CHAPTER IV

THE INFLUENCE OF THE ENVIRONMENT ON SYMBIOTIC EXPRESSION IN ARACHIS HYPOGAEA.

RESOLUTION OF ENVIRONMENTAL FACTORS:

Studies on strain variation in nitrogen fixation, host variability and symbiotic development must necessarily have reference to the factors of the environment. For, the environmental factors exert a decisive influence in symbiotic development through their effects on host physiology (Nutman, 1956; Norris, 1958).

The environmental factors constitute:

1. Physical factors: Air, Moisture, Light, Temperature and Reaction.
2. Nutritional factors: The supply of elements like nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, boron, zinc, copper, molybdenum, chlorine and cobalt.
3. Biological factors: Effects of micro-organisms
    a) Native rhizobia
    b) Bacteriophages
    c) Antagonists
    d) Effects of higher plants
    e) Effects of insects.

The influence of the environment on symbiotic nitrogen fixation has recently been summarised by Van Schreven (1958).

Under tropical conditions of plant growth the light factor as well as the acidity or alkalinity of soils are of great interest. Indeed recently Masfield (1958) says "Turning to the effect of the environment on nodulation, the physical factors may be considered first. Of these the light factor raises special problems in the tropics under atleast four headings.

1. The short day length of equatorial latitudes.
2. The reduction in light intensity in dense tropical forests, where many legumes grow.
3. The shading of leguminous crops which are interplanted with tall cereal crops - a very common practice in the tropics;
4. The extreme cloudiness of some tropical climates during the rainy season, when for example in southern Nigeria, the sun may not be visible for days on end."

I. THE EFFECT OF LIGHT:

The influence of day length and light intensity on nodulation and nitrogen fixation has been studied by a number of workers. The capacity of legumes to form nodules varies with the species of host plant. On lucerne and vetch, in the complete absence of light, a few nodules are formed while nodules degenerate and cease to develop if the plants are placed in darkness (Wilson, 1931; Thornton, 1930 a). Soyabeans and clovers do not form nodules in the dark unless the plants were supplied with sugar (Schweizer, 1932). In peas, the number of nodules formed in the dark deceases on a supply of sugar, although their size increases (McGongale, 1949). An optimum light intensity was found essential for maximum nodulation and nitrogen fixation by several workers (Fred and Wilson 1934; Orcut and Fred, 1935; Fred, Wilson and Wyss, 1938; Raju, 1936, 1939). Conversely, too high a light intensity might adversely affect fixation through excessive carbohydrate accumulation leading to decreased nitrogen fixation. A reduction in light intensity by less than 50% of the normal light intensity decreases the nodule number, although the size of the nodules is reduced by even a moderate reduction in light intensity. Rudakov (1961) found that long and short day varieties showed an effective response only when the plants were grown in their respective day lengths. Bonnier and Sironval (1956) found that soyabean plants could develop large pink and effective nodules in a 16 hour day, while in an 8 hour day the nodules were small or did not develop at all. Bonnier, Sironval and Verlinden (1957) related the effect of day length on nodule formation to the control of the growth rate of the host plant by day length.

Since the photoperiod controls morphogenesis in many plants, a study of the effect of day length with particular regard to nodule size and abundance, seemed desirable, especially in view of the short day length of tropic situations.

In the present work the influence of normal day light and day length for various periods on nodule size and abundance, nitrogen fixation and haemoglobin synthesis were studied in sand cultures in the bunch variety of groundnuts (Arachis hypogaea, var. TMV 2) using an effective and specific Rhizobium strain (R_4).

The process of sterilization, seed sowing, inoculation, provision of nutrient solution and sterile water and care of plant assemblies have been described earlier.

The total number of pots for each photoperiod was: 5 inoculated and 5 uninoculated pots with 6 plants in each pot. Thus, 40 pots were maintained under four photoperiodic treatments of 4, 6, 8 and 10 hours. The plants were given photoperiod treatments by placing them in the sun outside the green house on a raised clean platform for various lengths of time. After the photoperiodic treatments, the plants were removed to a dark chamber and kept there until a subsequent repetition of the treatment. The practice of providing natural illumination of the sun and growing plants outside the green house was in order to simulate natural conditions as much as possible. The efficiency of this procedure was shown in that the uninoculated controls belonging to each photoperiodic treatment did not show nodulation, whilst the inoculated plants nodulated as well as under field conditions (in the maximum light treatment).

