CHAPTER VII.

THE EFFECT OF VIRUS INFECTION ON NODULE FORMATION.

As pointed out in an earlier section, the influence of day length in regulating haemoglobin formation, nitrogen fixation and the parallel development of haemoglobin in nodules and chlorophyll in leaves suggest that nodulation proceeds parallel with the accumulation of chlorophyll in the leaves. Such a physiological connection in the development of haemoglobin and chlorophyll was also suggested by Bonnier et al., (1957) working on soya bean.

Since haemoglobin and chlorophyll are known to have common biosynthetic pathways of development (Granick, 1951; Rimington, 1957) a marked reduction in chlorophyll such as occur in mosaic virus diseases might affect nodule development through derangement in host function. For instance, a low carbohydrate content and a low C/N ratio are known to be the effects of virus infection in mosaic diseases (Bawden, 1950). This can impair nodule formation through inadequate carbohydrate supply to the roots and this, in spite of the fact that mosaic diseases do not affect the phloem as do the leaf rolls (Bawden, 1950).

Another aspect of nodule physiology in relation to virus infection hinges on the formation of haemoglobin in root nodules. Since haemoglobin bears a causal relationship to nitrogen fixation (Virtanen, 1955; Hamilton, Shrug and Wilson, 1957) one determinant of an effective symbiosis constitutes the synthesis of haemoglobin in root nodules. For this synthesis to take place it was suggested that pyrroles or their percursors may form in the shoot and be transported to the roots (Falk, Appleby and Porra, 1959). However, in view of the fact that viruses alter metabolism through their effects on translocation, ion balance, chlorophyll synthesis, respiration, carbohydrate formation, protein synthesis and growth in general, it appears tenable to state that virus infection might affect this process. It is all the more evident, since virus diseases are regarded essentially as a change in the protein metabolism of the host cells (Bawden and Pirie, 1956).

It was, therefore, deemed desirable to study the influence of virus infection by Dolichos Enation mosaic virus on nodulation in Dolichos lablab.

PLAN OF WORK:

Healthy seeds of the variety DL 231 of Dolichos lablab were sown in sterilized sand in glazed containers. The plants were inoculated with an effective strain of Rhizobium (R_4) at the time of germination of the seedling, as was the practice in previous work. Inoculation with the virus (DEMV) was done by rubbing the primary leaves, 10 days after germination with an extract prepared from young leaves of Dolichos lablab showing severe symptoms of mosaic from systemically infected plants (vide Materials and Methods).

The green house sterile sand culture procedures consisted of the maintenance of the following four series of pots.

A. Dolichos lablab plants (DL 231) alone.

B. DL 231 + inoculation with rhizobia (R_4)

C. DL 231 + R_4 + inoculation with virus (DEMV)

D. DL 231 + DEMV.

Each pot contained three plants and the four series replicated five times were maintained in the green house. The temperature of the green house ranged from 22°C to 27°C.

Field cultivation of plants for the study of the effects of virus infection on nodulation were done alongside green house sand culture work. In the field two plots, 20' x 10', were used, one each for growing healthy and virus infected plants. The two plots were spaced apart from one another and received separate routes of water supply from a single source. Although the plants meant for the infected series were inoculated with virus, the rhizobial inoculum was not given in order to study the impact of virus infection under natural conditions of field growth where nodulation of plants depended on the dual factors of (a) the number of rhizobia in the soil and (b)the changes of infection as occur under natural conditions.

Unsterile pot cultivation was again resorted to in order to study the influence of virus infection under conditions where soil factors could perforce be made more favourable by provision of an abundance of rhizobial inoculum and creating optimum conditions for the spread of the rhizobia in the rhizosphere.

Whilst in the field 50 plants were grown widely spaced apart in soil well sifted, in pots under unsterile pot cultivation the healthy and infected series included 30 pots equally divided among the healthy and infected treatments, with five plants in each pot.

While the green house grown plants received sterile tap water regularly and nutrient solution free from combined nitrogen once in 15 days, the field grown and unsterile pot bound plants outside the green house received water supply regularly at the full complement of field saturation capacity without however undue waterlogging. Thus, care was exercised in growing the plants under green house, field and pot culture conditions, in order to minimise factors that might introduce variation in uniformity of experimental conditions.

The methods of virus and rhizobial inoculation are as described earlier (vide Materials and Methods). Field experiments were repeated twice on two different occasions. The plants were harvested at the end of 80 days following sowing from the field, whilst a period of 100 days were allowed to elapse prior to harvesting the plants from sand cultures in the green house. These times were chosen since they corresponded to a time just prior to the flowering of the host plant.

At the time of harvest the root nodules from a batch of 20 plants each from the field, green house, sand culture and unsterile pot culture series were carefully removed from healthy and infected plants. Nodules were carefully removed and the soil around plant roots were sifted thoroughly to collect any nodule that might have got detached.

