PHYSICAL ENVIRONMENT AND SYMBIOTIC NITROGEN FIXATION

V.* EFFECT OF TIME OF EXPOSURE TO UNFAVOURABLE ROOT TEMPERATURES

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Summary

Symbiotic nitrogen fixation by *Trifolium subterraneum* L., inoculated with three strains of *Rhizobium trifolii* Dang., was examined over the range of root temperatures 8–28°C. The plants were transferred from an optimal temperature for nitrogen fixation (23°C) to other temperatures at three stages, namely (1) immediately after inoculation, 3 days after germination, (2) 14 days after germination, when nitrogen fixation had commenced, and (3) 21 days after germination, when the plants had been fixing nitrogen for at least 7 days. Nitrogen increase and dry weight accumulation were determined for two growth periods—days 14–21 (I) and days 21–28 (II).

The total amount of nitrogen fixed during any period was determined by four factors—the root temperature during the period, the amount of nitrogen previously fixed, the nitrogen percentage of the plants at the start of the period, and the bacterial strain that formed the nodules. The plants forming nodules at 8°C fixed little or no nitrogen during the course of the experiment, while those at 13°C did not fix any nitrogen until day 21. The longer the period at 23°C before transfer to a lower or higher temperature, the greater the amount of nitrogen in the plants at the start of any growth period, and the greater the amount of nitrogen fixed during that period. However, the root temperature controlled the relative nitrogen assimilation rate (R_N), and for plants transferred to 8–18°C on day 21, the additional nitrogen fixation during period I did not enable them to achieve a higher R_N than that of the plants growing at these temperatures from day 14. At all lower temperatures, differences in the rate of nodule establishment, as indicated by the amount of nitrogen fixed during the early stages, were found between strains, although the time to first visible nodule and rate of nodule appearance were the same. At 28°C, the R_N of plants transferred on day 21 was similar to that of the day 14 plants during period I. For all strains, the R_N of both day 3 and day 14 plants at 28°C declined markedly from period I to period II, and for strain NA30, and a lesser extent strain CC17, the plants appeared to be dying despite the relatively high R_N during period I.

Under some conditions it was observed that exceptionally high R_N values were achieved by both nodulated and nitrogen control plants, and this was accompanied by low relative growth rates (R_W). As the percentage nitrogen level in the plants increased, the R_N declined, and there was an increase in R_W, such that the ratio R_N/R_W approximated to unity. A form of regulatory mechanism ensuring that the plant is achieving the most efficient use of resources is proposed.

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I. Introduction

The preceding papers in this series showed that symbiotic nitrogen fixation by nodulated *Trifolium subterraneum* L. was retarded by root temperatures below 22°C, and depending on the strain of *Rhizobium trifolii* Dang., by root temperatures above 22–25°C (Gibson 1963, 1965). However, the effect of root temperature on nodule formation followed a different pattern; plants nodulated most rapidly, and with the highest rate, at 30°C root temperature while the maximum temperature at which nodules would form was 33°C, and the minimum was *c.* 7°C (Gibson 1967). The extent of the delay to nodulation increased disproportionately with each 5°C reduction in root temperature below 22°C.

Previous studies on the effect of root temperature on symbiotic nitrogen fixation may be divided into two broad groups. In one group, the plants were subjected to various constant root temperatures from the time of inoculation (Jones and Tisdale 1921; Mes 1959; Meyer and Anderson 1959; Pate 1962). Under these circumstances, the amount of nitrogen fixed was determined primarily by the effect of the root temperature on nodule formation rather than its effect on nitrogen fixation. In the second group, the plants were allowed to nodulate under favorable conditions before the different temperature conditions were imposed (Gibson 1963, 1965; Possingham, Moye, and Anderson 1964). As most of these studies did not contain combined nitrogen controls, it is difficult to compare the results obtained by the use of the two different approaches.

In this paper the two approaches are combined. Nitrogen fixation and dry weight increase by plants growing at suboptimal and supraoptimal root temperatures for fixation is compared with that of plants allowed to nodulate, and commence nitrogen fixation, under favourable conditions before exposure to adverse conditions. These results are also compared with those from plants kept a further 7 days at the favourable conditions before exposure to the lower and higher root temperatures. The plants are inoculated with each of three strains of *Rh. trifolii* which differ in their capacity for symbiotic nitrogen fixation, but not in the time taken to form nodules or the rate of nodulation.

II. Materials and Methods

(*a*) *Plant Culture*

The plants were grown on agar slants, with only the roots inside the test tubes, and the shoots freely exposed to the atmosphere (Gibson 1963). The plants were *T. subterraneum* cv. Mount Barker. They were inoculated with each of three strains of *Rh. trifolii* (TA1, CC17, and NA30) 3 days after sowing the germinated seeds. The inoculum was prepared from 3-day cultures grown on yeast extract mannitol agar slants and added to the seedling nutrient solution used to water the plants (10^6 bacteria/tube).

