broadcasted 30^x N in band
30^x	80^x	60	30 N and 80 P broadcasted, 60 N mixed in band
30	80^x	60^x	30 N and 80 P broadcasted, 60^xN in band

No bacterial treatment

At seeding time		During the vegetation period (N)	Method of application
o	80	–	In band
60^x	80^x	–	N and P broadcasted
o	80^x	60^x	In band

x) labelled N and P fertilizers – ^{15}N, ^{32}P

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13 - Effect of N and P fertilizers application method on the P uptake from fertilizer.

14 - 1971 EXPERIMENT WITH ^{15}N and ^{32}P with bacterial treatment.

R E F E R E N C E S

[1] BILȚEANU,GH. - Fitotehnie.Editura didactică și pedagogică, București.(1969).

[2] BONNIER,CH.,BRAKEL,J. - Lute biologique contre la faim. Edition J. Duculot S.A.Gembloux.(1969).

[3] AKADEMIA NAUK SSSR, Institut microbiologii,Biologhiceskii azot i evo rol v zemledelii - Izdatelstvo "Nauka" Moskova.(1967).

[4] BALAN,N. - Impoitanța bacterizării la culturile de plante leguminoase.Revista "Agricultura" nr.13.(1970).

[5] BERGERSEN,F.J. - Ammonia - an early stable product of nitrogen fixation by soybean root nodules.Aust.J.biol. Sci.18.(1965).

[6] BERGERSEN,F.J. - Some properties of nitrogen fixing breis prepared from soybean root nodules.Biochim. biophys.Acta.130.(1966).

[7] BERGERSEN,F.J. - Nitrogen fixation in the legume root nodule. Proc.9 th.int.Congr.Microbiol.Symp.Moskova. (1966).

[8] MOORE,J.W. - Le soja aux États - Unis.Oléagineaux.France. Vol.26,nr.3.(1971).

[9] DORNEANU,A. și colab. - Cercetări privind influența îngrășă- mintelor chimice asupra producției soiei în cultură irigată și neirigată pe sol brun roșcat de pădure.Probleme agricole nr.3.(1970).

[10] GUKOVA,M.M., - Fixarea azotului la plantele leguminoase în
cazul aplicării îngrășămintelor cu azot.
Cultura și protecția plantelor de cîmp vol.VII,
nr.14.(1971).

[11] NELSON,W.L. - Fertilization of soybeans.Oléagineaux. France.
vol.26. nr.2.(1971).

[12] SARPE,N.,POPA,FL.,HERA,CR. si colab. - Noi rezultate expe-
rimentale privind tehnologia culturii de soia
în condiții de neirigare.Probleme agricole
nr.3.(1971).

[13] DIACONESCU,O. - Unele măsuri privind îmbunătățirea proce-
sului tehnologic la cultura soiei.Probleme
agricole nr.4.(1970).

[14] DIACONESCU,O.,MICLEA,E. - Soia - Editura "Ceres"-București.
(1971).

[15] HERA,CR. și colab. - Cercetări cu ajutorul izotopilor ^{15}N și
^{32}P privind sistemul de fertilizare la soia.
Analele ICCPT-Fundulea,Seria B,vol.39.(1971).

[16] BALAN,N. și colab. - Selecția de tulpini bacteriene din
populațiile autohtone de Rhizobium Japonicum.
Analele ICCPT-Fundulea vol.XXXVI,Seria B.
(1968).

[17] DATE,R.A. - Microbiological problems in the inoculation and
nodulation of legumes-Plant and soil,vol.32
nr.3.(1971).

[18] HULPOI,N. și colab. - Irigarea soiei - Analele ICCPT-Fundulea
seria B,vol XXXVI.(1968).

[19] NULPOI,N. și colab. - Rezultate experimentale în cultura
soiei irigate.Probleme agricole nr.4.(1971).

[20] CANTER,F. și colab. - Cercetări cu privire la eficiența
unor tulpini de Rhizobium Japonicum,în culturi
de soia,pe solul cernoziom levigat din zona
Iași. Lucrări științifice- Institutul Agronomic
Iași,Seria Agronomie-horticultură.(1969).

[21] x x x   Rezultate experimentale obținute la cultura
leguminoaselor pentru boabe în rețeaua sta-
țiunilor experimentale ale ICCPT - Fundulea.

THE EFFECT OF NITROGEN AND SULPHUR TREATMENTS ON PHOSPHORUS ABSORPTION AND YIELD OF GROUNDNUTS ON SOILS OF SANDSTONE ORIGIN

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Abstract

Sulphur and nitrogen application together with phosphate fertilizer to groundnut crop grown on a soil of sandstone origin with a short fallow period showed no influence on phosphate absorption of the crop. Increasing rates of combined nitrogen application to groundnuts increased the proportion of combined nitrogen in the harvest but showed no significant difference in total nitrogen absorbed by the crop. The proportion of the combined nitrogen to total at the time of flowering reduced considerably at the stage of maturity.

Groundnut cultivation in West Africa has been predominantly on the sandy to loamy sandy soils in the savannah belt. These soils are mostly of low inherent fertility and are deficient in nutrients especially phosphorus. Nitrogen and Sulphur are also known to limit crop yields considerably.