PLATE IV

PLATE IV

The figure shows shoot development in groundnut in relation to day length.

PLATE V

Fig.a

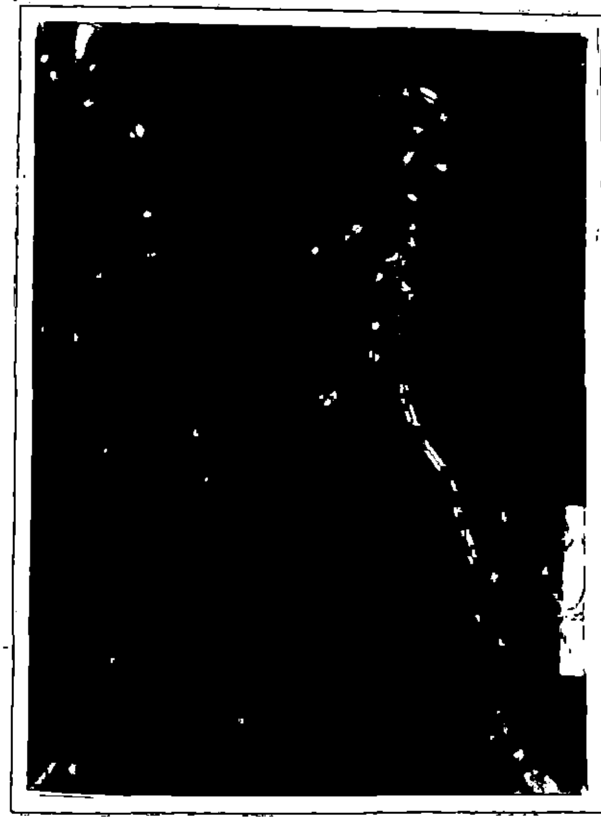

Fig.b

Fig.c

Fig.d

PLATE V

The figures illustrate root and nodule development in *Arachis hypogaea* grown under different day lengths:

a. 4 hours
b. 6 hours
c. 8 hours
d. 10 hours

(The white strip in fig. c. represents the scale: 1 inch).