The plants were grown in controlled-environment cabinets (Gibson 1965) which provide independent control of root and shoot temperature with diurnal fluctuation in shoot temperature. The root temperatures were 8, 13, 18, 23, and 28°C, and the shoot temperature regime 23/13°C, coinciding with a 16-hr daily light period. The light intensity was 2500 f.c.

(b) *Experimental Procedure*

The general outline of the experiment is shown in Figure 1. Forty plants were transferred to 8, 13, 18, and 28°C immediately after inoculation on day 3 (this is called the D3 treatment). The remaining plants were held at 23°C until they had nodulated and nitrogen fixation had commenced. On day 14, 20 plants were transferred from 23°C to each of the other temperatures (D14 treatment), and this procedure was repeated with 10 plants 7 days later (D21 treatment). On day 14, 10 replicates of each strain treatment at each temperature were transferred back to 23°C for 7 days.

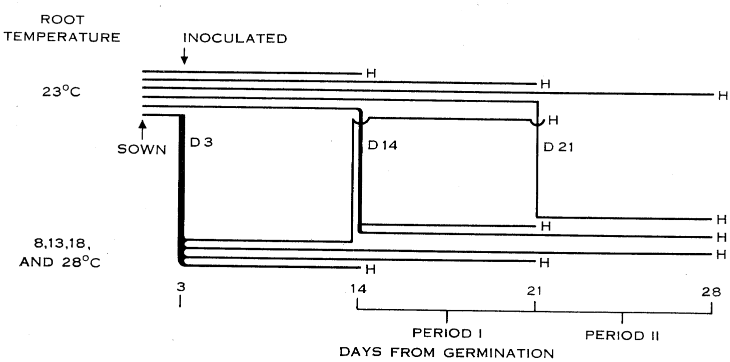

Fig. 1.—Diagrammatic representation of the experimental design.

On day 14 the plants in all strain × temperature treatments were ranked according to size, leaf development, and general appearance. They were then divided into 10 groups. One plant from each group was chosen at random for each of the treatment–harvest groups, e.g. for the D3 plants at 18°C, the treatment–harvest groups were (1) those harvested on day 14, (2) those kept at 18°C and harvested on day 21, (3) as for (2) but harvested on day 28, and (4) a treatment in which plants were transferred back to 23°C and harvested on day 21. In this way it was possible to calculate (Gibson 1965) relative growth rates (R_W) for individual plants, and relative nitrogen assimilation rates R_N for batches of three or four plants.

The nitrogen control plants received 0·2 mg nitrogen as ammonium nitrate on day 12, and a further 5 mg nitrogen on day 14.

The harvest interval day 14 to day 21 is referred to as period I, and that between day 21 and day 28 as period II.

III. Results

(a) *Initial Nitrogen Fixation*

By day 14 all inoculated plants at 23°C had commenced to fix nitrogen (Fig. 2; for the dry weight and total nitrogen values of plants at 23°C, see D14 and D21 data on day 14 and day 21 respectively at any root temperature). Plants nodulated by strain TA1 contained 0·52 mg nitrogen, an increase of 0·2 mg nitrogen over that in the seeds, whereas those nodulated by strains CC17 and NA30 had fixed 0·14 and