The importance of phosphorus in the production of groundnuts in West Africa is shown by its proportion in the fertilizer formular in these countries. Nye (1952), and Stephens (1960) obtained results which showed considerable yield increases of the crop in Northern Ghana by applying 1 cwt. per acre of single superphosphate. Recent results from extensive trials by Djokoto (1969) showed responses to phosphate to range from 40 to over 50 per cent when the fertilizer was applied at rates of 20 and 40 lb P_2O_5 per acre. Goldsworthy and Heathcote (1963) in Nigeria obtained large yield increases by applying half that quantity. Evelyn and Thornton (1964) also obtained similar results in the Gambia through the application of single super phosphate coupled with potash. Fertilizer

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recommendations in most of Senegal, the second largest groundnut producer in Africa,are based on this large proportion of phosphorus in the formular. In the Gambia, Ashrif et. al. (1962) also stressed the importance of this nutrient in the recommended formular.

Sulphur deficiency has been reported in a number of experiments in the groundnut growing belt of West Africa (Greenwood (1954); Bockelee-Morvan (1960) I.R.H.O. (1956); Stephens (1960).

This augmented the use of sulphur containing fertilizers as the nitrogen source generally. In some of the localities in Ghana however, results obtained on soils developed over the same parent material were not always consistent (Stephens 1961, Nye 1951) probably as a result of intensity of land use and management. There is also an indication that the rest-period of the land prior to cultivation may have an effect.

Soils of granite origin in the groundnut belt of northern Ghana are exhaustively cropped and nitrogen, phosphate and sulphur have been found essential for good crop yields. Nitrogen as "Starter dose" has often been applied to the crop. Results from 149 trials conducted in the savannah areas of Ghana by Djokoto (1969) showed an average increase of 12.9 per cent in Kernel yield when 10 lbs N per acre was applied. However, further investigations are required to establish the direct effects of nitrogen and sulphur on the crop on these soils. Furthermore, it is not very clear whether these nutrients directly enhance the efficiency of phosphorus uptake by the plant since the latter occupies a key position in the nutrition of groundnuts and legumes in general.

Results of field experiments using radio-active fertilizers containing N^{15}, P^{32} and S^{35} on groundnuts grown on soils developed over sandstone are presented in this paper.

Material and Method

Ejura series (Ghana nomenclature), a well drained upland soil consisting of dark brown to brown sandy topsoil over orange-brown sandy clays or clayey sands was chosen for the field experiment.

The chemical analysis of a sample from the experimental site is shown in the table below (Table 1A) and the rainfall data in table 1B.

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Table 1A. Soil chemical analysis. (0-15 cm depth)
(Ejura Series)

| pH | me/100g oven-dry soil | | | | | | % C | % N | % Org.M | C/N | Kg/ha |
	T(CEC)	Ca	Mg	Mn	K	Na					P_2O_5
6.6	2.85	2.10	.22	.01	0.1	.07	.56	.028	.96	20	55

The experimental site had been under elephant grass (Pennisetum purpureum) fallow for 2 years. The level of phosphorus (determined by Bray's P_1 method) was much higher than the average found in this soil series. The maize crop which was grown there previously might have received some amount of phosphate fertilizer application.

Table 1B. Rainfall data: September 1969-January 1970

M o n t h	September	October	November	December	January
Rainfall (in)	3.39	13.21	3.61	0.06	0.39
Mean for 45 years	8.16	8.78	3.18	0.87	0.51

The experiment consisted of 8 treatments laid out in a randomized block and replicated 4 times. A plot consisted of 5 rows of 2.40 m. in length. Distance between rows was 0.60 m. Plot width was therefore 3.00 m. and area of plot was $7.20m^2$. Treatments are shown in Table 2.

Phosphate was applied as labelled triple superphosphate (50% P_2O_5). Potassium treatment was omitted as from previous experiments groundnuts showed no response to this nutrient on this as well as many other soil series in the country.

Experimental area was ploughed and harrowed and groundnuts planted on the flat without ridging. Planting distance within row was 15 cm. with 2 seeds per hole; placed about 1 cm apart. Between rows planting distance was 0.60 m. Fertilizers were applied mixed and broadcast evenly in a groove of 6 cm. depth and 10 cm. in width and covered with soil about 2.0 cm thickness. The groundnut seeds were then placed on this soil layer and completely covered with soil. Groundnut variety "Kumawu" was planted, a local widely cultivated variety of medium kernel size. Plants were sampled about 4 days after flowering from the row receiving the radio active fertilizers for laboratory analysis. At harvest, three rows in each treatment were used for yield estimation.

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Total and radioactive phosphorus values were determined after ashing in an oven. Ammonium vanadate-molybdate method was used for the total phosphorus whilst the liquid G.M. Counter was used in P^{32} counting.

	Source	N-Fertilizer	P-Fertilizer (0-50-0) Kg/ha
A	None	--	--
B	None	-	50
C	$(NH_4)_2SO_4$	30 Kg/ha (S labelled)	50
D	$CO(NH_2)_2$	30 Kg/ha labelled	50
E	$CO(NH_2)_2$	30 Kg/ha unlabelled	50
F	$CO(NH_2)_2$	30 Kg/ha unlabelled	50*
G	$CO(NH_2)_2$	15 Kg/ha labelled	50

*In treatment F. an equivalent amount of S in sulphate of ammonia(treatment C) was added in the form of sodium sulphate $(Na2\ SO_4)$.

S^{35} was determined using end window counting. One gramme of ground plant material was taken in each case and the standard prepared by adding a known quantity of labelled Na_2SO_4 solution to a non-radioactive plant material thoroughly mixed, and dried in an oven at about $100^{o}C$. till the sample was well dried.

RESULTS

Dry Forage yield

At flowering, nitrogen and phosphate together increased the yield of dry forage but phosphate alone gave a slight depression in yield. Increases in yield with urea as the nitrogen source were not significant at the 5% level. Addition of sodium sulphate to 30 kg/ha N as urea lowered the nitrogen effect slightly. However, reducing the nitrogen rate to half, identical yield effect was obtained as the higher nitrogen rate applied together with sulphur.