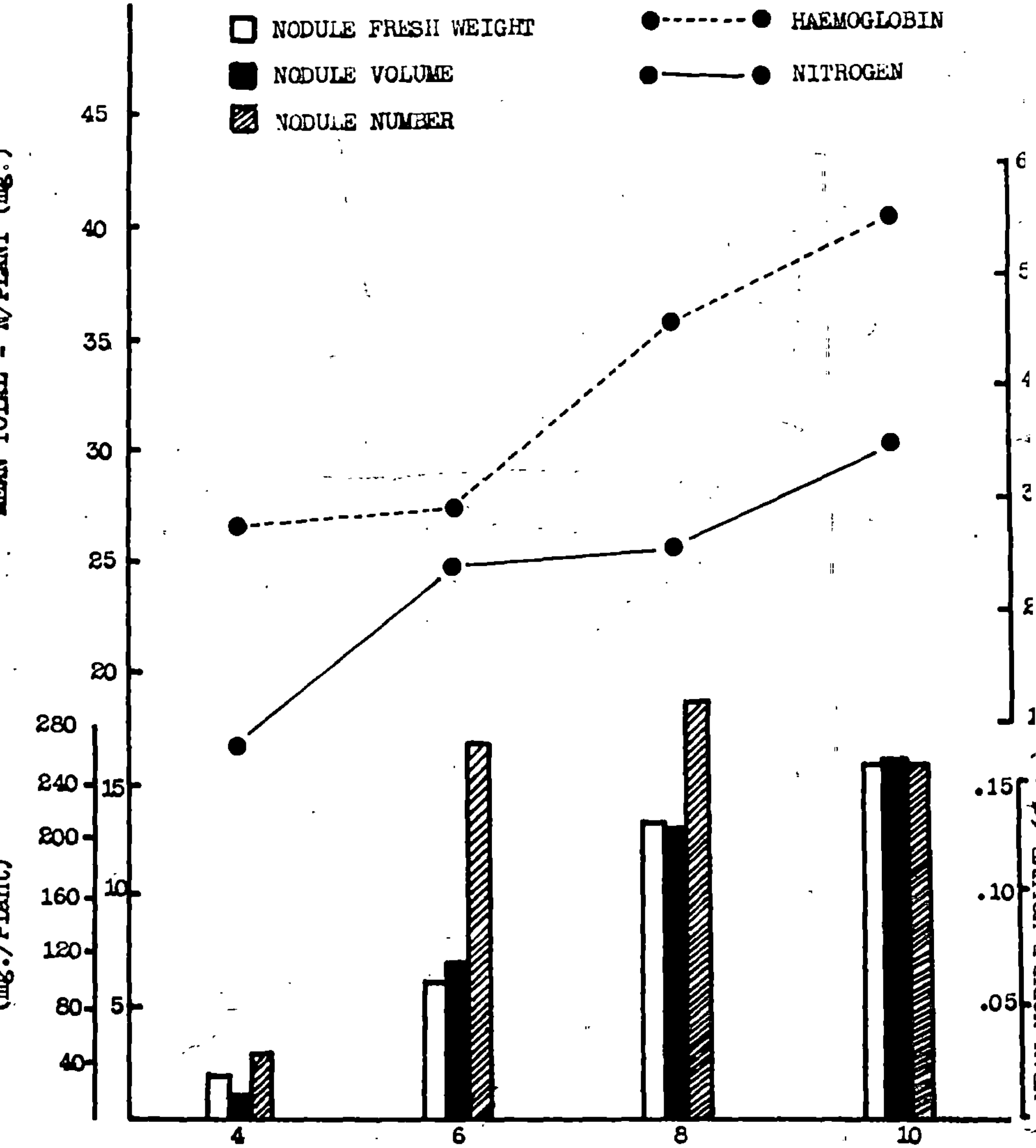

Fig.10. THE EFFECT OF DAY LENGTH ON NODULATION, NITROGEN FIXATION AND HAEMOGLOBIN CONTENT OF ROOT NODULES IN ARACHIS HYPOGA

The plants were harvested at the end of 45 days, for, at this time the peak of flowering is attained under field conditions of plant growth.

Nodules were carefully excised, washed, dried, weighed and their volume and haemoglobin concentrations were determined as described previously. The shoots of five plants from each treatment were dried to constant weight at 100°C, accurately weighed and nitrogen determinations made according to the Kjeldahl procedure (Humphries, 1956). Plates IV and V (a, b, c, d) illustrate the development of shoots, roots and nodules of plants grown at various day lengths.

<u>MEAN NODULE NUMBER:</u> A reduction in day length to 4 hours caused a very poor development of nodules and a few white nodules had developed on the roots (fig. 10).

<u>MEAN NODULE WEIGHT:</u> The mean nodule weight steadily increased with increasing photoperiod although a reduction in light to 4 hrs. has drastically reduced mean nodule weight to less than 50% of the 6 hour treatment. The mean nodule fresh weight showed an increase of 50% in the 8 hour treatment when compared to the 6 hour photoperiod. At 10 hours the mean fresh weight of nodules had attained a maximum (fig. 10)

MEAN TOTAL NITROGEN:

From the mean total nitrogen of plants, it was observed that a reduction in day length to 4 hours drastically decreased nitrogen fixation. There was no significant difference in terms of nitrogen fixed between the 6 hour and 8 hour treatments, whilst at 10 hours nitrogen fixation showed a fivefold increase, when compared to the 4 hour treatment (fig. 10)

ROOT NODULE HAEMOGLOBIN:

The haemoglobin content of root nodules increased only when the day length was more than 6 hours. Between 4 and 6 hours photoperiod the haemoglobin content of root nodules did not show a significant difference. An increase in photoperiod over six hours caused a pronounced and significant increase in nodule haemoglobin content, with a maximum formation of the pigment at 10 hours. The effect of day length on all these factors are discussed subsequently (fig. 10)

II. THE EFFECT OF SOIL REACTION:

While it is well known in temperate countries that extremes of soil reaction could adversely affect nodulation, the importance of this factor does not seem to have been studied widely in the tropics. However, the effects of soil acidity itself on the growth of plants have been studied widely.