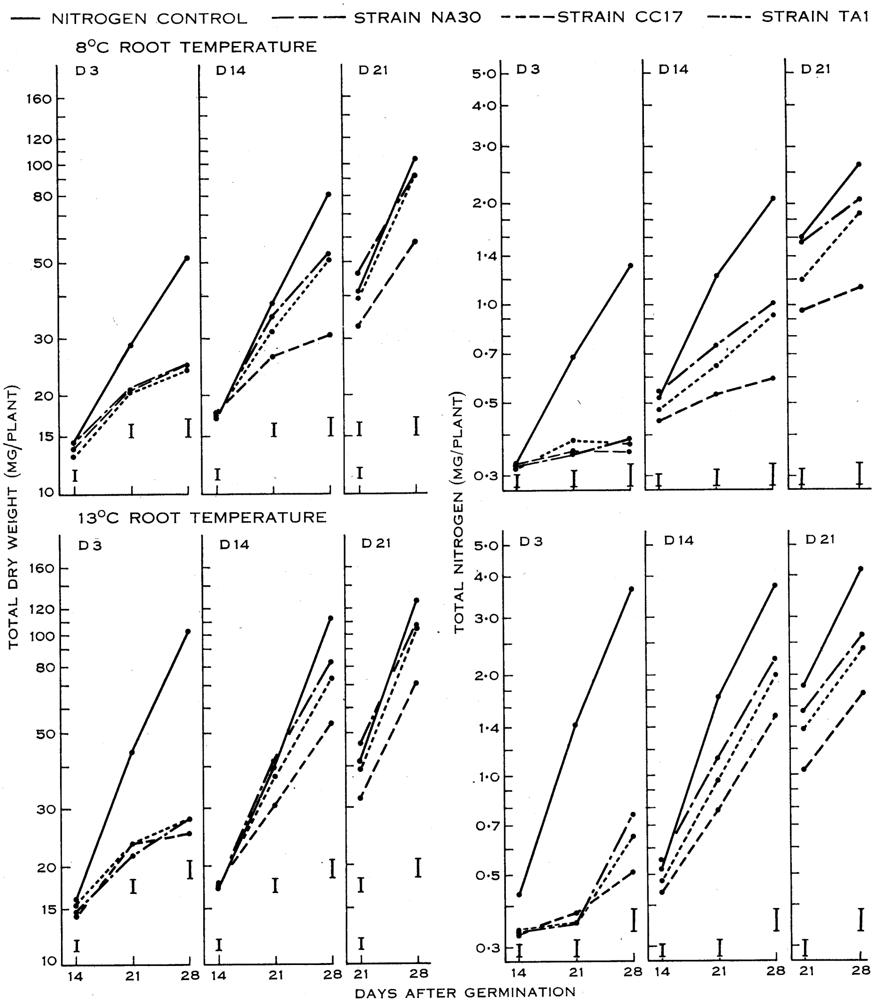

Fig. 2.—Dry weight and total nitrogen (mg/plant) values for inoculated (strain TA1, CC17, and NA30) and nitrogen control plants grown at 8, 13, 18, and 28°C root temperature from 3 (D3), 14 (D14), and 21 (D21) days after germination. Prior to transfer to these temperatures, the plants were grown at 23°C. The least significant difference values shown at the bottom of each figure also apply to comparisons between treatments harvested at the same time.

0·10 mg nitrogen respectively. The total amount of nitrogen fixed by plants at 28°C by day 14 was less than that fixed at 23°C (Fig. 2, D3 values). The strain effects were similar to those at 23°C. At 18°C there was a small but definite increase in total nitrogen over that in the seeds (0·32 mg nitrogen) but strain differences were not evident until day 21. At 13°C the plants formed nodules between days 10 and 16 (7–13 days after inoculation), and nitrogen fixation commenced on day 21 (approx-