The negative effect of the fertilizers on yield might have been due largely to the unfavourable amount of rainfall during the early growth of the crop. The crop was planted on 18th September, and the recorded rainfall for that particular month was only 42 per cent of the

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average for 45 years. This would be particular true of the phosphate effect which was shown to be negative at the time of flowering but showed increase in haulm yield at maturity.

Application of 30 kg/ha as sulphate of ammonia with 50 kg/ha P_2O_5 gave the highest increase in yield; the increase being significant at the 5% level compared with the control and phosphate treatment.

Nitrogen and phosphate application increased the haulm yield at harvest but these increases were not significant. The most effective treatment was the phosphate which resulted in an increase of 21.5 per cent. Nitrogen applied as ammonium sulphate was as effective as urea applied at equivalent rate with addition of corresponding amount of sulphur. With sulphur addition the yield in the urea treatment was slightly depressed by 2 per cent at equivalent nitrogen level. The effect of phosphate was reduced by 16.9 per cent when nitrogen was applied at 15 kg/ha.

Per cent P_2O_5

% P_2O_5 values in forage at flowering were higher in treatments with phosphates than in the control; the differences being significant at the 5% level. Source of nitrogen had no significant effect on these values. However, nitrogen application lowered the P_2O_5 content as a result of forage yield increase. Application of sulphur together with urea resulted in an increase of only 2.1 per cent in the P_2O_5 content compared with corresponding urea treatment without sulphur.

With the nitrogen rate reduced to 15 kg/ha, the % P_2O_5 increased by 9.5 per cent over the urea treatment with sulphur even though the forage yield remained unaffected.

Phosphate application significantly increased per cent P_2O_5 at the 5% level in the forage at maturity. Both sulphate of ammonia and urea applied together with sodium sulphate reduced the P_2O_5 value compared with an equivalent rate of nitrogen as urea without sulphur addition. Differences due to nitrogen source was not significant.

Per cent N

Application of combined nitrogen to the crop resulted in a slight increase in % N which did not reach statistical significance. There was no

Table 3. The effect of treatment on forage yield, and amount of P and N present

Treatment kg Nutrient/ha	Plants sampled at flowering				Plants at maturity			
	Dry wt/10 plants (g)	% P_2O_5	% N	P_2O_5/N Ratio	Dry forage yield Kg/ha	% P_2O_5	% N	P_2O_5/N Ratio
1. (-)	52.7	0.342	2.36	0.145	1946	0.370	1.81	0.204
2. 50 kg/ha P_2O_5	48.9	0.572	2.39	0.239	2365	0.478	2.32	0.206
3. 30 kg N + 50 kg P_2O_5 $(NH_4)_2SO_4$	71.6	0.483	2.49	0.194	2302	0.455	1.95	0.233
4. 30 kg N + 50 kg P_2O_5 $CO(NH_2)_2$	66.3	0.484	2.48	0.195	2341	0.490	2.03	0.241
5. 30 kg N + 50 kg P_2O_5 + 35.5 kg S	57.7	0.494	2.55	0.193	2295	0.453	2.09	0.217
6. 15 kg N + 50 kg P_2O_5 $CO(NH_2)_2$	57.5	0.541	2.45	0.221	1965	0.475	1.87	0.254
S.E.	5.3	0.059	0.123		67	0.024	0.18	
L.S.D.	15.8	0.128	N.S.		NS	0.073	NS	

difference in the % N due to source of nitrogen. The ratio of P_2O_5/N remained essentially the same at the same level of nutrient application, independent of source of nitrogen or addition of sulphur.

Utilization of P_2O_5 from Fertilizer Source.

Fertilizer phosphorus absorption increased considerably with the application of nitrogen. An increase in the rate of N application from 15 to 30 kg/ha resulted in an increase of 6.6% in the P_2O_5 derived from fertilizer source. Sulphur addition to urea increased % P_2O_5 derived from fertilizer only slightly.

Table 4. % P_2O_5 derived from fertilizer

T r e a t m e n t kg/ha	% P_2O_5 d.f. fertilizer
1. (-)	-
2. 50 kg/ha P_2O_5	27.8
3. 30 kg N + 50 kg P_2O_5 $(NH_4)_2SO_4$	41.9
4. 30 kg N + 50 kg P_2O_5 $CO(NH_2)_2$	38.9
5. 30 kg N + 50 kg P_2O_5 + 35.5 kg S $(CO(NH_2)_2$	40.4
6. 15 kg N + 50 kg P_2O_5 $(CO(NH_2)_2$	32.3
S.E. ±	4.3
L.S.D. (P = 0.05)	N.S.

Effect of treatment on yield of groundnuts

Differences due to treatment were very small and not significant. A slight increase of 6 and 7.4 per cent was obtained in pod and kernel yield respectively when sulphur was added to urea. Application of fertilizer nitrogen increased neither kernel nor pod yield. The general depression in yield in the fertilizer treatments could be explained by the dry weather at the time of planting and this most probably induced fertilizer toxicity on such a sandy soil.

Table 5. Fertilizer effect on groundnut yield
(Ejura Soil Series)(Kg/ha)

Treatment (Kg/ha)	Pod Yield	Kernel
50 kg/ha P_2O_5	1544	870
50 kg N + 50 kg P_2O_5 $(NH_4)_2SO_4$	1549	877
30 kg N + 50 kg P_2O_5 $CO(NH_2)_2$	1562	847
30 kg N + 50 kg P_2O_5 + (35.5 Kg s)	1669	910
15 kg N + 50 kg P_2O_5	1447	815
(-)	1877	1155
S.E. +	167	112
L.S.D. (P = 0.05)	N.S.	N.S.