The reaction of the soil not only influences the growth of the rhizobia and the formation of nodules but the growth and uptake of nitrogen by the host plant are affected as well. Soil acidity constitutes a complex of a number of factors, the more important of which are toxicity of aluminium, manganese or molybdenum (Hewitt, 1952). The problem is more complicated due to the fact that these elements not only act independently but also in some cases react with one another.

Besides these factors of the soil complex, rhizobia themselves are known to behave differently towards soil activity. A soil pH of 5.0, 4.5-4.7 and 3.7-3.9 bring about the inactivation of rhizobia from lucerne, red clover and soya bean (Bryan, 1923). Jensen (1943) observed the formation of nodules of Trifolium seedlings grown in agar at a pH of 4.5 and even at pH 4.2 while the natural soil nodules were formed at pH 4.9-5.0 and in sand medium even at 4.2 to 4.5. According to Oslen (1925) a sparse nodulation in Trifolium occurs at pH 4.0 while pH 5.0 (Sewell and Gainey, 1930) and pH 4.5 to 4.8 were found to be more favourable (Jensen, 1948). While nodulation was favoured within the entire range of pH suitable for plant growth (Joffee, 1920; Bryan, 1922), Virtanen (1928, 1953) and Fletcher (1958) reported that clover bacteria were more sensitive to soil reaction than clover plants. Working with thirteen different strains from different legumes Cabezas de Herera (1956) showed that many rhizobia were modified into bacteriod, coccal or L-shaped forms at pH 4.0 and these were unable to induce nodulation while the same rhizobia became infective when nutrient solution at pH 5.6 was supplied. In a seven course rotation of clovers extending over a period of two years Fedorov and Egorov (1957) found a decrease in virulence and nitrogen fixing ability of clover rhizobia at a soil pH 5.3-5.7. In the Netherlands, a study by Harmsen on the distribution in soil of effective and ineffective strains of clover rhizobia revealed that while diluvial soils favoured growth of rhizobia, the acid peat bog soils contained a great majority of ineffective strains.

It is, therefore, possible that the nitrogen fixing capacity as well as variation of rhizobial strains may be influenced by the reaction of the soil. Besides, many tropical legumes are unknown to nodulate well in soils of a low pH in contrast to temperate legumes. For these reasons an understanding of soil reaction effects was sought in relation to nitrogen fixation in *Arachis hypogaea*.

The effect of soil reaction on nitrogen fixation in Arachis hypogaea, var. TMV 2.

Preparation of soil and plant assemblies: River sand was freed from larger particles by sieving through a fine mesh (2 mm) and the sand was thoroughly washed several times with tap water. The sand was air dried and dispensed into glazed containers. A total of 48 pots for six reaction levels ranging from 3.0 to 8.0 with 8 pots equally divided for inoculated and uninoculated controls for each reaction level were maintained in the green house.