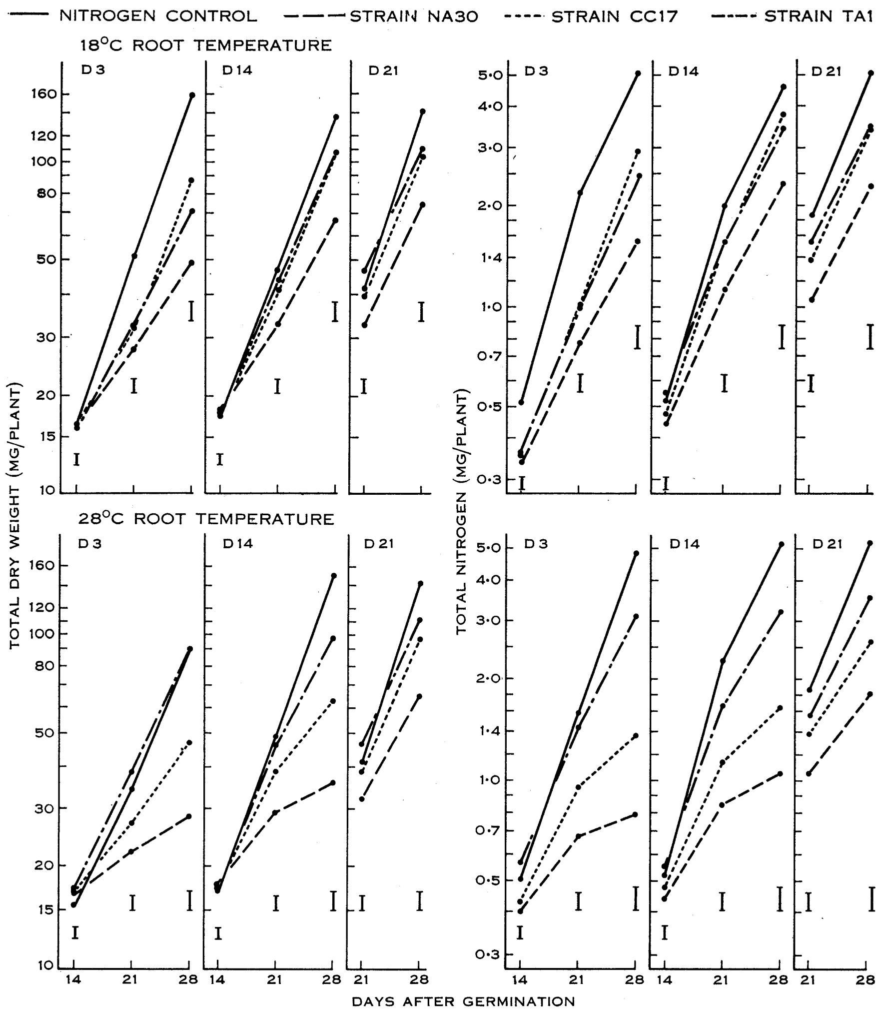

Fig. 2 (*Continued*).

imately). All plants at 8°C had nodulated by day 28, but little, if any, nitrogen was fixed during the course of the experiment. Nitrogen uptake by the nitrogen controls had commenced by day 14 at all temperatures except 8°C.

Dry weight differences were found between the D3 treatments on day 14, with the plants at 23 and 28°C being significantly heavier than those at 8 and 13°C. Differences in dry weight between strain treatments were small.

TABLE 1

RELATIVE GROWTH RATES, R_W (mg/mg/day), AND RELATIVE NITROGEN ASSIMILATION RATES, R_N (mg N/mg N/day), FOR PLANTS INOCULATED WITH THREE STRAINS OF *RH. TRIFOLII* (TA1, CC17 and NA30) OR PROVIDED WITH AMMONIUM NITRATE

The treatments at the various root temperatures started 3 (D3), 14 (D14), and 21 (D21) days after germination and the harvest periods were from day 14 to day 21 (I), and day 21 to day 28 (II). 10 replicates

Root Temp. (°C)	Treatment	Harvest Period	Nitrogen Control			Strain NA30			Strain CC17			Strain TA1		
			R_N ×100	R_W ×100	R_N/R_W	R_N ×100	R_W ×100	R_N/R_W	R_N ×100	R_W ×100	R_N/R_W	R_N ×100	R_W ×100	R_N/R_W
8	D3	I	10·8	9·7	1·11	1·2	5·9	0·20	2·8	6·4	0·44	0·5	5·1	0·10
		II	9·1	8·6	1·06	0·3	2·5	0·12	0·0	2·2	0·00	1·6	2·1	0·76
	D14	I	12·3	11·6	1·06	2·7	5·6	0·48	4·6	8·5	0·54	4·5	9·7	0·46
		II	7·9	10·7	0·74	2·1	2·3	0·91	5·1	5·7	0·89	4·3	6·3	0·68
	D21	II	7·7	13·2	0·58	3·6	8·4	0·43	5·0	12·3	0·41	4·2	9·7	0·43
13	D3	I	17·0	14·4	1·18	2·1	7·1	0·30	1·1	4·7	0·23	1·1	5·4	0·20
		II	13·4	12·1	1·11	4·1	1·2	3·42	8·8	3·7	2·38	11·1	3·8	2·92
	D14	I	14·4	12·2	1·18	8·4	7·7	1·09	10·2	11·0	0·93	10·3	12·3	0·84
		II	11·1	14·7	0·76	9·3	8·3	1·12	10·5	9·8	1·07	10·0	10·0	1·00
	D21	II	11·4	15·9	0·72	11·4	15·9	0·72	7·6	11·3	0·67	8·2	13·9	0·59
18	D3	I	20·8	16·5	1·26	11·9	7·3	1·63	15·0	9·4	1·60	14·6	10·0	1·46
		II	11·8	16·1	0·73	10·0	8·7	1·15	15·2	14·7	1·03	12·9	11·4	1·13
	D14	I	19·3	14·4	1·34	13·4	8·6	1·56	17·1	12·4	1·38	15·2	13·0	1·17
		II	11·7	15·2	0·77	10·3	10·4	0·99	12·5	13·6	0·92	11·0	12·9	0·85
	D21	II	14·1	17·6	0·80	11·3	12·0	0·94	13·1	13·7	0·96	11·2	12·1	0·93
23	D3	I	18·3	12·7	1·44	12·4	8·5	1·46	15·3	11·7	1·31	15·0	14·0	1·07
		II	15·0	17·6	0·85	7·6	10·9	0·70	12·0	13·4	0·90	13·2	13·2	1·00
28	D3	I	16·4	11·3	1·45	7·4	4·2	1·76	11·4	6·9	1·65	13·4	11·7	1·15
		II	15·8	17·8	0·89	2·2	3·7	0·59	5·2	7·9	0·66	8·5	12·0	0·71
	D14	I	21·1	15·2	1·39	7·2	9·4	0·77	12·5	11·6	1·08	16·0	13·9	1·15
		II	11·5	15·9	0·72	2·9	3·3	0·88	5·4	6·9	0·78	9·3	10·5	0·89
	D21	II	14·3	17·4	0·82	7·9	10·2	0·78	9·1	13·0	0·70	11·6	12·3	0·94