Per cent N derived from fertilizer

Combined nitrogen was actively absorbed by the groundnut crop and was as high as 30 per cent of the total nitrogen at flowering when 30 kg N per ha. was applied. Reducing the rate of application to half, the per cent nitrogen derived from fertilizer correspondingly reduced. There was a sharp reduction of the proportion of combined nitrogen in the leaves at the time of harvest. This reduction was as high as 75 per cent of the value at flowering. The distribution of the combined nitrogen between leaves and husk at the time of harvest seemed to be very similar; the per cent nitrogen from fertilizer source being only slightly higher in the forage than in the husk.

Table 6. % N derived from fertilizer

Treatment	Forage		Husk	
Kg Nutrient/ha	Flowering	Harvest	Flowering	Harvest
1. 30 kg N + 50 kg P_2O_5	30.0	7.34	-	6.33
2. 15 kg N + 50 kg P_2O_5	17.0	3.69	-	2.44

Per cent Sulphur in dry forage.

Application of Sulphur had no significant effect on the per cent sulphur in the forage; although treatments receiving this nutrient showed a slight increase. The sulphur derived from the fertilizer source was as high as 38 per cent of the total in the harvested forage.

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Table 7. % S in dry forage at harvest

Treatment kg/ha	% S	% S d.f.f.
1. 30 kg N + 50 kg P_2O_5 $(NH_4)_2SO_4$*	0.1720	38.6
2. 30 kg N + 50 kg P_2O_5 $CO(NH_2)_2$	0.1677	
3. 30 kg N + 50 kg P_2O_5 + 35.5 kg S	0.1716	
4. 15 kg N + 50 kg P_2O_5	0.1604	

* Sulphur in ammonium sulphate labelled.

Discussion

With few exceptions the response of groundnuts to phosphate fertilizers in the savannah areas in Ghana has been high as confirmed by recent extensive trials carried out by Djokoto (1969). Lack of response in the present experiment might be due largely to fertilizer toxicity on such a sandy soil under unfavourable weather condition. Thus the haulm yield was depressed at time of flowering when the weather was severest but increased by 21 per cent at harvest. Nevertheless kernel yield was adversely affected. Stephens (1961) obtained a significant yield increase due to phosphate application on groundnuts on this soil series in the same locality. The site had been under grass fallow for many years. Phosphate level of the Ejura Series is usually not as high as was found in the soil analysis in the present experiment; and this might be due to a previous fertilizer application. The response to this nutrient might, therefore, not have been high under favourable weather conditions.

Phosphate application significantly increased the per cent P_2O_5 in haulm even though the increase in yield was not significant. This may be important in the fertilization of forage legumes. Thorton (1964) also observed a significant increase in phosphorus content when phosphate fertilizer was applied to groundnuts. However, addition of nitrogen and sulphur did not significantly affect the per cent P_2O_5, probably because of lack of significant yield response to these two nutrients.

Per cent phosphorus derived from fertilizer source was above 30% at the time of flowering. Thornton (1964) observed a very low recovery of fertilizer P from a groundnut crop. This result might probably be due to the method of fertilizer application. The groundnut plant

rapidly develops a long radicle after germination. But the active absorbing rootlets are concentrated near to the cotyledons at the initial stages. Fertilizer placed in this region is therefore, actively absorbed. Thus Ofori (1965) using radioactive phosphate showed that fertilizer placement below groundnut seed gave the best result compared with banding to the side or broadcasting. On the other hand Burkhart and Collins (1941) observed that the degree of nodulation appeared to be an important factor in the utilization of phosphates by the peanut plant. Contradictory results might therefore, be explained by one or a combination of these various.factors.

Nitrogen application increased the proportion of phosphorus derived from fertilizer source. However, these increases were not significant. The small increases in per cent P_2O_5 when sulphate of ammonium was applied as compared with urea and an equivalent amount of sulphur, would suggest a slight superiority of the ammonium nitrogen source, even in the legume crop, as has been observed in many experiments with cereals.

Sulphur did not seem to be deficient in the soil and thus the yield of the groundnut crop in this experiment was not significantly affected. At flowering the addition of this nutrient to urea slightly reduced the yield of the forage. At maturity a slight increase in both pod and forage yields was obtained. The lack of effect of this nutrient might be partly due to the comparatively high rate at which it was applied. In another experiment with groundnuts on the same soil series and in the same locality, Ofori (1970), obtained an increase in forage yield with 15 kg S/ha whilst double that rate reduced it considerably. In many experiments on the groundnut crop in West Africa, sulphur has been found beneficial and its application either as ammonium sulphate or single super phosphate greatly increased crop yield (Stephens 1960; Greenwood (1954); Bockelee-Morvan (1966); I.R.H.O. (1956). Most of these responses were obtained on very intensively cropped soils where the fertility status had decreased to a low level.

Sulphur addition even though ineffective on yield, increased the per cent S in the forage. This could be of immense benefit to the livestock industry whereby some of the sulphur containing amino acids in the legume forage might be increased even though increase in forage yield might not occur.

Nitrogen did not give any significant response with kernel or haulm yield at maturity. However, the effect of sulphate of ammonia at

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flowering was significant. Stephens (1960) reported a highly signifi-
cant response of the crop to sulphate of ammonia on the same soil series.
Djokoto (1969) obtained an average of 13 per cent increase in kernel
yield over extensive savannah areas in Ghana by applying 10 lbs N per
acre. Since there was no inocculation with rhizobia in any of these
experiments, this effect might possibly be due to ineffective symbiotic
fixation. However, this would need further investigation.