The progress of virus infection under green house and field conditions of plant growth was determined serologically (vide Materials and Methods) and is represented in Table 10.

TABLE 10.

The progress of virus infection under green house and field conditions of plant growth in Dolichos lablab (DL 231). Temperature: 22°-27°C (November/January).

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Days after virus inoculation.	Relative Serological Virus Titre (~~Dilution end points~~).			
	Green House		Field.	
	RH + V	V	H	V
30	1/25	1/5	0	1/5
35	1/25	1/25	0	1/25
50	1/25	1/25	0	1/25
60	1/25	1/5	0	1/25
70	1/25	-	0	1/25

RH + V: Rhizobium inoculated, virus (DEMV) infected.

V: Virus infected.

H: Healthy

V: Virus infected.

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PLATE VIII

Fig.a

Fig.b

PLATE IX

Fig.a

Fig.b

PLATE VIII

The figures represents root and nodule development in relation to:

a) Healthy plants

b) Virus infected plants

PLATE IX

The figures illustrate the effects of virus infection (DEMV) on plant growth in Dolichos lablab infected with Dolichos enation mosaic virus.

Fig. a. shows the treatments:

A. Dolichos lablab. DL 231 alone.
B. DL 231 + inoculation with rhizobia (R_4).
C. DL 231 + R_4 + inoculation with virus (DEMV).
D. DL 231 + DEMV.

b. An infected leaf of Dolichos lablab showing cup-like laminar outgrowths and filiform enations.

The Dolichos Enation Mosaic Virus was serologically demonstrable in the infected plants of Dolichos lablab both under conditions of inoculation with Rhizobium as well as without it in the plants grown in the green house in sand cultures (RH + V and V). In those which were infected with the virus, but which did not receive Rhizobium inoculation, the serological virus titre showed a decrease after 60 days of growth in the host plant. In the field, the serological virus titre remained at a constant tissue level for a further period of time.

The methods adopted for nodule excision, determination of their number, weight and volume as well as haemoglobin in root nodules were the same as those described previously.

The observed effects of virus infection on nodulation in Dolichos lablab are presented in plates VIII & IX and Tables 11 and 12 and relate to

1. The increase in nodule number, weight and volume in virus infected plants in contrast to healthy plants.
2. The increase in root nodule haemoglobin consequent on systemic virus infection.

TABLE 11.

t of virus infection by Dolichos Enation Mosaic virus on Nodulation aemoglobin content in Dolichos lablab, grown under field conditions ll as under sterilised sand culture conditions (free from supply of ned nitrogen) and inoculated with an effective strain of Rhizobium(R_4).

	Green house sand culture.			Field cultivation.		
	Healthy.	Virus infected.	% increase on infection.	Healthy.	Virus infected.	% increase on infection.
ge nodule me cc/nodule	1.1	1.0	--	4.1	7.0	70.7
ge nodule ht mg/nodule	10.50	10.20	--	30.91	70.41	131.6
globin odule	2.10	5.30	152.3	19.10	23.61	23.0
globin/unit le volume	0.109	0.409	266.1	0.009	0.020	122.2

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The average nodule volume and weight do not exhibit any significant difference between the healthy and virus-infected plants grown in the green house in sand cultures with a specific Rhizobium inoculant. However, virus infection under field cultivation of the host plant resulted in an increase of 70.7 percent in mean nodule volume, whilst the mean nodule weight increased by 131.6 per cent. There was an increase of 152.3 percent in haemoglobin content per nodule in infected plant nodules over healthy ones. The increase in haemoglobin per unit nodule volume as a result of virus infection was 266.1 per cent in the green house grown plants. In the field this percentage was 122.2.

The increase in root nodule haemoglobin on a tissue weight basis (µg/g fresh weight of nodules) following virus infection of the host plant ranged from 16.7 to 36.2 as is shown in Table 12.

TABLE 12.

EFFECT OF VIRUS INFECTION BY DOLICHOS ENATION MOSAIC VIRUS ON ROOT NODULE HAEMOGLOBIN CONTENT IN DOLICHOS LABLAB.

Host plant.	Root nodule haemoglobin content (µg/g fresh weight of nodules).			
	Sterile sand cultures +	Pot culture unsterile soil.+	Field Soil I trial*	Field Soil II trial.*
Healthy	260	275	400	560
Virus-infected	330	340	545	655
% increase in heaemoglobin consequent on virus infection	26.9	23.6	36.2	16.7

+A pure culture of Rhizobium (R_4) was used as an inoculant.

*Inoculation with rhizobia was not done. Nodulation was a result of infection by the soil population of rhizobia.