Least significant differences ($P = 0\cdot05$) between any treatments within the groups indicated:

		R_N	R_W			R_N	R_W
8—23°C	I	2·9	2·2	23—28°C	I	2·0	1·3
	II	1·9	1·9		II	2·3	2·2

(b) *Effects of Suboptimal Temperatures*

Within the D14 treatments at 8 and 13°C, total nitrogen increased exponentially in the nodulated plants during periods I and II (Fig. 2). For any strain–temperature treatment, the R_N values were similar for both periods (Table 1). Furthermore, the R_N values for plants transferred to 8 and 13°C on day 21 (D21 treatment) were similar to those of the corresponding strains in the D14 treatment during period II. However, the total amount of nitrogen fixed by the D21 plants in this period was greater than that fixed by the D14 plants. These results indicate that the R_N is controlled by the root temperature, while the total amount of nitrogen fixed is a function of the amount of nitrogen present in the plants.

The R_N values for the nitrogen controls at 8 and 13°C were considerably higher than those for the nodulated plants, despite the decline in these values during period II. Unlike the results for the nodulated plants, the R_N values for the D3 treatments were at least equal to those for the D14 and D21 treatments at these temperatures.

At both 8 and 13°C the total plant dry weight of the D14 plants increased at a relatively high rate during period I (Fig. 2) and at 13°C, the values for TA1 and CC17 were similar to those for the nitrogen controls. During period II, the relative growth rate (R_W) declined, particularly at 8°C. As with the total nitrogen values, the D21 plants achieved the highest dry weight by the end of the experiment, with R_W values during period II greater than those for any other plants at these temperatures. However, this was associated with a marked fall in the percentage nitrogen level in the D21 plants (Fig. 3). By day 28 the nitrogen percentage for plants in the D21 treatment was similar to that being maintained by the plants in the D14 treatment.

During period II the plants nodulated by strains TA1 and CC17 in the D3 treatment at 13°C achieved R_N values similar to those of the corresponding D14 plants (Table 1). The rate of nitrogen fixation by the NA30 plants in the D3 treatment was less than that of the corresponding D14 plants in this period. For the D3 plants the increase in dry weight was retarded in relation to that observed in period I (Fig. 2), and there was a marked increase in the percentage nitrogen values, to a level similar to that of the D14 plants (Fig. 3).

The dry weight and nitrogen results for plants growing at 18°C were very similar to those plants at 23°C (Fig. 2; Table 1), apart from the initial difference in early nitrogen fixation [see Section III(*a*)].

Differences due to bacterial strain were found at both 8 and 13°C root temperatures. The total amount of nitrogen fixed by the strain NA30 plants was less than that of the plants nodulated by strains TA1 and CC17. Only with the D21 plants at 13°C did the R_N for the NA30 plants exceed that of the other strains, although the final total nitrogen value was still significantly lower than the value for TA1 and CC17. This indicates that plants nodulated by strain NA30 require a longer period at a favourable temperature before they will fix nitrogen at a high rate at a lower temperature.

(c) *Effects of Supraoptimal Temperature*

The chief features of the results for plants grown at 28°C was the differentiation between the strains, and the reduction in relative nitrogen assimilation rate during period II.

In period I strain TA1 plants in the D3 and D14 treatments at 28°C fixed nitrogen at a rate similar to each other and to that of plants growing at 23°C (Table 1); the differences in total nitrogen between the treatments reflect the difference in the initiation of nitrogen fixation after nodule formation (Fig. 2). The R_N values for the D3 and D14 plants inoculated with strains CC17 and NA30 were also similar to each