The soil in the present experiment showed a very high carbon-
nitrogen ratio of 20; an evidence of the short rest under grass fallow.
Some degree of response to nitrogen was therefore expected. A possible
explanation of the lack of response might be that with the adequate
phosphate level, conditions for the nitrogen fixation were favourable
and the plants quickly had sufficient nitrogen supply. Field
observations showed that the plants in the non-nitrogen treatments
were pale for about two weeks after germination. In the third week,
all plots were looking green showing no colour difference between
nitrogen and plots without this nutrient. Albrecht and McCalla (1937)
suggested that dressings of phosphate on legumes might greatly increase
fixation of nitrogen as this nutrient stimulates root growth. Ludecke
(1941) also observed that the rate of nitrogen fixation per gramme of
nodule was greatly increased by phosphorus. It would, therefore, seem
probably that soils adequately supplied with phosphorus might not
require much combined nitrogen on groundnuts provided other conditions
are favourable for active nitrogen fixation.

Some of the results obtained on groundnuts in West Africa showed
the existance of a positive interaction between sulphur and nitrogen
(I.R.H.O. (1956). Anderson and Spencer (1950) working with subterannean
clover, also obtained interaction between sulphur and combined nitrogen.
But this interaction was found only when nitrogen fixation was impaired
by molybdenum deficiency. As already stated elsewhere, it would seem
that nitrogen fixation was adequate hence combined nitrogen did not
increase crop yield.

The higher per cent of fertilizer nitrogen recovered in the
forage at flowering showed that combined nitrogen was actively absorbed
by the groundnut crop. When the nitrogen rate was reduced to half, the
per cent nitrogen derived from fertilizer source was proportionately
reduced. This showed clearly that the nitrogen fixation was decreased
with increasing rates of combined nitrogen application. On soils without

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favourable conditions for rapid nitrogen fixation, therefore, sufficient combined nitrogen would be required if the legume crop is to establish properly. Using labelled nitrogen, Allos and Bartholomew (1955), also showed the same relationship between nitrogen fixation in legumes and combined nitrogen application.

The per cent nitrogen derived from fertilizer source reduced considerably with maturity. At harvest only 25 per cent of the value found at flowering stage was in the haulm. However, there was no significant change in the total nitrogen. Again this confirmed an active nitrogen fixation even though combined nitrogen was applied at 30 kg N per ha.

The very close values of fertilizer nitrogen found at maturity in both haulm and husk in such a crop as a legume were worth noting; since per cent total nitrogen in the haulm at harvest was much higher than in the husk. Further work on the distribution of combined nitrogen in a grain legume such as groundnut could be of much value.

References

ALBRECHT W.A. and McCALLA T.M. J. Bact. 1937. 33 80.

ALLOS H.F. and BARTHOLOMEW W.V. Proc. Soil Sci. Am. 1955, 19. 182.

ANDERSON A.J. and SPENCER D. Aust. Jour. Sci. Res. 1950. B 111, 431.

ASHRIF M.I., EVELYN, S.H. and THORNTON I. Oleagineux 1962, 17, 611,

BOECKELEE-MORVAN A. Oleagineux 1966 21, 163.

BURKHART L. and COLLINS, E.R. Proc. Soil Sci. Soc. Amer. 1941 6, 272.

EVELYN, S.H. and THORNTON I. Emp. J. Exp. Agr. 1964. 32, 153.

DJOKOTO, R.K. Unpublished Report on Fertilizer Trials Under FAO (FFHC) 1969.

GOLDSWORTHY P.R. and HEATHCOTE R. Emp. J. Exp. Agr. (1963) 31, 351.

GREENWOOD M. Trans. 5th Int. Congr. Soil Sci. Leopoldville Vol. 111. 245. 1954.

I.R.H.O. Rapport Annual 1956, 53.

LUDECKE H. Die Phosphorsaeure 1941. 10, 196.

NYE P.H. Empire Journ. of Exp. Agric. 1952, 20.

OFORI C.S. 1970. Unpublished.

       —   L'agronomic Tropicale 1965 5, 490.

STEPHENS D. Empire Journ. of Exper. Agric. 1960. 28 151.

THORNTON I. Emp. J. Exp. Agric. 1964 32, 325.

Preliminary Field and Greenhouse Trials on
Nitrogen Fertilization and Symbiotic N Fixation
with Beans and Clover

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ABSTRACT

A sand culture experiment on beans and clover using ^{15}N labelled NH_4^+ and NO_3' nitrogen solutions showed no detectable symbiotic fixation of nitrogen in the presence of NH_4^+ and NO_3' nitrogen respectively – perhaps due to too short a growth period (4 weeks).

A field experiment on the same crops using ^{15}N labelled fertilizers showed that the application of N fertilizers did not inhibit the symbiotic fixation of N. Fertilizer N was utilized more efficiently when top dressed at flowering than when applied at seeding. There was no yield response to N.

Introduction

In agricultural practice, nitrogen fertilization of legume crops is rather an exception than a rule.

It is generally accepted that applications of nitrogen fertilizers may reduce the extent of symbiotic nitrogen fixation of root nodules.

Little is known about the nature of this "inhibiting" effect, i.e. whether the reduction of symbiotic fixation by fertilizer nitrogen is due to absence of nodules, ineffective nodules, the chemical nature of the nitrogen fertilizer, the timing of the applications etc.