PLATE VI

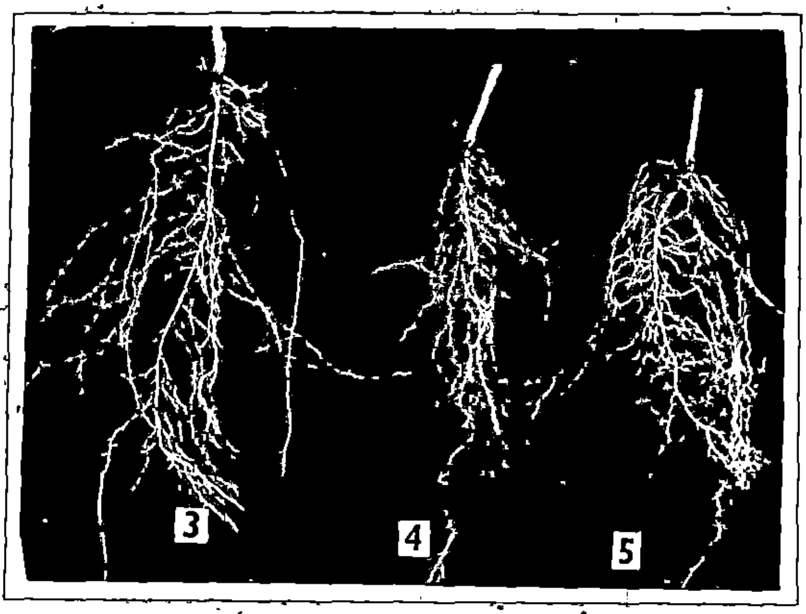

Fig.a

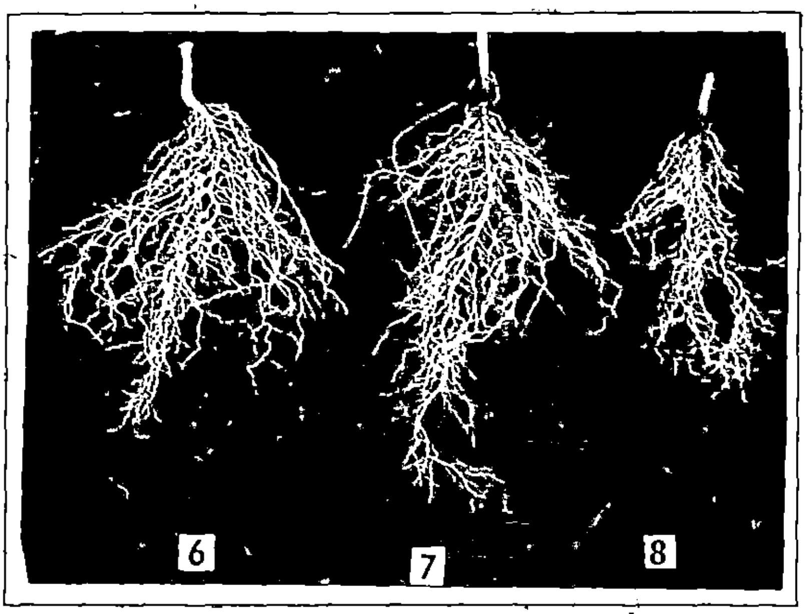

Fig.b

PLATE VI

Figs. a and b illustrate root and nodule growth in relation to soil reaction (pH 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0)

The sterilized sand in the pots were watered thrice daily for a period of 20 days with sterile distilled water adjusted to various reaction levels in order to sterilise the pH levels in the sand to the desired levels prior to sowing. At the end of this period, seeds were sown. Seed sterilisation, sowing, rhizobial inoculation and care of plant assemblies in the green house are as described previously. The plants received sterile distilled water adjusted to the various pH levels. Plant nutrient solution similarly treated was poured once in every week (*vide* Materials and Methods). The plants were grown in the green house under broad day light conditions for a period of 45 days. At the end of this period incoulated and uninoculated control plants at various reaction levels were carefully freed from sand and analysed.

Determinations of total nitrogen/plant and root nodule haemoglobin were made as described earlier. The mean values for each reaction level based on an analysis of 9 plants are presented in fig. 11. Plate VI (a & b) illustrate the effects of soil reaction on root and nodule development in *Arachis hypogaea*.