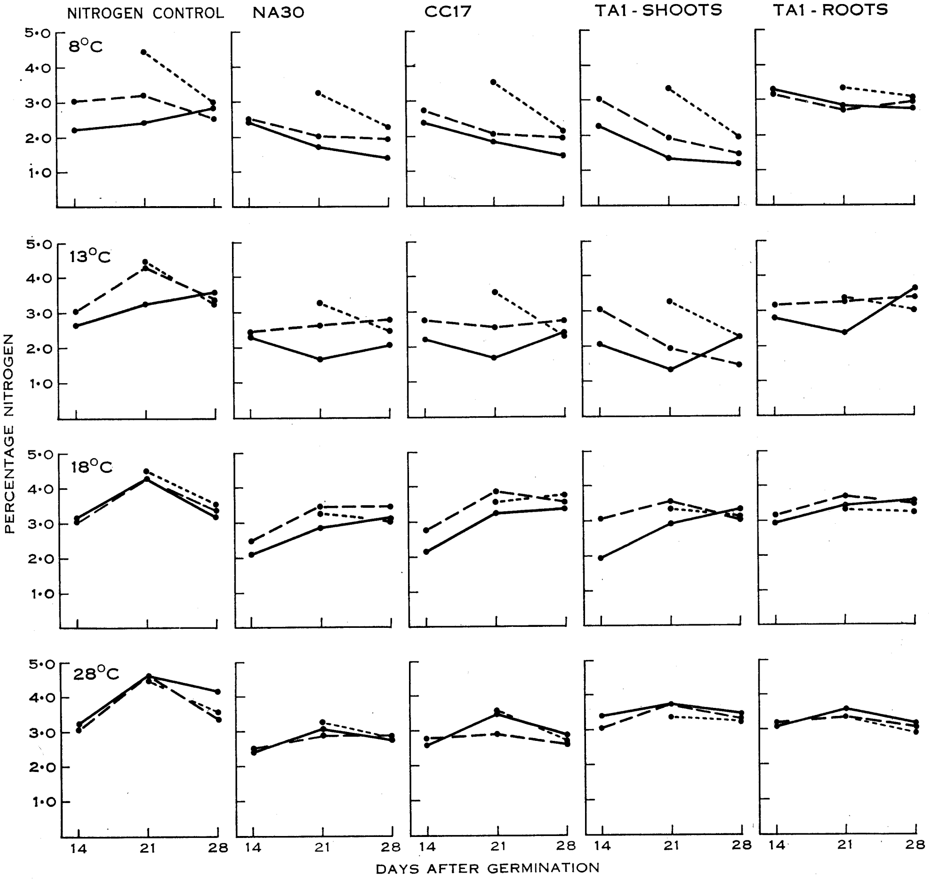

Fig. 3.—Percentage nitrogen values for whole plants (nitrogen controls, NA30- and CC17-inoculated plants) and shoots and roots of TA1-inoculated plants, grown at 8, 13, 18, and 28°C from 3 (——), 14 (– – –), and 21 (- - -) days after germination. Prior to transfer to these temperatures the plants were grown at 23°C.

other in period I, but they were less than the values for the corresponding plants at 23°C (80% for strain CC17, 60% for strain NA30). In period II the differentiation between the strains increased in the D3 and D14 treatments (Table 1, Fig. 2).

For the D3 and D14 plants at 28°C, the R_N values were lower in period II than period I, although within strains they were again similar. With the NA30 plants the R_N was 35% of the period I value, and the total amount of nitrogen fixed was also less than that fixed in period I (Fig. 2). The fall in R_N for the CC17 and TA1 plants was less (to 44 and 61% of the period I values respectively) but still considerable, especially for strain CC17. The R_N values for the D21 plants at 28°C were greater than those of the D3 and D14 plants during period II, but approximated the values of these treatments in period I.

(d) *Temperature Effects on the Distribution of Nitrogen and Dry Matter*

In Table 2 the increase in nitrogen in the shoots is expressed as a percentage of the increase in total plant nitrogen (nitrogen distribution index—Gates, Bouma, and Groenewegen 1961) for all TA1 plants during periods I and II. For the D3 plants the values were similar for both periods, provided the plants were fixing nitrogen. In three of the treatments (periods I and II at 8°C, and period I at 13°C) no nitrogen was fixed and nitrogen was translocated from the shoots to the roots. All D14 plants showed a similar distribution of the fixed nitrogen during period I (*c.* 70%), regardless of the temperature. In period II a lower proportion of the nitrogen increase was translocated to the shoots at temperatures below 23°C, so that at 8°C only 40% (0·28 mg nitrogen fixed) reached the shoots. A higher proportion of the fixed nitrogen reached the shoots at 28°C than at 23°C.

Table 2

Nitrogen and dry weight distribution indices between days 14–21 (period I) and days 21–28 (period II)

The plants were inoculated with strain TA1, and transferred to the various temperatures 3, 14, or 21 days after germination (D3, D14, and D21 treatments respectively)

Root Temperature (°C)	Harvest Period	Nitrogen Distribution Indices			Dry Weight Distribution Index		
		D3	D14	D21	D3	D14	D21
8	I	Negative	69	—	75	81	—
	II	Negative	40	61	48	76	83
13	I	Negative	67	—	64	76	—
	II	54	59	62	51	69	74
18	I	66	68	—	66	69	—
	II	69	67	68	69	72	70
23	I	72	—	—	71	—	—
	II	69	—	—	72	—	—
28	I	76	71	—	77	67	—
	II	76	81	79	74	79	74

For plants transferred from 23°C on day 21 (D21 treatment), the pattern for the distribution of nitrogen was similar to that found for D14 plants in period II, but the magnitude of the temperature effect was less.