In order to answer the question: "What is the effect of chemical nature of nitrogen fertilizer on symbiotic N fixation", a sand culture experiment using ^{15}N labelled nitrate and ammonium solutions, was carried out. In field and pot experiments it is not possible to ensure a continuous supply of either NO_3^- or NH_4^+ ions, since nitrification reactions are likely to transform part or all NH_4^+ into NO_3^-. The solution to the question: "What is the effect of nitrogen fertilizer on the magnitude of symbiotic N fixation under field conditions", requires an independant direct estimation of soil N supply, symbiotic N supply and fertilizer N supply. The use of ^{15}N labelled fertilizer enables an estimation of the sum of soil N and symbiotic N supply. If it is assumed that fertilizer nitrogen effects symbiotic N fixation, but not the rate of soil N supply, any change in amount of soil + symbiotic N induced by nitrogen fertilizer applications, could be interpretated as a change in symbiotic N fixation only. This approach was used for two field experiments with beans and clover respectively.

A. Sandculture Experiment

The objective of the experiment was to study the extent of symbiotic N fixation by clover and beans, as affected by the nature of the nitrogen source (NH_4^+ or NO_3^-).

Absence and presence of inocculum were taken as additional treatments. The composition of the nutrient solutions has been given in Table 1.

Concentrated stock solutions were made from which 25 1 culture solutions were prepared daily. The pH was adjusted to 6.8.

TABLE 1

Composition of Nutrient Solution (1 % ^{15}N atom excess).

NH_4^+ Solution		NO_3 Solution	
NH_4^+	3.5 me	NO_3^-	3.5 me
K^+	1.5 me	H_2PO_4'	1.5 me
Mg^{++}	1.5 me	SO_4''	1.5 me
Ca^{++}	2.0 me	K^+	1.5 me
SO_4''	7.0 me	Mg^{++}	1.5 me
H_2PO_4'	1.5 me	Ca^{++}	3.5 me

+ NaOH to make pH 6.8

micro nutrients in ppm: Fe 2.8; Cu 0.0032; Zn 0,032; Mn 0.275; Mo 0.024; B 0.165.

The solutions were supplied to pots containing about 1.5 kg sterilized sand, at a rate of 1 drop/second. The pots were allowed to drain freely. Inocculum was applied at the seeding of clover and beans. The plants were harvested 4 weeks after germination.

Results

The growth and colour of the plants were excellent and identical for all treatments. Nodules were observed on the roots of phaseolus for the NH_4^+ + inocculation treatment only. In the case of clover, nodules were observed for all treatments.

Table 2 lists the % N and % N derived from the nutrient solution in phaseolus and trifolium that were found for the treatments.

TABLE II

Results Sandculture Experiment.

Treatments		Phaseolus		Trifolium	
Source of N	Inocculation	% N	% NdFC[+]	% N	% NdFC[+]
$Ca(NO_3)_2$	-	4.0	92	4.3	93
	+	3.9	92	4.2	92
$(NH_4)_2SO_4$	-	3.8	91	4.2	91
	+	4.2	94	4.4	88

+ % NdfC = % N in the plants derived from the culture solution.

The nitrogen contents were not affected by the nature of the source. Also the % N in the plants that was derived from the nutrient solution was remarkably constant.

If no symbiotic nitrogen would have been fixed, the nitrogen from nutrient solution together with the original nitrogen in the seed would have been the only sources.

It may be seen that 6 - 12 % N was derived from other sources than the culture solution, which might have been symbiotic N and seed N.

The nitrogen content of the dry plant material was of the order of 4 % and 3 - 4 g of dry matter were produced per pot. This represents 120 - 160 mg N/pot in the above ground plant parts.

About 20 mg N were supplied with phaseolus seed and 25 mg with trifolium. It is evident that in this case the 6 - 12 % N not derived from the culture solution may well have been supplied by nitrogen from the seeds. From the sand culture experiment it may therefore be concluded that in the presence of NH_4 and NO_3 nitrogen, symbiotic N fixation either did not occur or was too low to be detected with the technique that was adopted. It may well be, of course, that the growing period of 4 weeks was too small to enable detection of symbiotic N fixation.

B.   Field Experiments.

The objective of the field experiments with clover and beans was to study the effect of fertilizer nitrogen on symbiotic N fixation. The treatments included absence and presence of Rhizobium inocculum and two times of application of N, i.e. a basic treatment and a top dressing at flowering.

In order to determine the soil + symbiotic N supply in the "absence" of nitrogen fertilizer, a small rate of nitrogen application of 5 kg N/ha was included. It was assumed that such a low rate of N application was unlikely to have a depressing effect on any symbiotic N fixation. The actual nitrogen fertilizer application was done with $NaNO_3$ at a rate of 100 kg N/ha. The nitrate supplied at a rate of 5 kg/ha was labelled with 20 % ^{15}N atom excess, whereas the nitrite applied at 100 kg/N/ha had 1 % ^{15}N atom excess.

The experiments were arranged as split plots with inocculation and absence of inocculation as main plots and the fertilizer - time of application treatment combinations as sub plots. Each treatment was replicated four times.

Phaseolus was seeded in rows 45 cm apart with 4 cm between plants. Each subplot consisted of 7 rows, 2 m long. ^{15}N labelled $NaNO_3$ was applied to the centre row only, while the remaining rows received non-labelled $NaNO_3$. The whole experimental area received 100 kg P_2O_5/ha as superphosphate and 100 kg K_2O as K_2SO_4; both fertilizers applied prior to seeding.

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The inocculum was applied on the field as a suspension, after germination of the seeds.

The trifolium was seeded as a broadcast application on treatment subplots of 1.5 x 2 m. ^{15}N labelled NaNO3 was applied as a band, 0.5 m in width and 2.0 m in length, through the centre of the plot.

The remaining plot area received non-labelled $NaNO_3$. After germination, inocculum was applied as a suspension.