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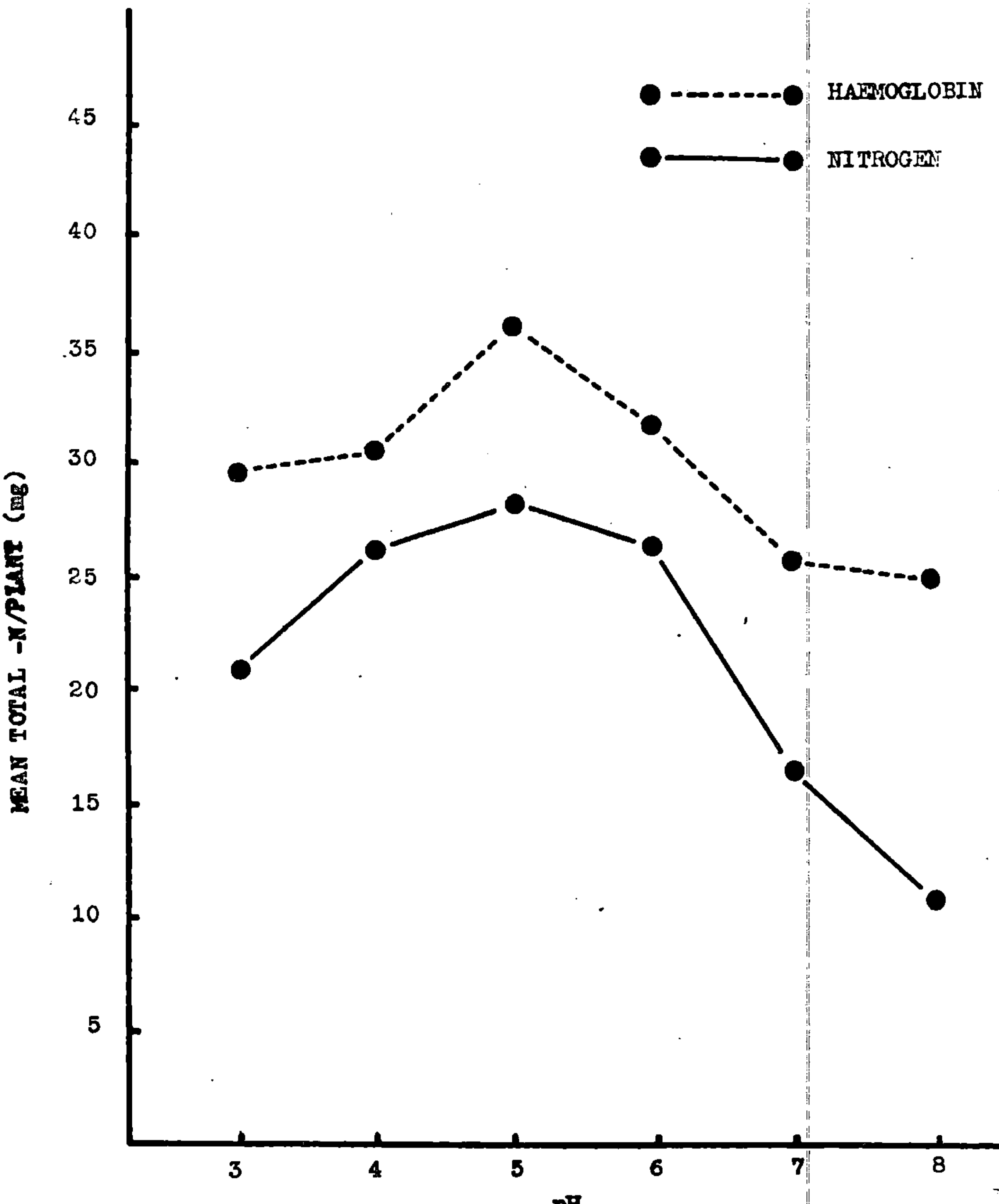

Fig. 11. INFLUENCE OF SOIL REACTION ON NITROGEN FIXATION AND HAEMOGLOBIN CONTENT IN <u>ARACHIS</u> <u>HYPOGAEA</u>

OBSERVATIONS:

Under the conditions of the experiment, the plants in symbiotic association fixed more nitrogen at pH 5.0 than at other reaction levels. The values for mean total nitrogen per plant showed a decrease at pH 3.0 while at pH 7.0 and 8.0 there were definite decreases of plant total nitrogen (fig. 11). At pH 7.0 and 8.0 the plants were distinctly chlorotic.

The concentration of root nodule haemoglobin was comparatively high at pH 5.0 than at pH 3.0, 4.0 and 6.0. The pigment concentrations decreased in root nodules at pH 7.0 and 8.0.

Thus pH levels of 4.0, 5.0 and 6.0 were found suitable for both plant growth and optimum nitrogen fixation and haemoglobin formation, with a most favourable effect at pH 5.0, while the performance deteriorated toward pH 3.0, 7.0 and 8.0.