The dry weight distribution indices were more variable than those for the distribution of nitrogen. A major difference between the two sets of values was that at 8°C a higher proportion of the increase in dry weight went into the shoots than it did at 23°C, at least during period I.

(e) *Nitrogen Fixation by Plants Transferred from Suboptimal to Optimal Temperatures*

The plants transferred from 8 and 13°C to 23°C rapidly formed nodules, and by day 21 there was an increase in total nitrogen over that for the plants remaining at the lower temperatures (Table 3). The results again show the superiority of strain TA1 over the other strains in establishing nodules and commencing to fix nitrogen. For plants at 18 and 28°C until day 14, transfer to 23°C had little effect on the amount of nitrogen fixed.

TABLE 3

TOTAL NITROGEN (MG/PLANT) FOR PLANTS TRANSFERRED FROM 8, 13, 18, AND 28°C TO 23°C ROOT TEMPERATURE 14 DAYS AFTER GERMINATION, OR LEFT AT THE ONE TEMPERATURE THROUGHOUT

Plants harvested 21 days after germination. 10 replicates

Treatment	Nitrogen Control	Strain NA30	Strain CC17	Strain TA1
8°C	0·70	0·36	0·36	0·35
8 → 23°C	1·41	0·38	0·40	0·49
13°C	1·43	0·38	0·36	0·35
13 → 23°C	2·21	0·45	0·50	0·61
18°C	2·20	0·78	1·02	1·00
18 → 23°C	2·34	0·61	1·06	0·90
23°C	1·89	1·06	1·41	1·58
28°C	1·63	0·69	0·97	1·46
28 → 23°C	1·52	0·76	0·91	1·30

IV. DISCUSSION

The results of the experiment reported show that the total amount of nitrogen fixed is controlled by the root temperature, the strain of bacteria forming the nodules, and the amount of nitrogen in the plants at the start of any growth period. Only the first two factors affect the relative nitrogen assimilation rate.

The effect of lower root temperatures is twofold. Firstly, nodule initiation and development is retarded at temperatures below 23°C (Gibson 1967), and as a consequence, the commencement of nitrogen fixation is delayed (Fig. 2). Secondly, the rate of nitrogen fixation by plants nodulated under favourable temperature conditions is reduced at temperatures lower than 23°C (Table 1). High root temperatures also have a twofold effect. Firstly, they stimulate a more rapid initiation of individual nodules, and a higher rate of nodule appearance, than is found at 23°C. Secondly, the commencement of nitrogen fixation is retarded (Fig. 2) and although the R_N is the same for these plants as those transferred from 23°C on day 14, there is a subsequent reduction in R_N (Table 1). The extent of this reduction is dependent on the bacterial strain forming the nodules.

(a) Relationship between R_N and R_W

In some treatments unusually high relative nitrogen assimilation rates (cf. Gibson 1965) are found for growth period I, which followed the commencement of nitrogen fixation or the assimilation of combined nitrogen. In this period the numerical value of the R_W of these plants is less than that of the R_N. For the subsequent growth period the R_N values are lower, but there is an increase in R_W, and the ratio of the two values approximates to 1·0. Examination of the percentage nitrogen values for the plants at 23°C throughout the experiment shows that on day 14 the values were 3·1, 2·8, and 2·5 for strains TA1, CC17, and NA30 respectively. By day 21 the respective values had risen to 3·3, 3·6, and 3·3, while the R_N/R_W ratios in this period were 1·07, 1·31, and 1·46. The percentage nitrogen values were maintained during period II, and except for strain NA30, the R_N/R_W values were close to unity. Similar comment applies to the D14 plants at 18°C.

These observations suggest that the rate of nitrogen fixation is so regulated that an overall balance is maintained between nitrogen fixation and dry weight increase. Below a particular nitrogen percentage, resources are diverted to increase the rate of nitrogen fixation, with a consequent fall in R_W. When the nitrogen percentage approaches a satisfactory level, the R_N declines, and R_W increases, so that the ratio R_N/R_W approximates to unity. Under favourable conditions, such as 18 and 23°C root temperature, the R_N during the period of adjustment may be higher than that achieved during the "balanced" growth of the plants. This indicates that factors other than root temperature are controlling the rate of nitrogen fixation during this period of growth.