Results

Phaseolus

The observations relevant to the experiment have been listed in Table III. No differences in bean yield were observed. The low average yield for treatment $100^+ - 0$ was due to the very low yield of some of the replications.

The nitrogen content of the beans was not affected by any of the treatments. This seems to suggest that Rhizobium must have been present in the soil of the experimental fields. Both yield and N content of the treatments were identical to those of the control plots. In accordance with this assumption is the fact that the % N in the plants derived from fertilizer was not affected by inocculation treatment. It is obvious that the low application of N resulted in a low and the high application of N in a high % N in the plant derived from fertilizer. It may be seen, however, that the fraction of the fertilizer that was taken up by the crop was not affected by the rate of application. For applications of 5 and 100 kg N at seeding, 12 – 15 % of the fertilizer nitrogen was taken up by the beans. When these applications were made at flowering time, 22 – 28 % of the fertilizer were utilized, irrespective of rate of application.

In order to compare the amount of soil N + symbiotic N corresponding with each treatment, the A values were calculated

TABLE III - Results of Phaseolus Experiment (Field)

Treatments in kg N/ha		Inocula-tion	Yield of beans in ton/ha	% N	% N^{++} dff	% N Fert. yield	"A" Value (kg N/ha)
Seeding	Flowering						
0	0	−	2.1	3.2	−	−	−
		+	2.3	3.1	−	−	−
5$^+$	0	−	2.2	3.2	0.8	12	748
		+	1.9	3.0	1.2	14	467
0	5$^+$	−	2.4	3.1	1.5	22	392
		+	2.4	3.1	1.9	28	270
100$^+$	0	−	1.9	3.0	21.3	12	512
		+	1.8	3.1	27.5	15	462
0	100$^+$	−	2.1	3.1	36.0	23	180
		+	2.1	3.1	33.6	22	202
5$^+$	100	−	2.2	3.2	0.8	12	622
		+	2.2	3.2	0.9	12	640
5	100$^+$	−	2.3	3.1	30.3	22	232
		+	2.2	3.2	28.9	20	255

+ labelled with ^{15}N   ++ % Ndff = % N in plant derived from fertilizer.

for each plot. The last column of Table III. gives the means of the A values. It is evident that inocculation had no effect on the A value and therefore the mean of the fertilizer treatments was calculated by bulking the data for presence and absence of inocculation.

Table IV. shows the mean "A" values of phaseolus experiment.

Table IV. seems to suggest that differences in "A" value due to rate of application are not significant. The differences due to time of application are highly significant. This could mean that rate of N application has little effect on the amount of nitrogen available from symbiotic N fixation. However, when nitrogen is

TABLE IV - Mean "A" Values of Phaseolus Experiment.

Treatment in kg N/ha			
Seeding	Flowering	"A" Value in kg N/ha	
5^+	0	609	LSDat P = 0.05
100^+	0	488	= 228
5^+	100	631	
0	5^+	331	LSD at P = 0.05
0	100^+	191	= 307
5	100^+	244	

$^+$labelled with ^{15}N.

applied as a fertilizer, the amount of N taken up from soil + symbiotic fixed N is reduced, but the rate of supply of N from soil + symbiotic N is not affected.

From Table III it is evident that much more fertilizer nitro-gen is taken up from top dressing than from application at seeding. The corresponding difference in "A" value due to this time effect (Table IV) is understandable but difficult to interprete because this difference may be due to several factors such as changes in the intensity of N fixation, root development, changes in soil N supply etc.

TABLE V - Results of Trifolium Experiment (Field).

Treatments kg N/ha			
at seeding	at flowering	inocculation	% Ndff
5^+	0	+	0.1
5^+	0	-	0.3
0	5^+	+	1.5
0	5^+	-	1.4
100^+	0	+	0
100^+	0	-	0
0	100^+	+	23.6
0	100^+	-	24.8
5^+	100	+	0.1
5^+	100	-	0
5	100^+	+	20.1
5	100^+	-	24.4

TABLE VI

Mean "A" Value for Clover Experiment.

Treatments in kg N/ha		% N derived from fertilizer	"A" Value in kg/ha
Seeding	Flowering		
0	5⁺	1.5	330
0	100⁺	23.7	320
5	100⁺	22.3	360

Trifolium

The % Ndff of the clover experiment is shown in Table V.
Due to the extremely dry spring, irrigation had to be given
continuously during germination of the crop.

This explains why the applications of $NaNO_3$ at seeding
failed to give any response. Apparently all NO_3 had been leached
out of the rooting zone when the plants had established their
root system.

The "A" values that were determined for the top-dressed
treatments (Table VI) confirm the findings of the bean experi-
ment: i.e. rate of N application had no effect on the availability
of soil + symbiotic N.

Conclusions

From the results of preliminary sandculture experiments with
beans, it can be concluded that root nodules developed
only when nitrogen was applied in the NH_4 form. There was no indication
of any symbiotic fixation in the presence of abundant NH_4 supply during
a one month growing period.

The field experiment with beans did not show any yield responses
to 100 kg N/ha. Although the N fertilizer is taken up by the crop at
the expense of soil + symbiotic N, it can be inferred from the "A"
values that the applications of N did not affect the availability of soil
+ symbiotic N. This would mean that the process of symbiotic N fixation
is not inhibited by N fertilization. The utilization of topdressed N
is higher than that of N applied at seeding. The experiments do not allow
for an interpretation of the drop in "A" value between seeding and flowering.
The experiment with trifolium failed to show any nitrogen uptake from the
application at seeding, due to removal of NO_3 with irrigation water during
germination of the seeds. The "A" values found for topdressing confirm
the findings of the bean experiment.