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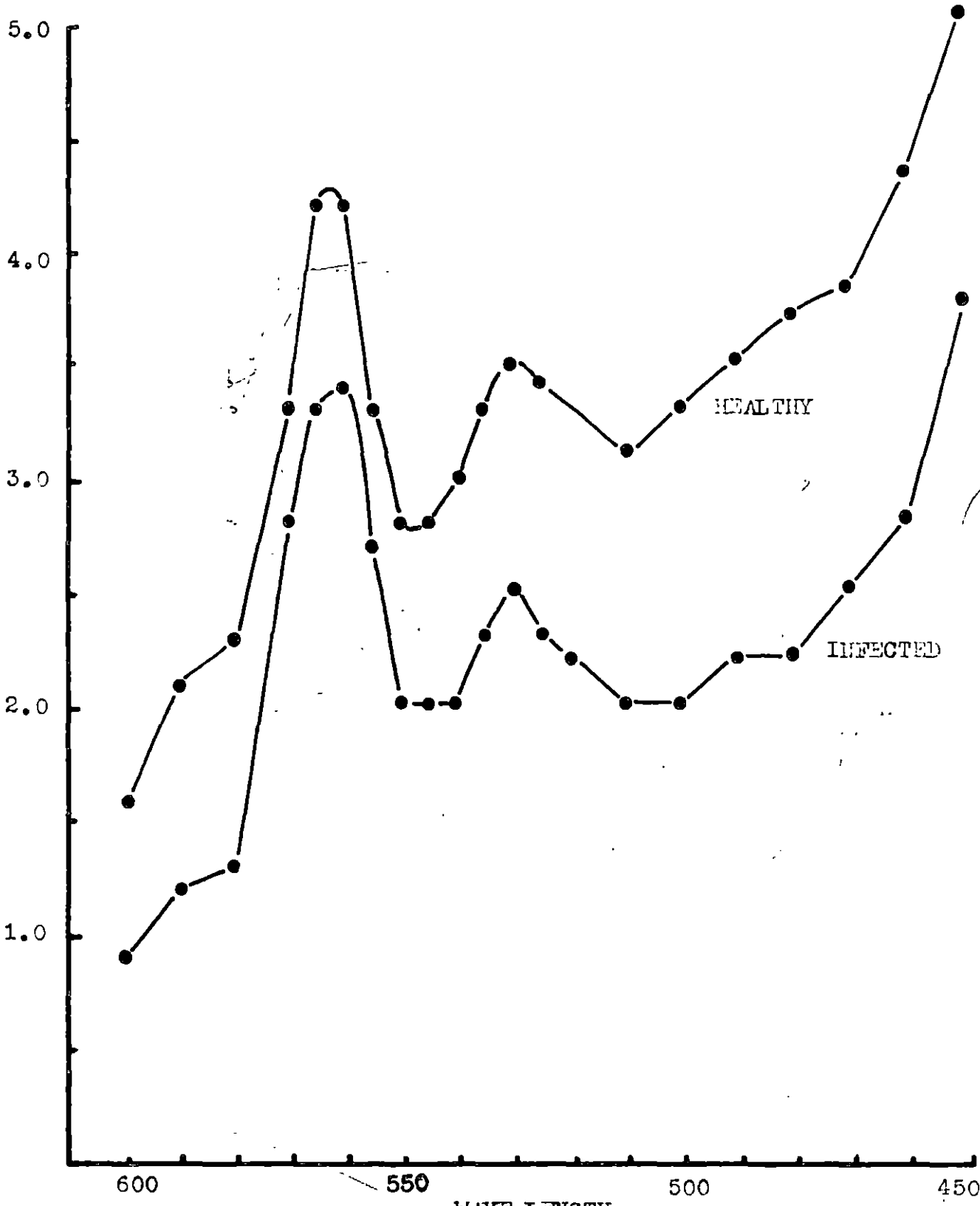

Fig. 17. SPECTRA OF PYRIDINE HAEMOCHROMOGENS OF PURIFIED HAEMOGLOBIN FROM THE ROOT NODULES OF HEALTHY AND VIRUS INFECTED PLANTS

$$\text{SP.EX}^{\circ}.K = \frac{\text{Optical Density}}{\text{mM Concentration of Haematin} \times \text{Optical Depth}}$$

Thus, an increase in root nodule haemoglobin content was associated with virus infection in Dolichos lablab.

The pigments contained in the healthy and virus-infected plant nodules were extracted as described previously and their absorption spectra were studied after conversion of the haemoglobin into solutions of pyridine haemochromogen. The absorption spectrum of the pigment (fig. 16) from the healthy plant nodules differed from that of the infected plant nodules in the presence of a band at 610 mμ in the former and its absence in the latter. Subsequently, the haemoglobin from the healthy and virus-infected plant nodules were obtained in a purified state through ammonium sulphate precipitation (vide Materials and Methods). The purified pigments from the healthy and infected plant nodules were converted to solutions of pyridine haemochromogen and their absorption spectra were studied. The specific extinction against wave lengths of the two pigments are presented in fig. 17. The spectra show that the pigments obtained on purification from healthy and virus infected plant nodules were essentially identical.