Under other circumstances (e.g. at 8 and 13°C), the root temperature places a limitation upon the rate of nitrogen fixation, and the R_W falls to a numerical value comparable to that of the R_N. This limitation to the growth of nodulated plants at low root temperatures is a consequence of the reduced rates of nitrogen fixation. In period II the nitrogen percentage values remained at *c.* 2·7 at 13°C, and at *c.* 2·0 at 8°C, and the R_N/R_W was close to unity. This indicates that the plants are maintaining a "balanced" growth, albeit at a low rate, and that this will continue indefinitely. The actual value of the nitrogen percentage at which the plants will maintain a "balanced" growth varies with the root temperature. Furthermore, it is likely to vary with different symbiotic combinations (due to difference in the nitrogen percentage in the roots—Gibson 1966*a*), and different host species, while shoot temperature, light period, and light intensity may also exert an influence.

At 28°C root temperature the R_N/R_W ratio is greater than 1·0 for TA1 and CC17, especially in the D3 treatment where the percentage nitrogen values were low on day 14 (Fig. 3). In period II the R_N/R_W ratios vary between 0·8 and 1·0; however, there was a marked reduction in the R_N for all strains in this period. For strain NA30 and strain CC17 the reduction was more than 50% of the period I value and the absolute amount of nitrogen fixed was also less than during period I. Hence there has been a marked deterioration in the nitrogen-fixing capacity of the plants nodulated by these strains. This is possibly an effect of the length of exposure to the higher temperature rather than the age of the nodules. Plants transferred to 28°C on day 21 achieved R_N values in period II similar to those for the corresponding D3 and D14 treatments in period I.

The observation that the R_W values for many treatments rose in period II while the R_N values declined included the nitrogen control plants receiving ammonium nitrate. This supports the contention (Gibson 1966*b*) that the assimilation of combined nitrogen requires the utilization of significant quantities of carbohydrate that would otherwise increase plant dry weight. The same conclusion may be drawn for the nodulated plants. The present data do not permit a further comparison of the relative carbohydrate requirements for nitrogen assimilation by nodulated and nitrogen control plants.

(*b*) *Distribution of Nitrogen*

The retention of nitrogen within the roots of plants growing at low root temperatures, and its rapid translocation to the shoots under higher root temperature conditions (Table 3) confirms previous observations (Gibson 1966*a*). That this is not an effect associated with recently nodulated plants is shown by the fact that a lower proportion of the total nitrogen increase was translocated to the shoots in the D21 plants at 8 and 13°C, and a higher proportion went to the shoots in the D21 at 28°C. The translocation of nitrogen from the shoots to the roots in non-nodulated plants at low root temperature extends an earlier observation with uninoculated plants growing at 22°C (Gibson 1966*b*).

Apart from their physiological significance, the results have considerable practical implications. Plants which nodulate under favourable root temperature conditions are able to commence nitrogen fixation earlier than plants whose nodulation is delayed through the adverse effects of lower root temperature. With strains TA1 and CC17, which may be regarded as "effective" in their symbiotic association with the host, the R_N of plants nodulated at 8 and 13°C is similar to that of plants transferred to these temperatures after nodules have developed and nitrogen fixation commenced at a more favourable temperature. However, the results for strain NA30 ("intermediate effectiveness") at 13°C root temperatures suggest that it does not achieve the same R_N as NA30 plants nodulated under more favourable conditions. This may be due to additional delay to the commencement of nitrogen fixation (although nodule initiation and development was similar to that for strain TA1 —Gibson 1967) but if it is indicative of the response of the majority of the strains in this category of effectiveness, it would raise serious practical problems.

The results also show the considerable advantage to be gained where plants nodulate and grow under favourable conditions for a period before root temperatures decline. Although the subsequent R_N of such plants is the same as that of plants exposed to the less favorable conditions immediately after nitrogen fixation commences, there are large differences on the amount of nitrogen fixed, and growth made, during any period.

Previous work (Gibson 1967) showed that nodule initiation and development is greatly retarded at 7 and 12°C root temperature. The present results indicate that nitrogen fixation is very slow to commence at 8°C where the plants have nodulated under these conditions. Even at 13°C the commencement of nitrogen fixation is delayed until the plants are 3 weeks old, although the subsequent rates of nitrogen fixation are adequate to ensure continued growth.

Although higher temperatures stimulate nodule initiation, they delay the commencement of nitrogen fixation, while the subsequent rates of fixation are less than that found at 23°C. There is an indication that the symbiotic association deteriorates under continued exposure to 28°C, with the extent of this effect being dependent on the bacterial strain forming the nodules.

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VI. References

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