HBroeshart/pe   11 May 1972

CONCLUSIONS AND RECOMMENDATIONS

1. Panel Objectives

    (i)   To advise on the potential for a research project to study how
          fertilizer utilization by grain legume crops can be maximised
          without losing any economic benefits from their nitrogen fixing
          capacity,

    (ii) Should such a programme be considered justified, to define its
          scope and direction, and

    (iii) To formulate draft proposals for implementing the project.

2. Conclusions

    The information presented at the meeting led to the following con-
clusions:

    (i)   Grain legume crops such as beans, groundnut, soya beans, chick peas,
          pigeon peas etc. have shown economic yield responses to the appli-
          cation of phosphorus (50 - 80 kg P_2O_5/ha) and starter nitrogen
          (10 - 30 kg N/ha).

    (ii) Sometimes economic yield responses are obtained to heavier appli-
          cations of nitrogen, e.g. in the acid soils of the humid tropics
          (pulses — India, beans — Latin America, groundnuts — West
          Africa), in areas newly planted to legume crops and in some
          areas where they are grown in rotation with non-leguminous
          crops (soya bean — Romania, beans — Latin America), and under
          conditions of intensive farming (soya bean — U.S.A.)

(iii)   Inoculation with effective nitrogen fixing strains of rhizobium
          is often necessary for satisfactory grain legume production
          though not always practiced.

(iv)   Economic yield responses have also been obtained to potassium,
          magnesium, sulphur, calcium, molybdenum, zinc and vanadium.

(v)   Inadequate information is available on the value of nitrogen
          applications later in the growth stage of legume crops and the
          effect of such applications on nitrogen fixation. There is some
          indication that late application, at flowering time, can increase
          seed nitrogen content and yields (soya bean).

(vi)   Inadequate information is available on how and when fertilizers
          should be applied for maximum efficiency of utilization.

(vii)   Under conditions suitable for rhizobium survival and activity,
          nodule functioning and host plant growth, legume crops can derive
          most, if not all, of their nitrogen requirements through fixation
          of atmospheric nitrogen provided that an effective rhizobium
          strain is present. If such conditions are not met (e.g. liming
          materials may not be economically available for improving soil
          reaction, innoculants may be ineffective), legume crops may
          have to depend on fertilizer nitrogen for maximising production.

(viii)   While recognising that higher grain production from legume crops
          may involve a breeding and selection programme, it is considered
          that from an immediate practical point of view the question of
          efficient fertilizer practice merits serious study.

(ix)   The tracer technique offers the most effective means of investigating
          the fertilizer problems indicated above.

3.   Recommendations

(i)   The panel recommends that in view of economic yield responses obtained
          to fertilizer application in various grain legume crops in different
          regions of the world, a coordinated research programme be initiated
          using tracers to study:

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a)   The impact of fertilizer application on nitrogen fixation.

b)   Factors influencing the efficiency of fertilizer utilization
     with particular reference to nitrogen and phosphate fertilizers.

The object of this study would be to see how maximum use could be
made of the nitrogen fixation process towards meeting the demands of
legume crops for nitrogen and how best fertilizer should be applied
when this is necessary.

(ii)   The panel recommends that the choice of crops to be studied and the
       countries participating be determined by the economic importance of
       the crops to the respective countries - e.g. soya bean (glycine max)
       in the East European region, broad bean (Vicia faba) in the Middle
       East, groundnut (Arachis hypogaea) in West Africa, bean (Phaseolus
       vulgaris) in Latin America, and chick pea (Cicer arietum) and pigeon
       pea (Cajanus Cajan) in India.

(iii) The panel recommends a basic field experimental programme to study
       the effects and interactions of inoculant fertilizer nitrogen and
       phosphorus.

       In planning the experiments, the need for examining the following
       factors should also be borne in mind:

a)   Rates, time, method of application and source of N and P.

b)   The effects of other nutrients on nitrogen fixation.

c)   The effects of other cultural practices including irrigation, and
     liming of acid soils.

(iv)   Nodule observations on selected individual plants to be done as
       indicated in the appendix.

(v)   The panel recommends that in addition to the usual data on soil
       characteristics and climate (including meteorological data for evalu-
       ating potential evapotranspiration), the following observations should
       be made at each site:

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a)   Maximum soil temperature at seed level daily for 7 to 10 days from sowing - (desirable), for a further 7 to 10 days (desirable) and then maximum weekly temperatures if possible.

b)   Soil moisture measurements or at least observations at the same positions and frequency as for soil temperature.

(vi)   The following microbiological observations should be made on selected plots:

a) 1.   Rhizobium counts in soil before and after sowing.

2.   Rhizobium counts in rhizosphere at late seedling stage - about 4 weeks [*]

b) 1.   Type of rhizobia in soil at sowing, and at late seedling stage.

2.   Type of rhizobia in rhizosphere at late seedling stage.[*]

3.   Type of rhizobia in nodule at late seedling stage[*], flowering and mid-pod stage.

[*] These measurements are essential for studying the establishment of the inoculum in the soil and nodule.

A P P E N D I X

Nodule Observations on Selected Individual Plants

t_2 late seedling (about 4 weeks when nodules are clearly visible but plants are not too large).

t_3 flowering

t_4 middle pod

Parameter	Time		
	t_2	t_3	t_4
Nodule number per plant a) crown b) laterals	e	d	d
Nodule weight per plant	d	d	d
Nodule N content	p	p	p
Nodule structure	p	p	p
Nodule colour when sliced	e	d	d
Acetyline reduction of whole or part of root	d	d	d

e = absolutely essential

d = desirable

p = possible

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